

Programa de Pós Graduação em Ecologia e Conservação Centro de Ciências Biológicas e da Saúde Universidade Federal de Mato Grosso Do Sul

### DINÂMICAS ECO-EVOLUTIVAS E EVOLUÇÃO DE ATRIBUTOS FENOTÍPICOS EM POPULAÇÕES DE ANUROS AO LONGO DE UM GRADIENTE DE URBANIZAÇÃO

Marcos Rafael Severgnini



Campo Grande, MS Fevereiro, 2025

### DINÂMICAS ECO-EVOLUTIVAS E EVOLUÇÃO DE ATRIBUTOS FENOTÍPICOS EM POPULAÇÕES DE ANUROS AO LONGO DE UM GRADIENTE DE URBANIZAÇÃO

Marcos Rafael Severgnini

Tese apresentada como requisito para a obtenção do título de **Doutor em Ecologia**, pelo Programa de Pós Graduação em Ecologia e Conservação, Universidade Federal de Mato Grosso do Sul.

Orientador: Prof. Dr. Diogo Borges Provete



### Banca avaliadora

Titulares

Dra. Monique Nouailhetas Simon [University of California]

Dra. Ana Paula Aprígio Assis [Universidade de São Paulo]

Dra. Tiana Kohlsdorf [Universidade de São Paulo]

Suplementes

Dra. Liliana Piatti [Universidade Federal de Mato Grosso do Sul]

Dr. Arthur Porto [Louisiana State University]

Dedico essa tese aos meus pais Laudir Severgnini e Neila Maria Severgnini que vieram da roça no interior do Paraná para tentar a vida na cidade. Ambos nem chegaram a completar o ensino fundamental, mas sempre souberam a importância dos estudos. Obrigado pelo trabalho duro no passado para que eu pudesse chegar até aqui no presente. Amo vocês!

Ao meu tio Leonir Severgnini in memoriam.

### Agradecimentos

Agradeço aos meus pais Laudir Severgnini e Neila Maria Severgnini. Sei que é difícil entender o motivo de um filho passar horas na frente de um computador "estudando" ou indo a campo para pesquisar sapos na cidade (é loucura, eu sei), por isso agradeço imensamente o apoio e o amor de vocês por me permitirem sonhar!

Às minhas irmãs agradeço ao apoio de alguma forma, inclusive a ajuda nas coletas.

À minha namorada e parceira Heloisa Medeiros, por me ajudar nas coletas, por entender minhas ausências e sempre estar ao meu lado nas conquistas e nas frustrações. Lhe amo <3

Aos meus colegas do laboratório de síntese em biodiversidade e da ecologia, Moroti, Adriana, Philip, Leandro, Daiene, Daniele e Jeane. As conversas diárias serviram com um alívio a intensa rotina da pós e os conselhos ajudaram muito no meu crescimento.

Agradeço a Bruna Yoshida, Philip, Klysman e muitos outros que dedicaram uma parte do seu tempo para me ajudar nos trabalhos de campo. Muito obrigado! Sem a ajuda de vocês isso seria impossível.

Aos proprietários das áreas rurais e urbanas onde realizamos as pesquisas, meu profundo agradecimento pela confiança e respeito pelo nosso trabalho.

A banca, agradeço pelas futuras sugestões que com certeza acrescentarão muito para melhorar o trabalho.

Agradeço também a minha bolsa CAPES que me sustentou nesses quatro anos e permitiu que essa pesquisa fosse possível.

A Lilian Franco-Belussi, muito obrigado pela paciência e gentileza ímpar em dedicar seu tempo para me explicar os procedimentos laboratoriais e me acompanhar no desenvolvimento do terceiro capítulo.

Ao Diogo Borges Provete, digo que esses seis anos de convivência (mestrado e doutorado) foram de muito aprendizado, respeito e admiração. Eu cheguei no laboratório como um girino e após essa longa metamorfose, hoje consigo dar meus primeiros saltos graças aos seus ensinamentos. Lições tais que ultrapassam a academia ou as vias científicas, são lições de vida, princípios, ética, e acima de tudo, de humanidade. É um privilégio ter tido você como orientador nessa jornada.

### Sumário

RESUMO GERAL	8
GENERAL ABSTRACT	10
Organização desta tese	12
INTRODUÇÃO GERAL	14
Ecological dynamics of frogs in tropical cities: Uncovering bias using literature review	a systematic 14
Abstract	15
Introduction	16
Material and Methods	18
Results	19
Discussion	21
Concluding remarks	30
References	32
Figures and Table legends	51
Supplementary material	55
CAPÍTULO 1	63
The effect of urbanisation and local environmental heterogeneity on variability of a tropical treefrog	n phenotypic 63
Abstract	64
Introduction	65
Methods	67
Results	69
Discussion	70
References	76
Figures and Table legends	85
Supporting Information	87
Supplementary Methods	87
Supplementary tables	93
Supplementary figures	96
CAPÍTULO 2	112
Frog call selection surface along an urbanisation gradient	112
Abstract	112
Introduction	113
Material and Methods	118
Results	125

Discussion126
Conclusion and Caveats
References
Figures and Table legends143
Supporting Information
CAPÍTULO 3159
How the Pace-of-Life Syndrome across multiple morphophysiological and behavioral traits in a tropical frog is affected by urban environments
Abstract160
Introduction161
Material and Methods166
Results173
Discussion
Conclusion
References
Figures and Table legends194
Supporting Information
CONCLUSÃO GERAL

SEVERGNINI, Marcos Rafael. **Dinâmicas eco-evolutivas e evolução de atributos fenotípicos em populações de anuros ao longo de um gradiente de urbanização**. 208f. 2025. Tese de Doutorado (Programa de Pós Graduação em Ecologia e Conservação) - Universidade Federal de Mato Grosso do Sul, Campo Grande, MS.

#### **RESUMO GERAL**

## Dinâmicas eco-evolutivas e evolução de atributos fenotípicos em populações de anuros ao longo de um gradiente de urbanização.

O processo de urbanização aumenta a fragmentação de habitats, produz ilhas de calor, poluição sonora, luminosa e química, e introduz novos patógenos. Essas modificações no ambiente provocam alterações em atributos fenotípicos, que por sua vez alteram as dinâmicas eco-evolutivas, impactando negativamente o fitness de organismos. No entanto, estudos sobre como a urbanização impacta a biodiversidade em países tropicais permanecem incipientes. Além disso, pouco se sabe como animais com baixa capacidade de dispersão, como os anfíbios respondem à urbanização nos trópicos. Os anfíbios ocupam ambientes aquáticos e terrestres, são ectotérmicos, possuem respiração cutânea e utilizam a vocalização para comunicação e encontro de parceiras. Portanto, são altamente propensos a responder às alterações ambientais associadas à urbanização. Para entender o impacto da urbanização em atributos morfológicos, acústicos, fisiológicos e comportamentais de anuros, essa tese foi dividida em três capítulos. No primeiro capítulo, testamos como efeitos diretos e indiretos da taxa de urbanização, heterogeneidade ambiental local, temperatura da superfície terrestre e gradiente espacial afetam a média e a variância de atributos morfológicos relacionados à aquisição de recursos, uso do ambiente, e capacidade de dispersão. Encontramos que o tamanho médio do corpo aumentou em poças mais urbanizadas, enquanto a variabilidade do tamanho do corpo e da forma da cabeça diminuiu. A variabilidade do comprimento da perna diminuiu com o aumento da temperatura da superfície. Entretanto, não há um padrão espacial claro de variação dos atributos ao longo do gradiente de urbanização, o que sugere uma seleção natural mais relaxada que pode ser explicada por um processo de urbanização recente. No segundo capítulo, estávamos interessados em testar como parâmetros acústicos do canto de anúncio variam ao longo de um gradiente de urbanização e se esses atributos estavam sob diferentes regimes de seleção natural. A média da frequência dominante (FD) não mudou entre as áreas, porém a variabilidade foi menor em áreas urbanas. Ainda, as populações rurais e urbanas parecem estar sob um fraco regime de seleção direcional em que indivíduos que vocalizam em altas frequência tiveram menor fitness. Nossos resultados apontam para um fraco regime de seleção estabilizadora em áreas urbanas, apesar da menor variabilidade da FD. Ainda, um menor fitness em vocalizações com altas frequências sugere que a urbanização pode impactar diretamente a seleção sexual nesses organismos. No terceiro capítulo, investigamos como o comportamento e atributos morfofisiológicos mudam em ambientes rurais e urbanos. Encontramos que animais de área urbana exibem comportamento de fuga menos intenso, indicando uma possível habituação à presença humana. Valores elevados do índice hepatosomático e glicose sugerem estresse crônico e exposição a xenobióticos. A coloração mais escura e as camadas mais espessas da pele em áreas urbanas conferem uma maior resposta desidratação e proteção contra patógenos. Ainda, animais mais velhos nas áreas urbanas tiveram uma diminuição das células brancas e vermelhas, o que pode estar associado a estresse oxidativo e possível imunossenescência precoce. Em conjunto, nossos resultados contribuem para elucidar os efeitos da urbanização sobre diferentes eixos do fenótipo em cidades tropicais, e como eles possivelmente alteram dinâmicas eco-evolutivas em populações naturais.

#### **GENERAL ABSTRACT**

## Eco-evolutionary dynamics and the evolution of phenotypic traits in frog populations along an urbanization gradient.

The urbanization process increases habitat fragmentation, produces heat islands effect, noise, light, and chemical pollution, and introduces new pathogens. These environmental alterations can change phenotypic traits, which in turn affect ecoevolutionary dynamics, with potential negative effects on organismal fitness. However, it remains unclear how urbanization impacts biodiversity in tropical countries. Furthermore, little is known about how animals with low dispersal abilities, such as amphibians, respond to urbanization in the tropics. Amphibians occupy both aquatic and terrestrial environments, are ectothermic, have cutaneous respiration, and their calling behavior is used in mate attraction. Therefore, they are highly prone to respond to changes inherent to cities. To study the impact of urbanization on the morphological, acoustic, physiological, and behavioral traits of anurans, this thesis was divided into three chapters. In the first chapter, we tested how the direct and indirect effects of urbanization rate, local environmental heterogeneity, land surface temperature, and spatial gradient affect the mean and variance of morphological traits related to resource acquisition and dispersal ability. We found that in more urbanized ponds, the mean body size increased, while body size and head shape variability decreased. Also, the leg length variability has decreased with increasing surface temperature. However, there was no clear spatial pattern of trait variation along urbanization gradient, suggesting a relaxed natural selection, which could be explained by a recent urbanization process. In the second chapter, we were interested in testing how acoustic traits vary along an urbanization gradient and whether these traits are under different natural selection regimes. The mean dominant frequency (DF) did not change between areas, but the variability was lower in urban ones. Additionally, rural and urban populations seem to be under a weak directional selection regime, in which individuals that vocalize at higher frequencies had lower fitness. Our results suggest a weak selection regime in urban areas despite the lower variability in DF. Furthermore, lower fitness in highfrequency calling suggests that urbanization may directly impact sexual selection in these organisms. In the third chapter, we investigated how behavior and morphophysiological traits change in response to divergent pace of life environments. We found that animals from urban areas displayed less intense escape behavior, indicating possible habituation to human presence. Elevated values in hepatosomatic indices and glucose suggest chronic stress and exposure to xenobiotics. Darker coloration and thicker skin layers in urban areas provide a stronger response to dehydration and protection against pathogens. Additionally, older animals in urban areas had a decrease in white and red blood cells, which may be associated with oxidative stress and potential immunosenescence. Our results together contribute to enlighten the effects of urbanization on different phenotype axes in tropical cities, especially those that change eco-evolutionary dynamics.

### Organização desta tese

Para entender como o processo de urbanização impacta as características morfológicas, acústicas, fisiológicas e comportamentais de anfíbios, esta tese foi organizada em três capítulos para satisfazer os diferentes objetivos propostos e facilitar a compreensão dos resultados obtidos.

A introdução geral da tese intitulada: "Ecological dynamics of frogs in tropical cities: Uncovering bias using a systematic literature review" consiste em uma revisão sistemática de literatura sobre ecologia urbana com anfíbios na região tropical, aponta vieses e propõe perspectivas para a ecologia urbana tropical. Portanto, além de fornecer um panorama geral dos efeitos da urbanização sobre a biodiversidade, a introdução geral também é um produto da tese que se encontra em fase de publicação. O capítulo estará disponível em Abril no livro "Ecology of Tropical Cities: Biodiversity, People & Places" publicado pela Springer Nature (https://link.springer.com/book/9783031708664).

O primeiro capítulo: "The effect of urbanisation and local environmental heterogeneity on phenotypic variability of a tropical treefrog" investigou como a taxa urbanização, a heterogeneidade ambiental local, a temperatura da superfície terrestre e o gradiente espacial afetam atributos fenotípicos associados à dispersão, aquisição de recursos, uso do ambiente, e performance de salto na perereca *Dendropsophus nanus* (Hylidae). Esse capítulo está em processo de revisão no periódico Ecology. O segundo capítulo: "Frog call selection surface along an urbanisation gradient" investigou como os parâmetros espectrais (e.g., frequência dominante) da vocalização em *Dendropsophus nanus* variam ao longo do gradiente de urbanização e testou se estes atributos estão sob diferentes regimes de seleção. Por fim, o terceiro capítulo: "How pace-of-life syndrome across

multiple morphophysiological and behavioral traits in frogs is affected in urban environments?" avaliou como atributos comportamentais e morfofisiológicos, especialmente aqueles correlacionados a habituação à presença humana, termorregulação, osmorregulação, imunidade e reprodução mudam em áreas rurais e urbanas utilizando o arcabouço de teoria de história de vida.

1	INTRODUÇÃO GERAL
2	
3	Capítulo 11 do livro "Ecology of Tropical Cities: Biodiversity, People & Places"
4	Springer Nature, DOI: 10.1007/978-3-031-70867-1_11
5	Ecological dynamics of frogs in tropical cities: Uncovering bias using a
6	systematic literature review
7	
8	Marcos R. Severgnini <sup>1</sup> , Carolina C. Ganci <sup>2</sup> , Franco L. Souza <sup>3</sup> , Mauricio Almeida-
9	Gomes <sup>3</sup> , Fábio H. S. Angeoletto <sup>4</sup> , Diogo B. Provete <sup>3,5</sup>
10	
11	<sup>1</sup> Graduate program in Ecology and Conservation, Institute of Biosciences, Federal
12	University of Mato Grosso do Sul, Campo Grande, 79002970, Mato Grosso do Sul,
13	Brazil
14	<sup>2</sup> Biology Department, University of Massachusetts Dartmouth, 285 Old Westport
15	Rd, Dartmouth, MA, 02747, USA.
16	<sup>3</sup> Institute of Biosciences, Federal University of Mato Grosso do Sul, Campo Grande,
17	79002970, Mato Grosso do Sul, Brazil
18	<sup>4</sup> Graduate Program in Geography, Federal University of Rondonópolis,
19	Rondonópolis, 78735000, Mato Grosso, Brazil.
20	<sup>5</sup> Gothenburg Global Biodiversity Centre, SE-405 30, Box 100, Göteborg, Sweden
21	
22	
23	
24	

- 25 Abstract
- 26

Earth is becoming increasingly urbanized. However, we are still lagging behind on 27 understanding the consequences of urbanization to biodiversity. This is essential if 28 we want to plan cities that are good for both people and nature. Here, we conduct a 29 systematic literature review on how ecological aspects of frogs respond to 30 urbanization in the tropical cities, including changes at behavioral, populational, and 31 community levels. We also, collected data on the gender of first and senior authors 32 to identify gender bias in authorship. We found a large geographical bias in research 33 34 on this topic, with most papers published by authors from Brazil and Australia. Most 35 studies were observational, investigating ecological aspects at population and community scale using adult frogs, while evolutionary aspects and tadpoles were 36 neglected. Urban environments from the tropical cities have different aspects than 37 those from the North. Most papers were authored by men in total and in both as first 38 and senior authors. We need to address knowledge gaps and improve research on 39 ecological dynamics of frogs in cities from the South if we want to build solid and 40 plural knowledge that can effectively be used to design conservation programs to 41 42 protect urban biodiversity in the Anthropocene.

43

Key-words: Urbanization, conservation, global change, gender bias, women in
 STEM;

- 46
- 47
- 48

49 Introduction

50

Urbanization is an anthropic process that generates significant changes in 51 the chemical, physical, and ecological conditions of the natural environment (Gerten 52 53 et al. 2019). It is estimated that around 54% of the world's population lives in urban environments, and that this proportion will be even higher in the future, especially in 54 developing countries, which will harbor 84% of the world urban population by 2030 55 (UNFPA 2007; Salmón et al. 2018; Tagil et al. 2018; UN-Habitat 2022). Given the 56 unprecedented growth of urban population, urbanization is currently one of the main 57 causes of biodiversity loss worldwide (G. Li et al. 2022; Simkin et al. 2022). 58 However, we are lagging behind in understanding how urbanization affects 59 biodiversity now and in the future. Given our dependence on ecosystem services 60 61 provided by nature (Díaz et al. 2018), we must take action to address this problem urgently. 62

Amphibians are among the most affected organisms by land use change 63 driven by urbanization (Hamer and McDonnell 2008; Beninde et al. 2015; Catenazzi 64 2015; G. Li et al. 2022), with around 35% of species being at some level of threat 65 (IUCN 2022). Characteristics shared by the group, such as a biphasic life cycle 66 (present in most amphibians), low dispersal ability (table 6.3 in Wells 2007) 67 compared to birds or mammals, and a highly permeable skin make amphibians 68 69 more prone to extinction due to processes associated with urbanization (Katzenberger et al. 2012; Hamer 2016; B. Li et al. 2016). Urbanization can change 70 both aquatic and terrestrial environments (Beninde et al. 2015), and consequently 71 affect amphibians in different ways. For example, Smallbone et al., (2011) found 72 that frog species richness decreased with increasing isolation of wetlands and 73 reduction of terrestrial vegetation cover caused by urbanization. Ganci et al., (2022) 74

also showed that frog species richness decreased as urbanization increased in a 75 tropical city, with high turnover between highly urbanized and less urbanized ponds. 76 In general, studies show that urbanization not only affects the survival rate of 77 individuals, but also greatly influences species richness and community structure of 78 amphibians in urban environments (Knutson et al. 1999; Rubbo and Kiesecker 79 2005: Hamer and McDonnell 2008: Pillsbury and Miller 2008: Yang et al. 2022). 80 However, most studies have been conducted in the Northern Hemisphere (see 81 Szulkin et al. 2020) and data about the ecological and evolutionary dynamics of 82 amphibian communities in tropical urban areas are still scant. 83

Tropical environments may respond to urbanization differently than 84 85 temperate ones, due to their distinct climate, geologic, and geomorphology conditions (Boulton et al. 2008). For example, environmental changes resulting from 86 urbanization tend to be intensified in the tropics (e.g., Marcacci et al. 2021), mostly 87 because they are experiencing unprecedented urban growth. Tropical cities have 88 different ages, development, growth rate, and history than those in the temperate 89 countries. While Europe took nearly two hundred years to transform itself from a 90 rural to a predominantly urban society (Landsberg 1981), tropical regions will do so 91 92 in less than sixty years (Oke et al. 1990; Givoni 1991; Jauregui 1997). Additionally, tropical zones harbor greater biodiversity than temperate ones (Brown 2014), which 93 makes the effect of urbanization even more worrying. 94

Here, we present a systematic literature review to summarize the effects of urbanization on amphibian ecological aspects focusing on tropical cities. We address aspects of individual (e.g., behavior), population dynamics, and community structure, as well as ecology, evolution, and conservation of amphibians in tropical urban areas. We also present a summary/consensus of proposals found in the literature for the conservation of amphibians and their associated aquatic habitats
in a growing urbanized planet. Plurality in knowledge production can be beneficial
for everyone, since more plural groups can have different yet complementary points
of view (Halpern et al. 2023). As a way to assess how equal authorship is on anuran
urban ecology, we also quantified gender bias on authorship.

- 105 Material and Methods
- 106

We conducted a literature search from June 2022 to January 2023 in 107 English on Scopus (www.scopus.com) to find papers related to the ecology, 108 evolution, and conservation of amphibians in tropical urban areas. We used the 109 following key terms combined to Boolean operators and asterisks as wildcard 110 111 symbol: amphibia\* OR anura\* OR frog\* AND urban\* OR cities OR city OR anthropogenic AND \*tropic\*. We followed the PRISMA 2.0 protocol (Page, 112 McKenzie, et al. 2021; Page, Moher, et al. 2021) (see Appendix 1 for a PRISMA 113 114 flow diagram). We also used a snowballing approach (Biernacki and Waldorf 1981; Johnson 2014; Naderifar et al. 2017) to track papers from the references or citations 115 of the papers found. Moreover, we used the Connected papers website 116 (www.connectedpapers.com) and Harzing's publish or perish software (Harzing 117 2007) using the same key terms to find additional papers not retrieved by Scopus. 118

Afterwards, we screened papers based on the title, abstract, main text, and reference list. The criteria for including papers were those that: (i) evaluated urbanization or some process related to it, such as anthropogenic noise, roads, buildings; (ii) related either ecological (e.g., composition, diversity, predation) or evolutionary patterns (e.g., phenotypic or genetic changes) to urbanization; (iii) related urbanization to other biological aspects (e.g., body condition, reproduction).

We excluded review papers, short notes, and papers that did not evaluate 125 126 urbanization effects (i.e., studies conducted in cities, but that did not test urbanization per se) (see Appendix 1 for a PRISMA flow diagram). Then, we built a 127 table to summarize characteristics of the papers selected, including: (a) authors and 128 year of study, (b) country, (c) study type, (d) organization level, (e) response 129 variable, (f) urbanization measure, impact or result, (g) evolutionary response, (h) 130 ecological response, (i) if evolution was detected, (j) spatial grain and extent, (k) 131 biological sample size; and (I) gender of authors and their position as first or senior. 132

We used author names in each paper to collect information about gender 133 134 identity. This approach based on names is limited and may be biased, since we cannot identify nonbinary people. To overcome this problem, we searched for 135 researchers' names in ResearchGate and their personal websites to confirm their 136 gender. We removed any name to which we could not confirm the gender. Then, we 137 calculated the proportion of female and male among authors and their position (first 138 or senior author). Finally, we explored these data visually and extracted quantitative 139 aspects. All graphs were made in R v. 4.2.1 (R Core Team 2023), with packages 140 ggplot2 (Wickham 2016), wordcloud (Fellows 2018), and tm (Feinerer et al. 2008). 141

- 142 **Results**
- 143

We screened 230 papers (202 from Scopus; 28 came from other search methods), of which 42 met the established criteria (Appendix 1). The most frequent words were: species, urbanization, habitat, frogs, diversity, richness, and ecology (Fig. 1). The number of studies published *per year* evaluating aspects related to ecology and evolution in tropical urban areas were low, with exception of 2019, 2021, and 2022 (Fig. 2). Most papers evaluating frogs and urbanization are concentrated in Brazil (N = 12), Australia (N = 7), China (N = 4), and South Africa (N = 4) (Fig. 3). Also, papers had more men (N = 129) than women (N = 62) as authors (fig. 4a). Moreover, gender differences were more remarkable when we compared the proportion of females and males in relation to authorship (16 papers were led by women; and 26 by men) and senior authorship (12 by women; and 30 by men) (fig. 4b, 4c).

Studies were mostly observational in nature (N = 37), two have experimental 156 and observational characteristics, and three had an experimental approach. Most 157 studies evaluated ecological and evolutionary aspects at population and community 158 159 levels (N= 21 and N = 20, respectively), and at least one study assessed a metacommunity. Only three out of 42 studies detected an evolutionary pattern. 160 Furthermore, the main response variables evaluated were abundance, species 161 richness, diversity, and composition (N = 17); calling behavior (N = 8); diet (N = 3); 162 reproduction (N = 1); habitat use (N = 1); morphology and phenotype (N = 6); genetic 163 diversity (N = 3); and body condition (N = 3). Many studies used adult frogs (N = 37)164 and only five used tadpoles. Most studies focused on diversity (alpha and beta), and 165 abundance (N = 17); and phenotypic changes following urbanization (N = 17), while 166 167 a minor part evaluated genetic diversity (N = 3) and niche dimensions (N = 5).

Most studies evaluating phenotypic changes focused mainly on shifts in calling behavior and temporal and spectral call parameters. These studies incorporated anthropogenic stressors (e.g., light pollution, vehicle noise, airplane noise, roads, engines) as explanatory variables. Conversely, few studies focused on morphological traits, such as body size, color patterns, parotoid gland, and health status (i.e., body condition). Studies that addressed how urbanization affects biodiversity evaluated mainly species richness and abundance (i.e., alpha diversity),

species composition (e.g., beta diversity - turnover and nestedness), probability of 175 176 occurrence, and occupancy. Furthermore, papers that assessed the impacts of cities on species niche focused mainly on predator-prey relationships, testing the 177 relationship between body size and mouth size and type of prev consumed (i.e., 178 impact niche sensu Chase and Leibold, 2003), and habitat use (requirement niche, 179 sensu Chase and Leibold, 2003). Finally, studies on genetic diversity assessed 180 181 population genetics and cytogenetics, focusing on genetic differentiation between populations (urban and rural anurans) (Appendix 2). 182

183

184 **Discussion** 

185

### 186 Ecological patterns on tropical cities

187

Studies with calling behavior showed that spectral parameters (e.g., calling 188 189 frequency) were higher and less variable in urban environments and sites close to roads, than in rural environments or sites distant from roads (e.g., Higham et al., 190 2021; Kruger and Du Preez, 2016; Leon et al., 2019). Conversely, three studies 191 found an opposite pattern, in which the dominant frequency decreased with 192 increasing urbanization (N. Liu et al. 2022) and anthropogenic noise (Caorsi et al. 193 194 2017) in some populations, but this pattern seems variable, since some studies did not detect any effect of noise (Lima et al., 2022). Call rate seems more variable and 195 consequently more prone to be affected by anthropogenic noise or urbanization 196 (e.g., Caorsi et al., 2017; Kaiser and Hammers, 2009). Also, some studies (Fig. 5) 197 found shorter calls and higher amplitude (Leon et al. 2019) that were longer (N. de 198 199 A. P. Lima et al. 2022), with either lower (Caorsi et al. 2017) or higher rate (Kaiser and Hammers 2009) in the presence of urban noise. Moreover, a paper found that
under light pollution, the peak calling season is anticipated (i.e., phenological shift).
Due to continuous light, frogs change their calling activity, calling all night (Dias et
al. 2019). In conclusion, both temporal and spectral call parameters of frogs seem
variable in relation to anthropogenic noise, light pollution in urban environments.

Papers evaluating morphological traits (Fig. 5), comparing rural (less 205 disturbed) and urban populations (highly disturbed) detected significant changes in 206 body size. A study found that parotoid glands in bufonids were smaller in urban than 207 in rural populations, while tibiofibula length of males in urban sites were longer than 208 209 rural populations. However, females showed a different pattern (Komine et al. 2022). 210 A paper evaluating body size and body condition found that species in undisturbed habitats were larger than in intermediate and in highly disturbed habitats, but had a 211 212 higher fluctuating asymmetry in tibia-fibula in natural habitats (Matías-Ferrer and Escalante 2015). Another paper found that frogs with larger body size and calling at 213 low dominant frequency were more tolerant to anthropogenic habitats, as well as 214 frogs that laid egg in water and have large or small clutches (G. Liu et al. 2021). 215 Moreover, closely-related species exhibited similar responses to anthropogenic 216 217 habitat in terms of their traits (G. Liu et al. 2021). One study found different color patterns in Engystomops pustulosus in urban and forest environments (Anderson et 218 al. 2019). Two studies evaluating behavior (Mühlenhaupt et al. 2022), and 219 morphology and performance (Mühlenhaupt et al. 2021) in tadpoles found no 220 differences between rural and urbanized populations. Overall, these studies show 221 that patterns related to phenotypic traits (e.g., body size) are highly variable in urban 222 environments when compared to undisturbed or natural habitats. Frogs usually have 223 better body condition in rural than urban habitats, with females heavier than males, 224

and males larger than females (Ofori et al. 2021). In general (e.g., Li et al., 2016;
Matías-Ferrer and Escalante, 2015) body condition (e.g., scaled mass index) is
negatively related to habitat disturbance.

Papers that evaluated trophic niche did not find a consensus when 228 comparing frog diet between urban and rural areas (Fig. 5). For example, a study 229 analyzing trophic niche (impact niche) showed that diversity of prey consumed by 230 anurans in urbanized areas was lower than in rural areas (Santana et al. 2019). 231 Conversely, another paper found a similar distribution of prey items between rural 232 and urban areas (Ofori et al. 2021). Also, a study that compared diet composition 233 234 (i.e., different prey taxa) across four cities found a large niche overlap and niche breadth between two frog species (Petrozzi et al. 2021). Finally, another study found 235 high diversity of ants in the diet of toads in urban sites (Oliveira-Souza et al. 2022). 236 A study analyzing niche dimensions related to habitat use (requirement niche) found 237 a strong effect of water body area and aquatic vegetation on the occurrence of frog 238 species in urban environments (Hamer and Parris 2011). Another study with 239 invasive frogs found that they were restricted to gardens (Ernst et al. 2011). Due to 240 the lower number of studies comparing diet or different niche dimension across 241 242 urban and rural areas, we did not find a clear pattern.

Several studies evaluating species diversity (Fig. 5) show that the levels of diversity (i.e., alpha and beta) and abundance decreased with urbanization (e.g., Bickford et al., 2010; Callaghan et al., 2021; Ganci et al., 2022; Kruger et al., 2015; MacGregor-Fors et al., 2013; Menin et al., 2019; Zhang et al., 2016). There was little consensus on patterns of beta diversity, when comparing urban and rural communities. Some studies found that beta diversity was driven by turnover along urbanization gradients (Holzer et al. 2017; Ganci et al. 2022), while others found it was driven by nestedness, in which species composition in urban sites are a subset
of non-urban sites (Lourenço-de-Moraes et al. 2018). Studies also showed a
negative influence of urbanization on species occupancy and occurrence probability
(e.g., Bajaru et al., 2020; Canessa and Parris, 2013; Holzer et al., 2017). Overall,
the main pattern was that all levels of diversity are negatively affected with
increasing urbanization.

256

### 257 The impact of geographical bias and knowledge gaps on urban ecology

258

259 In this review, most studies evaluating how urbanization impacts frogs focused on diversity patterns (e.g., alpha and beta). Overall, studies concluded that 260 species diversity and abundance decrease with increasing urbanization (e.g., more 261 generalists than specialist species in cities). Although these patterns have been 262 generated by different processes, the same overarching pattern emerged. Studies 263 evaluating how urbanization affects species richness increased over the years, but 264 not always added novel information. Also, some studies conducted in urban 265 environments did not discuss the importance of this novel ecosystem to ecological 266 or evolutionary aspects for the species evaluated. Some studies in South America 267 evaluated urban and rural sites as discrete categories (e.g., rural vs urban vs 268 suburban), instead of a gradient, as well as rural-urban gradient. This gradient 269 approach can bring interesting perspectives by allowing the detection of response 270 thresholds, for example, or at which percentage of urbanization level we find the 271 highest effect. 272

273 Most studies evaluating changes due to urbanization in tropical cities in the 274 tropical cities focused on adult frogs. Only five studies evaluated how urbanization

affected anuran larvae (Hamer and Parris 2011; Kruger et al. 2015; N. G. S. Lima 275 276 et al. 2019; Mühlenhaupt et al. 2021; Mühlenhaupt et al. 2022). Anurans have a complex life cycle (Wilbur 1980), and due to decoupled macroevolutionary 277 processes throughout ontogeny (Altig 2006; Sherratt et al. 2017), tadpoles and adult 278 frogs can respond in different ways to urbanization. Also, tadpoles are good models 279 to investigate adaptation to urban environments, since they have a highly plastic 280 phenotype (Levis and Pfennig 2016; Levis and Pfennig 2018; Levis and Pfennig 281 2020) that, in turn, can favor species to thrive in novel ecosystems, such as cities 282 (e.g., Stevens et al. 2023). Therefore, anuran larvae can reveal new ecological and 283 284 evolutionary patterns in urban areas that cannot be tested using only adult frogs.

285 Overall, studies that evaluated changes in calling behavior in response to urbanization or anthropogenic stressors, compared their findings to patterns from 286 the temperate countries (e.g., Caorsi et al. 2017; Dias et al. 2019). However, this 287 comparison can be misleading, because organisms can respond to environmental 288 change in different ways in the tropics. Thus, perhaps we might be comparing 289 "apples and oranges", i.e., patterns that cannot be compared since organisms have 290 distinct physiology and morphology (Bovo et al. 2023) in different global regions with 291 292 different evolutionary and ecological histories (see Marcacci et al. 2021). Also, tropical cities have particular building history and culture, distinct climatic conditions, 293 rapid rates of unplanned urban growth, introduction of invasive species by former 294 colonial powers, high native biodiversity living in cities, and effects of urban livestock 295 (McHale et al. 2013; Shackleton et al. 2021). All these factors invite us to change 296 our perspective when discussing ecological patterns found in tropical cities. Thus, 297 the main concerns and questions for ecology and evolution in tropical cities are: (i) 298 Are we building knowledge with empirical data that are biased? (ii) Are we 299

formulating hypothesis and building theories for the tropical cities based on patterns
 found in temperate countries (see McHale et al. 2013)? (iii) Do we need to adapt
 theories of urban ecology and evolutionary biology for the tropical cities?

303

### 304 **Overcoming Epistemic Injustice for a truly global urban ecology**

305

Epistemic injustice is a term coined by Fricker (2007) in the context of 306 feminist social epistemology. It refers to the systemic unfairness and discrimination 307 in knowledge production and dissemination. It has two components: testimonial and 308 309 hermeneutical injustice. Testimonial injustice happens when a speaker is given less 310 credit than she/he deserves due to prejudices of the hearer about a social group. Hermeneutical injustice on the other hand, happens when traditionally marginalized 311 groups lack the conceptual resources to make sense of certain distinctive social 312 experience (Grasswick 2018). For scientific knowledge production, hermeneutical 313 injustice can be further exacerbated by participatory epistemic injustice (sensu 314 Grasswick 2017), produced by the exclusion or poor representation of certain 315 groups in scientific research or marginalization of their knowledge systems (see 316 317 Nuñez et al. 2021; Primack et al. 2023).

This philosophical construct seems to apply to the field of urban ecology, given not only the strong gender bias in the authorship we found (Fig. 4), but also in the participation of tropical cities authors in the most prestigious journals, due to bias in peer review (Fox et al. 2023; Smith et al. 2023). This can have severe consequences for knowledge production in urban ecology, particularly in the tropical cities, where communities may have different and nuanced ways of understanding and interacting with their urban environments than those in the temperate countries

(see McHale et al. 2013). This type of injustice can manifest itself in various ways, 325 such as excluding local knowledge, dismissing the knowledge of marginalized 326 groups, or failing to recognize the expertise of scientists and researchers from 327 tropical cities (e.g., Smith et al. 2023, Primack et al. 2023). As a result, urban 328 ecology studies in tropical cities may be ignored or undervalued, which can lead to 329 incomplete or inaccurate understandings of urban areas and their challenges 330 (Nuñez et al. 2021). Epistemic injustice can further perpetuate existing power 331 imbalances (Kamath et al. 2022; Martínez-Blancas et al. 2023), where knowledge 332 production is dominated by those in positions of privilege and power (Nuñez et al. 333 334 2021; Hirschfeld et al. 2023). Power and privilege can manifest in different forms, be they in terms of the composition of editorial boards of journals, which have a 335 strong gender bias (Liévano-Latorre et al. 2020) that somehow impacts the fate of 336 manuscripts authored by women (Fox et al. 2016; Cássia-Silva et al. in press), or in 337 the centrality of knowledge production based on the temperate countries and 338 dominated by a Western view of science (Santos 2016). This highlights the 339 importance of acknowledging and valuing diverse forms of knowledge and expertise 340 in the field of urban ecology to ensure a more comprehensive and equitable 341 342 understanding of the complex issues facing urban areas in the tropical cities to ensure more accurate and holistic knowledge production. One way to overcome this 343 bias is to promote inclusive, diverse, and collaborative networks (Rayadin and 344 Buřivalová 2022; Halpern et al. 2023) based on respect and deprived of colonialist 345 practices (Haelewaters et al. 2021; Trisos et al. 2021). For example, many scientists 346 born in the tropical cities work in institutions in the temperate countries. Previous 347 studies have demonstrated that these expatriates tend to collaborate more 348 frequently with other fellow nationals, either in their home country or working in a 349

third country (Scellato et al. 2015). We must take advantage of the expertise of those expatriate individuals and the absence of language barriers to foster collaborative networks and secure funding to increase the scope and reduce the geographical bias of urban ecology studies. Only by recognizing the need to fight against epistemic injustice by increasing the participation of scientists from the tropical cities in the conversation about urban ecology we will make this a truly global science.

356

# 357 Summary of conservation actions to protect urban frog populations in the 358 tropical cities

359

The continuous increase of urban areas poses a serious threat to 360 amphibians worldwide, particularly in tropical regions. Therefore, establishing 361 conservation actions to maintain urban populations is urgent. For example, Ganci 362 et al. (2022) found that conserving large ponds in highly urbanized environments 363 might be a feasible strategy to maintain a higher number of species. Lee et al. (2022) 364 proposed an eight-step conservation framework to protect amphibian populations in 365 urban environments. They identify that the lack of public support for amphibian 366 conservation and limited monitoring data can harm efforts to promote amphibian 367 conservation. Furthermore, urban landscapes impose dispersal barriers to 368 amphibians (Fig. 6), such as gravel tracks and paved roads (Cayuela et al. 2019). 369 370 Finally, ecological findings must be applied in citywide planning processes (Fig. 6), management and restoration plans, policies and decision tools, in order to obtain 371 better results for amphibian conservation initiatives (Lee et al. 2022). 372

However, the incorporation of urban ecology and urban evolutionary biology
 into planning faces several obstacles. Tropical cities usually have a low capacity for

planning, environmental management or the elaboration of municipal environmental policies. For example, most medium-sized cities in Brazil, where 52 million people live, have few employees with academic training in the municipal environmental secretariats. Furthermore, the municipal secretariats for the environment are not autonomous: most of them are linked and subordinated to other municipal secretariats, such as municipal secretariats for agriculture (Angeoletto et al. 2016; Souza et al. 2023).

A concrete measure (Fig. 6) to mitigate the effects of urbanization on frogs 382 in the tropical cities must focus not only on green (e.g., parks), but also on blue 383 384 infrastructure (e.g., lakes, ephemeral and permanent ponds) (Beninde et al. 2015). 385 In order to improve the potential movement of frogs among urban wetlands, establishing green corridors connecting parks and ponds is essential (Lambert and 386 Donihue 2020; Lee et al. 2022). In addition, using anurans as surrogate taxa allow 387 designing more effective conservation planning in cities. Frogs can be a good target 388 taxa for freshwater conservation (Tisseuil et al. 2013) given their notably low 389 dispersal ability (Emerson 1985; Phillips et al. 2006; Semlitsch 2008). By prioritizing 390 frogs in conservation programs and urban planning, priority actions would be to 391 392 increase connectivity (Beninde et al. 2015, Tisseuil et al. 2013). This can potentially benefit species with high dispersal ability too, such as birds and mammals by 393 increasing gene flow and dispersal among patches, preventing genetic drift, a 394 common consequence of habitat fragmentation and isolation in cities (Miles et al. 395 2019; Rivkin et al. 2019). In this sense, it is essential to incorporate knowledge of 396 urban evolutionary biology in the environmental management of tropical cities, at 397 the risk of producing flawed conservation strategies that will result in the decline of 398 biodiversity (Lambert and Donihue 2020). 399

Geographical bias in studies of urban ecology, where research is 400 401 predominantly conducted in the temperate countries, can have significant impacts on conservation practice (McHale et al. 2013). This type of bias can lead to a limited 402 understanding of tropical urban areas and result in misguided conservation 403 practices that may not be effective in those regions. For example, a study that 404 focuses on the ecology of urban parks in the temperate countries may not be directly 405 applicable to the conservation of urban green spaces in the tropical cities, where the 406 ecology and social dynamics may be vastly different (McHale et al. 2013; Dobbs et 407 al. 2021). Moreover, if conservation practices are based on incomplete or biased 408 409 knowledge, they may exacerbate existing social and environmental inequalities in 410 the tropical cities. For instance, conservation measures that prioritize certain species or ecosystems without considering the needs and perspectives of local 411 412 communities (Valente-Neto et al. 2021) can lead to conflicts and further marginalization of these communities. Addressing geographical bias in studies of 413 urban ecology is thus crucial for ensuring more effective and equitable conservation 414 practices. This can be achieved by actively seeking out and incorporating diverse 415 perspectives, experiences, and knowledge systems from researchers and 416 417 communities in the tropical cities. Collaboration and partnerships between researchers in the temperate countries and south can also facilitate the exchange 418 of knowledge and promote more inclusive and informed conservation practices. 419

420

### 421 Concluding remarks

422

423 Overall, there are many studies on urban ecology in tropical cities, but few 424 about urban evolutionary biology when compared to temperate countries. Also,

studies evaluating changes in frog morphology driven by urbanization are still 425 incipient and share space with other phenotypic changes (e.g., calling behavior) or 426 studies focusing on the health status of frogs (e.g., body condition). Thus, we need 427 more innovative and daring studies that evaluate urbanization impacts on frogs in 428 the tropical cities and test hypothesis at local and regional scales, focusing on micro-429 and macroevolutionary process and patterns. We need to use tropical cities as 430 testbeds to study changes in phenotypes and genotypes, and use results and 431 discoveries to reformulate and expand urban evolutionary biology and ecology. 432

Furthermore, we need plurality when quantifying urbanization by using 433 434 multiple metrics, combining quantitative and multivariate approaches that 435 incorporate multifaceted measures of urbanization (e.g., human population density, house density, light pollution, anthropogenic noise; Moll et al. 2019), and gualitative 436 measures, such as social, economy, political, and cultural information (see Lembi 437 et al. 2020). Using combined information instead of only treating sites as rural vs 438 urban might be more useful to evaluate how much urbanization impacts biodiversity 439 and can help creating better urban planning for species conservation. 440

To conclude, urbanization introduces new challenges to species (Liker 441 442 2020). Amphibians are amid a global extinction crisis (Green et al. 2020) and their communities are undergoing biotic homogenization (Reboucas et al. 2021), mainly 443 due to land use change. In contrast, the rate of land cover change has increased 444 over the years and global urban population is expected to reach 6.5 billion people 445 by 2050 (United Nations, Department of Economic and Social Affairs, Population 446 Division 2019). Thus, understanding how species adapt to urban environments is 447 key to planning cities that are good for humans and biodiversity alike. 448

449

- 450 **References**
- 451
- Altig, Ronald. 2006. Tadpoles Evolved and Frogs Are the Default. *Herpetologica*62: 1–10. https://doi.org/10.1655/05-23.1.
- Anderson, Nigel K, Stephanie O Gutierrez, and Ximena E Bernal. 2019. From
- forest to city: urbanization modulates relative abundance of anti-predator
   coloration. *Journal of Urban Ecology* 5: juz016.
- 457 https://doi.org/10.1093/jue/juz016.
- Angeoletto, F., Santos, J. W. M. C., Ruiz Sanz, J. P., Silva, F. F. D., & Albertín, R.
- M. (2016). Tipología socio-ambiental de las ciudades medias de Brasil:
  aportes para un desarrollo urbano sostenible. Urbe. Revista Brasileira de
  Gestão Urbana, 8, 272-287.
- 462 Bajaru, Sameer, Saunak Pal, Mrugank Prabhu, Pinal Patel, Rahul Khot, and
- 463 Deepak Apte. 2020. A multi-species occupancy modeling approach to
- 464 access the impacts of land use and land cover on terrestrial vertebrates in
- 465 the Mumbai Metropolitan Region (MMR), Western Ghats, India. PLOS
- 466 ONE 15. Public Library of Science: e0240989.
- 467 https://doi.org/10.1371/journal.pone.0240989.
- Beninde, Joscha, Michael Veith, and Axel Hochkirch. 2015. Biodiversity in cities
- 469 needs space: A meta-analysis of factors determining intra-urban
- biodiversity variation. *Ecology Letters* 18: 581–592.
- 471 https://doi.org/10.1111/ele.12427.
- Bickford, David, Tze How Ng, Lan Qie, Enoka P Kudavidanage, and Corey J A
  Bradshaw. 2010. Forest Fragment and Breeding Habitat Characteristics
  Explain Frog Diversity and Abundance in Singapore. *Biotropica* 42. John

475 Wiley & Sons, Ltd: 119–125. https://doi.org/10.1111/j.1744-

476 **7429.2009.00542.x**.

- 477 Biernacki, Patrick, and Dan Waldorf. 1981. Snowball Sampling: Problems and
- 478 Techniques of Chain Referral Sampling. Sociological Methods & Research
- 47910. SAGE Publications Inc: 141–163.
- 480 https://doi.org/10.1177/004912418101000205.
- Boulton, Andrew J., Luz Boyero, Alan P. Covich, Michael Dobson, Sam Lake, and
- 482 Richard Pearson. 2008. Are Tropical Streams Ecologically Different from
- 483 Temperate Streams? *Tropical Stream Ecology*. Academic Press: 257–
- 484 284. https://doi.org/10.1016/B978-012088449-0.50011-X.
- Bovo, R P, M N Simon, D B Provete, M Lyra, C A Navas, and D V Andrade. 2023.
- 486 Beyond Janzen's Hypothesis: How Amphibians that Climb Tropical
- 487 Mountains Respond to Climate Variation. *Integrative Organismal Biology*.
  488 obad009. https://doi.org/10.1093/iob/obad009.
- Brown, James H. 2014. Why are there so many species in the tropics? *Journal of Biogeography* 41. John Wiley & Sons, Ltd: 8–22.
- 491 https://doi.org/10.1111/JBI.12228.
- 492 Callaghan, Corey T, Gracie Liu, Brittany A Mitchell, Alistair G B Poore, and Jodi J
- 493 L Rowley. 2021. Urbanization negatively impacts frog diversity at
- 494 continental, regional, and local scales. *Basic and Applied Ecology*.
- 495 https://doi.org/10.1016/j.baae.2021.04.003.
- 496 Canessa, Stefano, and Kirsten M Parris. 2013. Multi-Scale, Direct and Indirect
- 497 Effects of the Urban Stream Syndrome on Amphibian Communities in 498 Streams. *PLOS ONE* 8. Public Library of Science: e70262.
- 499 Caorsi, Valentina Zaffaroni, Camila Both, Sonia Cechin, Rógger Antunes, and

Márcio Borges-Martins. 2017. Effects of traffic noise on the calling 501 behavior of two Neotropical hylid frogs. PLOS ONE 12. Public Library of Science: e0183342. 502

Cássia-Silva. Overcoming the gender bias in Ecology and Evolution: is the double-503 anonymized peer review an effective pathway over time? PeerJ Accepted. 504

Catenazzi, Alessandro. 2015. State of the World's Amphibians. Annual Review of 505

Environment and Resources. Annual Reviews. 506

https://doi.org/10.1146/annurev-environ-102014-021358. 507

Cayuela, Hugo, Éric Bonnaire, Guillelme Astruc, and Aurélien Besnard. 2019. 508

Transport infrastructure severely impacts amphibian dispersal regardless 509

of life stage. Scientific Reports 9: 8214. https://doi.org/10.1038/s41598-510

019-44706-1. 511

500

Chase, Jonathan M., and Mathew A. Leibold. 2003. Ecological Niches Linking 512

Classical and Contemporary Approaches. 1st ed. Chicago: University of 513 Chicago Press. 514

515 Dias, Karina Soares, Elisa Stuani Dosso, Alexander S Hall, André Passaglia

Schuch, and Alexandro Margues Tozetti. 2019. Ecological light pollution 516

affects anuran calling season, daily calling period, and sensitivity to light in 517

natural Brazilian wetlands. The Science of Nature 106: 46. 518

https://doi.org/10.1007/s00114-019-1640-y. 519

Díaz, Sandra, Unai Pascual, Marie Stenseke, Berta Martín-López, Robert T. 520

Watson, Zsolt Molnár, Rosemary Hill, et al. 2018. Assessing nature's 521

contributions to people: Recognizing culture, and diverse sources of 522

knowledge, can improve assessments. Science 359. American 523

524 Association for the Advancement of Science: 270–272.

- https://doi.org/10.1126/SCIENCE.AAP8826/SUPPL\_FILE/AAP8826-DIAZSM.PDF.
- 527 Dobbs, Cynnamon, Alexis Vasquez, Pilar Olave, and Magdalena Olave. 2021.
- 528 Cultural Urban Ecosystem Services. In Urban Ecology in the Global South,
- 529 ed. Charlie M. Shackleton, Sarel S. Cilliers, Elandrie Davoren, and Marié
- J. du Toit, 245–264. Cham: Springer International Publishing.
- 531 https://doi.org/10.1007/978-3-030-67650-6\_10.
- 532 Emerson, Sharon B. 1985. Jumping and leaping. In *Functional vertebrate*
- 533 morphology, ed. M. Hildebrand, D. M. Bramble, K. F. Liem, and D. B.
- 534 Wake, 58–72. Cambridge: Harvard University Press.
- 535 Ernst, Raffael, David Massemin, and Ingo Kowarik. 2011. Non-invasive invaders
- 536 from the Caribbean: the status of Johnstone's Whistling frog
- 537 (*Eleutherodactylus johnstonei*) ten years after its introduction to Western
- 538 French Guiana. *Biological Invasions* 13: 1767–1777.
- 539 https://doi.org/10.1007/s10530-010-9930-5.
- 540 Feinerer, Ingo, Kurt Hornik, and David Meyer. 2008. Text Mining Infrastructure in
- 541 R. Journal of Statistical Software 025.
- 542 Fellows, I. 2018. wordcloud: Word Clouds. R package version 2.6,
- 543 https://CRAN.R-project.org/package=wordcloud.
- 544 Fox, Charles W., C. Sean Burns, and Jennifer A. Meyer. 2016. Editor and reviewer
- 545 gender influence the peer review process but not peer review outcomes at
- an ecology journal. *Functional Ecology* 30. John Wiley & Sons, Ltd: 140–
- 547 153. https://doi.org/10.1111/1365-2435.12529.
- Fox, Charles W., Jennifer Meyer, and Emilie Aimé. 2023. Double-blind peer review
   affects reviewer ratings and editor decisions at an ecology journal.

- *Functional Ecology*. John Wiley & Sons, Ltd. https://doi.org/10.1111/1365 2435.14259.
- Fricker, Miranda. 2007. *Epistemic injustice: power and the ethics of knowing*.
  Oxford ; New York: Oxford University Press.
- 554 Ganci, Carolina Cunha, Diogo B Provete, Thomas Püttker, David Lindenmayer,
- and Mauricio Almeida-Gomes. 2022. High species turnover shapes
- anuran community composition in ponds along an urban-rural gradient.
- 557 Urban Ecosystems 25: 633–642. https://doi.org/10.1007/s11252-021-
- 558 **01174-8**.
- 559 Gerten, Christian, Stefan Fina, and Karsten Rusche. 2019. The Sprawling Planet:
- 560 Simplifying the Measurement of Global Urbanization Trends. *Frontiers in*
- 561 Environmental Science 7. Frontiers Media S.A.: 140.
- 562 https://doi.org/10.3389/FENVS.2019.00140.
- 563 Givoni, B. 1991. Impact of planted areas on urban environmental quality: A review.
- 564 Atmospheric Environment. Part B. Urban Atmosphere 25. Elsevier: 289–
- 565 299. https://doi.org/10.1016/0957-1272(91)90001-U.
- 566 Grasswick, Heidi. 2017. Epistemic Injustice in Science. In The Routledge
- 567 *Handbook of Epistemic Injustice*. Routledge.
- <sup>568</sup> https://doi.org/10.4324/9781315212043.ch30.
- 569 Grasswick, Heidi. 2018. Feminist Social Epistemology. In *The Stanford*
- 570 *Encyclopedia of Philosophy*, ed. Edward N. Zalta, Fall 2018. Metaphysics
   571 Research Lab, Stanford University.
- 572 Green, David M, Michael J Lannoo, David Lesbarrères, and Erin Muths. 2020.
- 573 Amphibian Population Declines: 30 Years of Progress in Confronting a
- 574 Complex Problem. *Herpetologica* 76: 97–100.
https://doi.org/10.1655/0018-0831-76.2.97.

Haelewaters, Danny, Tina A Hofmann, and Adriana L Romero-Olivares. 2021. Ten
simple rules for Global North researchers to stop perpetuating helicopter
research in the Global South. *PLOS Computational Biology* 17. Public
Library of Science: e1009277.

Halpern, Benjamin S, Carl Boettiger, Michael C Dietze, Jessica A Gephart, Patrick
 Gonzalez, Nancy B Grimm, Peter M Groffman, et al. 2023. Priorities for
 synthesis research in ecology and environmental science. *Ecosphere* 14.

583 John Wiley & Sons, Ltd: e4342. https://doi.org/10.1002/ecs2.4342.

584 Hamer, Andrew J. 2016. Accessible habitat delineated by a highway predicts

585 landscape-scale effects of habitat loss in an amphibian community.

586 *Landscape Ecology* 31. Springer Netherlands: 2259–2274.

587 https://doi.org/10.1007/S10980-016-0398-2/FIGURES/3.

588 Hamer, Andrew J, and Mark J McDonnell. 2008. Amphibian ecology and

589 conservation in the urbanising world: A review. *Biological Conservation* 

590 141: 2432–2449. https://doi.org/10.1016/j.biocon.2008.07.020.

591 Hamer, Andrew J, and Kirsten M Parris. 2011. Local and landscape determinants

of amphibian communities in urban ponds. *Ecological Applications* 21.

John Wiley & Sons, Ltd: 378–390. https://doi.org/10.1890/10-0390.1.

Harzing, A.W. 2007. Publish or Perish, available from

595 https://harzing.com/resources/publish-or-perish.

Higham, V., N. D.S. Deal, Y. K. Chan, C. Chanin, E. Davine, G. Gibbings, R.

- 597 Keating, et al. 2021. Traffic noise drives an immediate increase in call
- 598 pitch in an urban frog. *Journal of Zoology* 313. Blackwell Publishing Ltd:

599 307–315. https://doi.org/10.1111/JZO.12866.

600	Hirschfeld, María N.Clerici, Luiz R.R. Faria, and Carlos Roberto Fonseca. 2023.
601	Avoid the reproduction of coloniality in decolonial studies in ecology.
602	Nature Ecology & Evolution 2023 7. Nature Publishing Group: 306–309.
603	https://doi.org/10.1038/s41559-022-01971-0.
604	Holzer, Katie A, Robert P Bayers, Thien Tao Nguyen, and Sharon P Lawler. 2017.
605	Habitat value of cities and rice paddies for amphibians in rapidly
606	urbanizing Vietnam. Journal of Urban Ecology 3: juw007.
607	https://doi.org/10.1093/jue/juw007.
608	IUCN. 2022. The IUCN Red List of Threatened Species. Version 2022-2.
609	https://www.iucnredlist.org.
610	Jauregui, Ernesto. 1997. The last Ms for 40th anniversary issue. Aspects of urban
611	human biometeorology. International Journal of Biometeorology 40.
612	Springer New York: 58–61.
613	https://doi.org/10.1007/BF02439413/METRICS.
614	Johnson, Timothy P. 2014. Snowball Sampling: Introduction. In Wiley StatsRef:
615	Statistics Reference Online.
616	https://doi.org/10.1002/9781118445112.stat05720.
617	Kaiser, Kristine, and Jessica L Hammers. 2009. The Effect of Anthropogenic Noise
618	on Male Advertisement Call Rate in the Neotropical Treefrog,
619	Dendropsophus triangulum. Behaviour 146. Brill: 1053–1069.
620	Kamath, Ambika, Beans Velocci, Ashton Wesner, Nancy Chen, Vince Formica,
621	Banu Subramaniam, and María Rebolleda-Gómez. 2022. Nature, Data,
622	and Power: How Hegemonies Shaped This Special Section. The
623	American Naturalist 200. The University of Chicago Press: 81–88.
624	https://doi.org/10.1086/720001.

Katzenberger, Marco, Miguel Tejedo, Helder Duarte, Federico Marangoni, and 625 Juan Francisco Beltrán. 2012. Tolerância e sensibilidade térmica em 626 anfíbios. Revista da Biologia 8. Universidade de Sao Paulo, Agencia USP 627 de Gestao da Informacao Academica (AGUIA): 25-32. 628 https://doi.org/10.7594/REVBIO.08.05. 629 Knutson, M G, J R Sauer, D A Olsen, M J Mossman, L M Hemesath, and M J 630 Lannoo. 1999. Effects of landscape composition and wetland 631 fragmentation on frog and toad abundance and species richness in Iowa 632 and Wisconsin, USA. Conservation Biology 13: 1437–1446. 633 634 Komine, Hirotaka, Kiyomi Yasumiba, and Lin Schwarzkopf. 2022. The country 635 toad and the city toad: comparing morphology of invasive cane toads (Rhinella marina) from rural and urban environments. Biological Journal of 636 the Linnean Society: blac100. https://doi.org/10.1093/biolinnean/blac100. 637 Kruger, Donnavan J.D., and Louis H. Du Preez. 2016. The effect of airplane noise 638 on frogs: a case study on the Critically Endangered Pickersgill's reed frog 639 (Hyperolius pickersgilli). Ecological Research 31. Springer Tokyo: 393-640 405. https://doi.org/10.1007/S11284-016-1349-8/FIGURES/9. 641 Kruger, Donnavan J D, Andrew J Hamer, and Louis H Du Preez. 2015. 642 Urbanization affects frog communities at multiple scales in a rapidly 643 developing African city. Urban Ecosystems 18: 1333–1352. 644 https://doi.org/10.1007/s11252-015-0443-y. 645 Lambert, Max R, and Colin M Donihue. 2020. Urban biodiversity management 646 using evolutionary tools. *Nature Ecology & Evolution* 4: 903–910. 647 https://doi.org/10.1038/s41559-020-1193-7. 648 Landsberg, H. 1981. The Urban Climate. The Urban Climate. Academic Press: 649

**275**.

651	Lee, Tracy S, Lea A Randall, Nicole L Kahal, Holly L Kinas, Vanessa A Carney,
652	Heather Rudd, Tyne M Baker, et al. 2022. A framework to identify priority
653	wetland habitats and movement corridors for urban amphibian
654	conservation. Ecological Solutions and Evidence 3. John Wiley & Sons,
655	Ltd: e12139. https://doi.org/10.1002/2688-8319.12139.
656	Lembi, Rafael Cavalcanti, Cecilia Cronemberger, Caroline Picharillo, Sheina
657	Koffler, Pedro H Albuquerque Sena, Jéssica Francine Felappi, Alice
658	Ramos de Moraes, Adnan Arshad, Jessie Pereira dos Santos, and
659	Andressa Vianna Mansur. 2020. Urban expansion in the Atlantic Forest:
660	applying the Nature Futures Framework to develop a conceptual model
661	and future scenarios. Biota Neotropica . scielo .
662	Leon, Evelina, Paola M Peltzer, Rodrigo Lorenzon, Rafael C Lajmanovich, and
663	Adolfo H Beltzer. 2019. Effect of traffic noise on Scinax nasicus
664	advertisement call (Amphibia, Anura) . Iheringia. Série Zoologia . scielo .
665	Levis, Nicholas A, and David W Pfennig. 2016. Evaluating 'Plasticity-First '
666	Evolution in Nature: Key Criteria and Empirical Approaches. Trends in
667	Ecology & Evolution 31: 563–574.
668	https://doi.org/10.1016/j.tree.2016.03.012.
669	Levis, Nicholas A, and David W Pfennig. 2018. Phenotypic plasticity, canalization,
670	and the origins of novelty: Evidence and mechanisms from amphibians.
671	Seminars in Cell & Developmental Biology 88: 80–90.
672	https://doi.org/10.1016/j.semcdb.2018.01.012.
673	Levis, Nicholas A, and David W Pfennig. 2020. Plasticity-led evolution: A survey of
674	developmental mechanisms and empirical tests. Evolution & Development

675	22:71-87.	John Wiley	& Sons.	https://doi.org	g/10.1111/ede.123	309.
		,				

- Li, Ben, Wei Zhang, Xiaoxiao Shu, Enle Pei, Xiao Yuan, Yujie Sun, Tianhou Wang,
   and Zhenghuan Wang. 2016. The Impacts of Urbanization on the
- Distribution and Body Condition of the Rice-paddy Frog (*Fejervarya*
- 679 *multistriata*) and Gold-striped Pond Frog (*Pelophylax plancyi*) in Shanghai,
- 680 China. Asian Herpetological Research 7: 200–209.
- 681 https://doi.org/10.16373/j.cnki.ahr.150061.
- Li, Guangdong, Chuanglin Fang, Yingjie Li, Zhenbo Wang, Siao Sun, Sanwei He,
- 683 Wei Qi, et al. 2022. Global impacts of future urban expansion on terrestrial
- 684 vertebrate diversity. *Nature Communications 2022 13:1* 13. Nature
- 685 Publishing Group: 1–12. https://doi.org/10.1038/s41467-022-29324-2.
- Liévano-Latorre, Luisa F, Rafaela Aparecida da Silva, Raísa R S Vieira, Fernando
- 687 M Resende, Bruno R Ribeiro, Fábio J A Borges, Lilian Sales, and Rafael
- 688 Loyola. 2020. Pervasive gender bias in editorial boards of biodiversity
- conservation journals. *Biological Conservation* 251: 108767.
- 690 https://doi.org/10.1016/j.biocon.2020.108767.
- Liker, András. 2020. Biologia Futura: adaptive changes in urban populations.
- 692 Biologia Futura 71: 1–8. https://doi.org/10.1007/s42977-020-00005-9.
- Lima, Natalya de Araujo Pedrosa, Raoni Rebouças, Luís Felipe Toledo, and
- 694 Cybele Sabino Lisboa. 2022. Influence of urban noise in call traits of the
- 695 Atlantic Forest treefrog Bokermannohyla hylax. Zoologischer Anzeiger
- 696 **300:** 41–46. https://doi.org/10.1016/j.jcz.2022.07.002.
- Lima, Nathália G S, Ubirajara Oliveira, Rafael C C Souza, and Paula C Eterovick.
   2019. Dynamic and diverse amphibian assemblages: Can we differentiate
   natural processes from human induced changes? *PLOS ONE* 14. Public

- Liu, Gracie, Jodi J L Rowley, Richard T Kingsford, and Corey T Callaghan. 2021.
- 702 Species 'traits drive amphibian tolerance to anthropogenic habitat
- 703 modification. Global Change Biology 27. John Wiley & Sons, Ltd: 3120-
- 704 3132. https://doi.org/10.1111/gcb.15623.
- Liu, Ningning, Shurong Zhong, Tianhou Wang, Xiuzhen Li, Li Wei, Chunjing Zou,
- <sup>706</sup> Shanshan Zhao, and Ben Li. 2022. Advertisement Call Variation of Two
- <sup>707</sup> Frog Species along an Urban-Rural Gradient in Shanghai, China.
- 708 *Diversity*. https://doi.org/10.3390/d14070550.
- Lourenço-de-Moraes, Ricardo, Leo R Malagoli, Vinicius Guerra, Rodrigo B
- Ferreira, Igor de Paiva Affonso, Célio F B Haddad, Ricardo J Sawaya, and
- 711 Rogério P Bastos. 2018. Nesting patterns among Neotropical species
- assemblages: can reserves in urban areas be failing to protect anurans?
- 713 Urban Ecosystems 21: 933–942. https://doi.org/10.1007/s11252-018-
- 714 **0767-5**.
- 715 MacGregor-Fors, Ian, Omar Hernández Ordoñez, and Rubén Ortega-Álvarez.
- 2013. Urban croaking: diversity and distribution of anurans in a neotropical
- city. Urban Ecosystems 16: 389–396. https://doi.org/10.1007/s11252-012-
- 718 **0267-y**.
- 719 Marcacci, Gabriel, Catrin Westphal, Arne Wenzel, Varsha Raj, Nils Nölke, Teja
- Tscharntke, and Ingo Grass. 2021. Taxonomic and functional
- homogenization of farmland birds along an urbanization gradient in a
- tropical megacity. *Global Change Biology* 27: 4980–4995. John Wiley &
- 723 Sons, Ltd. https://doi.org/10.1111/gcb.15755.
- 724 Martínez-Blancas, Alejandra, Arona Bender, Verónica Zepeda, Rosa McGuire,

Olivia Tabares, Priyanga Amarasekare, Alicia Mastretta-Yanes, Maria 725 726 Miriti, Ana M. C. Santos, and Marcel C. Vaz. 2023. Surviving Racism and Sexism in Academia: Sharing Experiences, Insights, and Perspectives. 727 The Bulletin of the Ecological Society of America 104. John Wiley & Sons, 728 Ltd: e02033. https://doi.org/10.1002/BES2.2033. 729 Matías-Ferrer, Noemí, and Patricia Escalante, 2015, Size, body condition, and 730 limb asymmetry in two hylid frogs at different habitat disturbance levels in 731 Veracruz, México. Herpetological Journal 25: 169-176. 732 https://doi.org/British Herpetological Society. 733 734 McHale, Melissa R, David N Bunn, Steward T A Pickett, and Wayne Twine. 2013. Urban ecology in a developing world: why advanced socioecological 735 theory needs Africa. Frontiers in Ecology and the Environment 11. John 736 Wiley & Sons, Ltd: 556–564. https://doi.org/10.1890/120157. 737 Menin, Marcelo, Rafaela Fernanda Batista Ferreira, Izomar Barbosa Melo, 738 Marcelo Gordo, Gustavo Yomar Hattori, and Bruno Sampaio Sant'anna. 739 740 2019. Anuran diversity in urban and rural zones of the Itacoatiara municipality, central Amazonia, Brazil. Acta Amazonica 49: 122-130. 741 https://doi.org/10.1590/1809-4392201800284. 742 Miles, Lindsay S, L Ruth Rivkin, Marc T J Johnson, Jason Munshi-South, and 743 Brian C Verrelli. 2019. Gene flow and genetic drift in urban environments. 744 Molecular Ecology 28. John Wiley & Sons, Ltd: 4138–4151. 745 https://doi.org/10.1111/mec.15221. 746 Moll, Remington J, Jonathon D Cepek, Patrick D Lorch, Patricia M Dennis, Eric 747 Tans, Terry Robison, Joshua J Millspaugh, and Robert A Montgomery. 748 2019. What does urbanization actually mean? A framework for urban 749

750	metrics in wildlife research. Journal of Applied Ecology 0. John Wiley &
751	Sons, Ltd: 1–12. https://doi.org/10.1111/1365-2664.13358.
752	Mühlenhaupt, Max, James Baxter-Gilbert, Buyisile G Makhubo, Julia L Riley, and
753	John Measey. 2021. Growing up in a new world: trait divergence between
754	rural, urban, and invasive populations of an amphibian urban invader.
755	NeoBiota 69. Pensoft Publishers: 103–132.
756	Mühlenhaupt, Max, James Baxter-Gilbert, Buyisile G Makhubo, Julia L Riley, and
757	John Measey. 2022. No evidence for innate differences in tadpole
758	behavior between natural, urbanized, and invasive populations. Behavioral
759	Ecology and Sociobiology 76: 11. https://doi.org/10.1007/s00265-021-
760	03121-1.
761	Naderifar, Mahin, Hamideh Goli, and Fereshteh Ghaljaei. 2017. Snowball
762	Sampling: A Purposeful Method of Sampling in Qualitative Research.
763	Strides in Development of Medical Education 14: e67670.
764	https://doi.org/10.5812/sdme.67670.
765	Nuñez, Martin A., Mariana C. Chiuffo, Aníbal Pauchard, and Rafael D. Zenni.
766	2021. Making ecology really global. Trends in Ecology & Evolution 36.
767	Elsevier Current Trends: 766–769.
768	https://doi.org/10.1016/J.TREE.2021.06.004.
769	Ofori, Benjamin Yeboah, John Bosu Mensah, Roger Sigismund Anderson, and
770	Daniel Korley Attuquayefio. 2021. Diet composition, body condition and
771	sexual size dimorphism of the common African toad Amietophrynus
772	regularis) in urban and agricultural landscape. bioRxiv:
773	2021.01.25.428067. https://doi.org/10.1101/2021.01.25.428067.
774	Oke, T. R., Roger Taesler, and Lars E. Olsson. 1990. The tropical urban climate

775	experiment (TRUCE). Energy and Buildings 15. Elsevier: 67–73.
776	https://doi.org/10.1016/0378-7788(90)90117-2.
777	Oliveira-Souza, Aline Emanuele, Maria Madalena Salviano Santana, Maria
778	Jeovana Lima Martins, Jessica Stefany Costa Anaissi, Patrick Ribeiro
779	Sanches, and Carlos Eduardo Costa-Campos. 2022. Diversity of ants in
780	the diet of <i>Rhinella major</i> (Anura: Bufonidae) in an urban area in North
781	Brazil. Herpetology Notes 15: 663–670.
782	Page, Matthew J, Joanne E McKenzie, Patrick M Bossuyt, Isabelle Boutron,
783	Tammy C Hoffmann, Cynthia D Mulrow, Larissa Shamseer, et al. 2021.
784	The PRISMA 2020 statement: an updated guideline for reporting
785	systematic reviews. BMJ 372: n71. https://doi.org/10.1136/bmj.n71.
786	Page, Matthew J, David Moher, Patrick M Bossuyt, Isabelle Boutron, Tammy C
787	Hoffmann, Cynthia D Mulrow, Larissa Shamseer, et al. 2021. PRISMA
788	2020 explanation and elaboration: updated guidance and exemplars for
789	reporting systematic reviews. BMJ 372: n160.
790	https://doi.org/10.1136/bmj.n160.
791	Petrozzi, F, G C Akani, E A Eniang, S N Ajong, S M Funk, J E Fa, N Amadi, D
792	Dendi, and L Luiselli. 2021. Generalist, selective or 'mixed 'foragers?
793	Feeding strategies of two tropical toads across suburban habitats. Journal
794	of Zoology 315. John Wiley & Sons, Ltd: 288–300.
795	https://doi.org/10.1111/jzo.12925.
796	Phillips, Benjamin L, Gregory P Brown, Jonathan K Webb, and Richard Shine.
797	2006. Invasion and the evolution of speed in toads. Nature 439: 803.
798	https://doi.org/10.1038/439803a.
799	Pillsbury, Finn C, and James R Miller. 2008. Habitat and Landscape

800	Characteristics Underlying Anuran Community Structure along an Urban-
801	Rural Gradient. Ecological Applications 18. Ecological Society of America:
802	1107–1118.
803	Primack, Richard B., Tara K. Miller, Carina Terry, Erika Marín-Spiotta, Pamela H.
804	Templer, Asmeret Asefaw Berhe, Emily J. Diaz Vallejo, et al. 2023.
805	Historically excluded groups in ecology are undervalued and poorly
806	treated. Frontiers in Ecology and the Environment. John Wiley & Sons,
807	Ltd. https://doi.org/10.1002/fee.2613.
808	R Core Team. 2023. R: A language and environment for statistical computing. R
809	Foundation for Statistical Computing, Vienna, Austria. Vienna, Austria:
810	URL https://www.R-project.org/.
811	Rayadin, Yaya, and Zuzana Buřivalová. 2022. What does it take to have a
812	mutually beneficial research collaboration across countries? Conservation
813	Science and Practice 4. John Wiley & Sons, Ltd: e528.
814	https://doi.org/10.1111/CSP2.528.
815	Rebouças, Raoni, Marcileida M Dos Santos, Ana Glaucia da Silva Martins, Adão
816	Henrique Rosa Domingos, Isaias Santos, and Luís Felipe Toledo. 2021.
817	Warming drives cryptic declines of amphibians in eastern Brazil. Biological
818	Conservation 256: 109035. https://doi.org/10.1016/j.biocon.2021.109035.
819	Rivkin, L. Ruth, James S. Santangelo, Marina Alberti, Myla F.J. Aronson, Charlotte
820	W. de Keyzer, Sarah E. Diamond, Marie Josée Fortin, et al. 2019. A
821	roadmap for urban evolutionary ecology. Evolutionary Applications 12:
822	384–398. https://doi.org/10.1111/eva.12734.
823	Rubbo, Michael J, and Joseph M Kiesecker. 2005. Amphibian Breeding
824	Distribution in an Urbanized Landscape. Conservation Biology 19. Wiley,

- Society for Conservation Biology: 504–511.
- 826 Salmón, Pablo, Emilie Stroh, Amparo Herrera-Dueñas, Maria von Post, and
- 827 Caroline Isaksson. 2018. Oxidative stress in birds along a NOx and
- <sup>828</sup> urbanisation gradient: An interspecific approach. *Science of The Total*
- *Environment* 622–623. Elsevier: 635–643.
- https://doi.org/10.1016/J.SCITOTENV.2017.11.354.
- 831 Santana, Diego José, Vanessa Gonçalves Ferreira, Gabriel Nassif Crestani, and
- 832 Matheus Oliveira Neves. 2019. Diet of the Rufous Frog *Leptodactylus*
- *fuscus* (Anura, Leptodactylidae) from two contrasting environments.
- *Herpetozoa* 32. Pensoft Publishers: 1–6.
- https://doi.org/10.3897/herpetozoa.32.e35623.
- Santos, Boaventura de Sousa. 2016. *Epistemologies of the South: justice against epistemicide*. London New York: Routledge.
- 838 Scellato, Giuseppe, Chiara Franzoni, and Paula Stephan. 2015. Migrant scientists
- and international networks. *Research Policy* 44: 108–120. https://
- doi.org/10.1016/j.respol.2014.07.014.
- 841 Semlitsch, Raymond D. 2008. Differentiating Migration and Dispersal Processes
- for Pond-Breeding Amphibians. *Journal of Wildlife Management* 72: 260–
  267. https://doi.org/10.2193/2007-082.
- 844 Shackleton, Charlie M., Sarel S. Cilliers, Marié J. du Toit, and Elandrie Davoren.
- 2021. The Need for an Urban Ecology of the Global South. In *Urban*
- *Ecology in the Global South*, 1–26. Springer, Cham.
- 847 https://doi.org/10.1007/978-3-030-67650-6\_1.
- 848 Sherratt, Emma, Marta Vidal-García, Marion Anstis, and J. Scott Keogh. 2017.
- Adult frogs and tadpoles have different macroevolutionary patterns across

850	the Australian continent. Nature Ecology and Evolution 1: 1385–1391.
851	https://doi.org/10.1038/s41559-017-0268-6.
852	Simkin, Rohan D, Karen C Seto, Robert I McDonald, and Walter Jetz. 2022.
853	Biodiversity impacts and conservation implications of urban land
854	expansion projected to 2050. Proceedings of the National Academy of
855	Sciences 119. Proceedings of the National Academy of Sciences:
856	e2117297119. https://doi.org/10.1073/pnas.2117297119.
857	Smallbone, Lisa T, Gary W Luck, and Skye Wassens. 2011. Anuran species in
858	urban landscapes: Relationships with biophysical, built environment and
859	socio-economic factors. Landscape and Urban Planning 101: 43–51.
860	https://doi.org/10.1016/j.landurbplan.2011.01.002.
861	Smith, Olivia M., Kayla L. Davis, Riley B. Pizza, Robin Waterman, Kara C.
862	Dobson, Brianna Foster, Julie C. Jarvey, et al. 2023. Peer review
863	perpetuates barriers for historically excluded groups. Nature Ecology and
864	Evolution. Nature Publishing Group: 1–12. https://doi.org/10.1038/s41559-
865	023-01999-w.
866	Souza, F. L., Fenoglio, M. S., & Angeoletto, F. (2023). To Be a Brazilian City
867	Dweller, Sometimes We Must Learn to Say Enough!. Sustainability, 15:
868	3699.
869	Stevens, Dale R., Matthew A. Wund, and Kaitlyn A. Mathis. 2023. Integrating

- 870 environmental complexity and the plasticity-first hypothesis to study
- responses to human-altered habitats. *Animal Behaviour*. Academic Press.

872 https://doi.org/10.1016/J.ANBEHAV.2022.12.005.

873 Szulkin, M., Munshi-South, J., and Charmantier, A. (Eds.). (2020). Urban

evolutionary biology. Oxford University Press, USA.

875	Tagil, Sermin, Sevgi Gormus, and Serhat Cengiz. 2018. The Relationship of Urban
876	Expansion, Landscape Patterns and Ecological Processes in Denizli,
877	Turkey. Journal of the Indian Society of Remote Sensing 46. Springer:
878	1285–1296. https://doi.org/10.1007/S12524-018-0801-3/FIGURES/5.
879	Tisseuil, Clement, Jean François Cornu, Olivier Beauchard, Sebastien Brosse,
880	William Darwall, Robert Holland, Bernard Hugueny, Pablo A. Tedesco,
881	and Thierry Oberdorff. 2013. Global diversity patterns and cross-taxa
882	convergence in freshwater systems. Journal of Animal Ecology 82. John
883	Wiley & Sons, Ltd: 365–376. https://doi.org/10.1111/1365-2656.12018.
884	Trisos, Christopher H., Jess Auerbach, and Madhusudan Katti. 2021. Decoloniality
885	and anti-oppressive practices for a more ethical ecology. Nature Ecology
886	& Evolution 5. Nature Publishing Group: 1205–1212.
887	https://doi.org/10.1038/s41559-021-01460-w.
888	UN-Habitat, United Nations Human Settlements Programme. 2022. World Cities
889	Report 2022: Envisaging the Future of Cities.
890	UNFPA. 2007. State of the World Population 2007: Unleashing the Potential of
891	Urban Growth, United Nations Population Fund.
892	United Nations, Department of Economic and Social Affairs, Population Division.
893	2019. World Urbanization Prospects: The 2018 Revision
894	(ST/ESA/SER.A/420). New York: United Nations. Available at
895	https://population.un.org/wup/. New York: United Nations.
896	Valente-Neto, Francisco, Fabio de Oliveira Roque, Carolina Ferreira Pauliquevis,
897	Ademir Kleber Morbeck de Oliveira, Diogo B. Provete, Judit K. Szabo, and
898	Franco Leandro Souza. 2021. Loss of Cultural and Functional Diversity
899	Associated with Birds Across the Urbanization Gradient in a Tropical City.

900	Frontiers in Ecology and Evolution 9. Frontiers Media S.A.: 331.
901	https://doi.org/10.3389/FEVO.2021.615797.
902	Wells, Kentwood David. 2007. The ecology and behavior of amphibians. Chicago:
903	University of Chicago Press.
904	Wickham, Hadley. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-
905	Verlag New York.
906	Wilbur, H M. 1980. Complex Life Cycles. Annual Review of Ecology and
907	Systematics 11: 67–93.
908	https://doi.org/10.1146/annurev.es.11.110180.000435.
909	Yang, Lu, Shuqing Zhao, and Shuguang Liu. 2022. A global analysis of
910	urbanization effects on amphibian richness: Patterns and drivers. Global
911	Environmental Change 73. Pergamon: 102476.
912	https://doi.org/10.1016/J.GLOENVCHA.2022.102476.
913	Zhang, Wei, Ben Li, Xiaoxiao Shu, Enle Pei, Xiao Yuan, Yujie Sun, Tianhou Wang,
914	and Zhenghuan Wang. 2016. Responses of anuran communities to rapid
915	urban growth in Shanghai, China. Urban Forestry & Urban Greening 20:
916	365–374. https://doi.org/10.1016/j.ufug.2016.10.005.
917	
918	
919	
920	



Figure 1. Word cloud of the most frequent words mentioned in the 42 studies
remaining after screening. Word size and color represent how often it appears.
Grey: word mentioned more than 2,500 times; Yellow: more than 1,500 times;
Purple: more than 750 times; Orange: more than 500 times; Green: more than 300
times.



**Figure 2.** Barplot showing the number of published papers per year on tropical frogs

- 931 in urbanized areas from 2008 to 2022.
- 932



Figure 3. World map showing the number of published papers evaluating urbanization impact on frogs in tropical countries. The size of circles represents the number of papers published per country.



Figure 4. Pie chart showing proportional gender differences in research papers
evaluating urbanization impact on frogs in tropical countries. A) proportion of women
and men in the 42 papers evaluated. B) proportion as first author. C) proportion as
senior author.



Figure 5. Landscape showing a rural-urban contrast that depicts the most relevant 944 results we found in the literature search. Notice the difference in Artificial Light At 945 Night (ALAN) between regions, with urban areas being much more lit. Urban areas 946 have also more impervious surfaces and polluted water bodies and are more prone 947 to sound pollution (notice the car horns and music). Water bodies available in rural 948 areas have varied sizes, while those in the urban area are small. A) scene showing 949 that frog species richness is higher in rural than in urban areas, diet of frogs is also 950 more diverse, with more arthropod species, frog species are usually smaller in rural 951 than in urban areas; B) species in urban areas are larger but may have 952 953 malformations (notice the lack of eye and leg), while feeding upon a less diverse set 954 of arthropods; C) frogs call at higher rates for extended periods at urban than rural areas due to ALAN. 955

956



957

**Figure 6.** Contrast between two urban landscapes. Right one is more frog friendly, than the one on the left. Notice the differences between landscape in terms of imperviousness, variety of pond sizes, proximity between ponds, potential refugia during the dry season, and landscape permeability.

## 963 Supplementary material

Appendix 1. PRISMA 2020 Flow Diagram showing the process of literature search







- Anderson, Nigel K, Stephanie O Gutierrez, and Ximena E Bernal. 2019. From
   forest to city: urbanization modulates relative abundance of anti-predator
   coloration. *Journal of Urban Ecology* 5: juz016.
- 999 https://doi.org/10.1093/jue/juz016.
- 1000 Arruda, M. P., and E. Morielle-Versute. 2008. Cytogenetic and random amplified
- 1001 polymorphic DNA analysis of *Leptodactylus* species from rural and urban
- 1002 environments (Anura, Amphibia). Genetics and Molecular Research 7: 161–
- 1003 176. https://doi.org/10.4238/vol7-1gmr402.
- 1004 Bajaru, Sameer, Saunak Pal, Mrugank Prabhu, Pinal Patel, Rahul Khot, and
- 1005 Deepak Apte. 2020. A multi-species occupancy modeling approach to access
- the impacts of land use and land cover on terrestrial vertebrates in the
- 1007 Mumbai Metropolitan Region (MMR), Western Ghats, India. *PLOS ONE* 15.
- 1008 Public Library of Science: e0240989.
- 1009 Bickford, David, Tze How Ng, Lan Qie, Enoka P Kudavidanage, and Corey J A
- 1010 Bradshaw. 2010. Forest Fragment and Breeding Habitat Characteristics
- 1011 Explain Frog Diversity and Abundance in Singapore. *Biotropica* 42. John
- 1012 Wiley & Sons, Ltd: 119–125. https://doi.org/10.1111/j.1744-
- 1013 **7429.2009.00542.x**.
- 1014 Burbano-Yandi, Carlos E., Wilmar Bolívar-García, and Alan Giraldo. 2015.
- 1015 Ensambla jes de anuros en tres zonas con intervención humana en el Parque
- 1016 Nacional Natural Los Katíos (Colombia). Boletin Científico del Centro de
- 1017 *Museos* 19: 157–170. https://doi.org/10.17151/bccm.2015.19.1.11.
- 1018 Canessa, Stefano, and Kirsten M Parris. 2013. Multi-Scale, Direct and Indirect
- 1019 Effects of the Urban Stream Syndrome on Amphibian Communities in
- 1020 Streams. *PLOS ONE* 8. Public Library of Science: e70262.

1022	Márcio Borges-Martins. 2017. Effects of traffic noise on the calling behavior of
1023	two Neotropical hylid frogs. PLOS ONE 12. Public Library of Science:
1024	e0183342.
1025	Dias, Karina Soares, Elisa Stuani Dosso, Alexander S Hall, André Passaglia
1026	Schuch, and Alexandro Marques Tozetti. 2019. Ecological light pollution
1027	affects anuran calling season, daily calling period, and sensitivity to light in
1028	natural Brazilian wetlands. The Science of Nature 106: 46.
1029	https://doi.org/10.1007/s00114-019-1640-y.
1030	Ernst, Raffael, David Massemin, and Ingo Kowarik. 2011. Non-invasive invaders
1031	from the Caribbean: the status of Johnstone's Whistling frog
1032	(Eleutherodactylus johnstonei) ten years after its introduction to Western
1033	French Guiana. Biological Invasions 13: 1767–1777.
1034	https://doi.org/10.1007/s10530-010-9930-5.
1035	Ferreira, Cláudia Márcia Marily, Augusto Cesar de Aquino Ribas, and Franco
1036	Leandro Souza. 2017. Species composition and richness of anurans in
1037	Cerrado urban forests from central Brazil. Acta Herpetologica 12: 157–165.
1038	https://doi.org/10.13128/Acta_Herpetol-18179.
1039	Ganci, Carolina Cunha, Diogo B Provete, Thomas Püttker, David Lindenmayer,
1040	and Mauricio Almeida-Gomes. 2021. High species turnover shapes anuran
1041	community composition in ponds along an urban-rural gradient. Urban
1042	Ecosystems 25: 633–642. https://doi.org/10.1007/s11252-021-01174-8.
1043	Gersava, Jacky Roselle, Reynaldo Abad, Fritzie Camino, Mae A Responte, Marion
1044	John Michael Achondo, and Lief Erikson Gamalo. 2020. Native and invasive
1045	alien anuran species in urbanized areas in Davao City, Philippines, with

Caorsi, Valentina Zaffaroni, Camila Both, Sonia Cechin, Rógger Antunes, and

1021

1046 preliminary study of feeding biology. *Journal* 13: 1–8.

- 1047 Getelina, M A, J B Schwantes, D A S Graichen, and A P Schuch. 2022. Influence
- 1048 of anthropogenic pressure on the genetic diversity and chromosomal
- instability of an endangered forest-specialist anuran. *Hydrobiologia* 849:
- 1050 2463–2475. https://doi.org/10.1007/s10750-022-04840-w.
- 1051 Hamer, Andrew J, and Kirsten M Parris. 2011. Local and landscape determinants
- 1052 of amphibian communities in urban ponds. *Ecological Applications* 21. John
- 1053 Wiley & Sons, Ltd: 378–390. https://doi.org/10.1890/10-0390.1.
- Hamer, Andrew J, Phoebe J Smith, and Mark J McDonnell. 2012. The importance
- 1055 of habitat design and aquatic connectivity in amphibian use of urban
- stormwater retention ponds. *Urban Ecosystems* 15: 451–471.
- 1057 https://doi.org/10.1007/s11252-011-0212-5.
- Higham, V., N. D.S. Deal, Y. K. Chan, C. Chanin, E. Davine, G. Gibbings, R.
- 1059 Keating, et al. 2021. Traffic noise drives an immediate increase in call pitch in
- an urban frog. *Journal of Zoology* 313. Blackwell Publishing Ltd: 307–315.
- 1061 https://doi.org/10.1111/JZO.12866.
- Holzer, Katie A, Robert P Bayers, Thien Tao Nguyen, and Sharon P Lawler. 2017.
- 1063 Habitat value of cities and rice paddies for amphibians in rapidly urbanizing
- 1064 Vietnam. *Journal of Urban Ecology* 3: juw007.
- 1065 https://doi.org/10.1093/jue/juw007.
- 1066 Kaiser, Kristine, and Jessica L Hammers. 2009. The Effect of Anthropogenic Noise
- 1067 on Male Advertisement Call Rate in the Neotropical Treefrog, *Dendropsophus*
- *triangulum. Behaviour* 146. Brill: 1053–1069.
- 1069 Komine, Hirotaka, Kiyomi Yasumiba, and Lin Schwarzkopf. 2022. The country
- 1070 toad and the city toad: comparing morphology of invasive cane toads

- 1071 (*Rhinella marina*) from rural and urban environments. *Biological Journal of the* 1072 *Linnean Society*: blac100. https://doi.org/10.1093/biolinnean/blac100.
- 1073 Kruger, Donnavan J.D., and Louis H. Du Preez. 2016. The effect of airplane noise
- 1074 on frogs: a case study on the Critically Endangered Pickersgill's reed frog
- 1075 (*Hyperolius pickersgilli*). *Ecological Research* 31. Springer Tokyo: 393–405.
- 1076 https://doi.org/10.1007/S11284-016-1349-8/FIGURES/9.
- 1077 Kruger, Donnavan J D, Andrew J Hamer, and Louis H Du Preez. 2015.
- 1078 Urbanization affects frog communities at multiple scales in a rapidly
- developing African city. *Urban Ecosystems* 18: 1333–1352.
- 1080 https://doi.org/10.1007/s11252-015-0443-y.
- Leon, Evelina, Paola M Peltzer, Rodrigo Lorenzon, Rafael C Lajmanovich, and
- Adolfo H Beltzer. 2019. Effect of traffic noise on Scinax nasicus advertisement
   call (Amphibia, Anura). *Iheringia. Série Zoologia*. scielo.
- Li, Ben, Wei Zhang, Xiaoxiao Shu, Enle Pei, Xiao Yuan, Yujie Sun, Tianhou Wang,
- and Zhenghuan Wang. 2016. The Impacts of Urbanization on the Distribution
- and Body Condition of the Rice-paddy Frog (*Fejervarya multistriata*) and
- 1087 Gold-striped Pond Frog (*Pelophylax plancyi*) in Shanghai, China. Asian
- 1088 Herpetological Research 7: 200–209.
- 1089 https://doi.org/10.16373/j.cnki.ahr.150061.
- 1090 Lima, Natalya de Araujo Pedrosa, Raoni Rebouças, Luís Felipe Toledo, and
- 1091 Cybele Sabino Lisboa. 2022. Influence of urban noise in call traits of the
- 1092 Atlantic Forest treefrog *Bokermannohyla hylax*. *Zoologischer Anzeiger* 300:
- 1093 41–46. https://doi.org/10.1016/j.jcz.2022.07.002.
- Lima, Nathália G S, Ubirajara Oliveira, Rafael C C Souza, and Paula C Eterovick.
- 1095 2019. Dynamic and diverse amphibian assemblages: Can we differentiate

natural processes from human induced changes? *PLOS ONE* 14. Public
Library of Science: e0214316.

Liu, Gracie, Jodi J L Rowley, Richard T Kingsford, and Corey T Callaghan. 2021.

1099 Species' traits drive amphibian tolerance to anthropogenic habitat

modification. *Global Change Biology* 27. John Wiley & Sons, Ltd: 3120–3132.

1101 https://doi.org/10.1111/gcb.15623.

Liu, Ningning, Shurong Zhong, Tianhou Wang, Xiuzhen Li, Li Wei, Chunjing Zou,

1103 Shanshan Zhao, and Ben Li. 2022. Advertisement Call Variation of Two Frog

1104 Species along an Urban-Rural Gradient in Shanghai, China. *Diversity*.

1105 https://doi.org/10.3390/d14070550.

1106 Lourenço-de-Moraes, Ricardo, Leo R Malagoli, Vinicius Guerra, Rodrigo B

1107 Ferreira, Igor de Paiva Affonso, Célio F B Haddad, Ricardo J Sawaya, and

1108 Rogério P Bastos. 2018. Nesting patterns among Neotropical species

assemblages: can reserves in urban areas be failing to protect anurans?

1110 Urban Ecosystems 21: 933–942. https://doi.org/10.1007/s11252-018-0767-5.

1111 MacGregor-Fors, Ian, Federico Escobar, Rafael Rueda-Hernández, Sergio

Avendaño-Reyes, Martha L Baena, Víctor M Bandala, Santiago Chacón-

III3 Zapata, et al. 2016. City "Green" Contributions: The Role of Urban

1114 Greenspaces as Reservoirs for Biodiversity. *Forests*.

1115 https://doi.org/10.3390/f7070146.

1116 MacGregor-Fors, Ian, Omar Hernández Ordoñez, and Rubén Ortega-Álvarez.

1117 2013. Urban croaking: diversity and distribution of anurans in a neotropical

city. Urban Ecosystems 16: 389–396. https://doi.org/10.1007/s11252-012-

1119 **0267-y**.

1120 Matías-Ferrer, Noemí, and Patricia Escalante. 2015. Size, body condition, and

- limb asymmetry in two hylid frogs at different habitat disturbance levels in
- 1122 Veracruz, México. *Herpetological Journal* 25: 169–176. https://doi.org/British

Herpetological Society.

- 1124 Menin, Marcelo, Rafaela Fernanda Batista Ferreira, Izomar Barbosa Melo,
- 1125 Marcelo Gordo, Gustavo Yomar Hattori, and Bruno Sampaio Sant'anna. 2019.
- Anuran diversity in urban and rural zones of the Itacoatiara municipality,

1127 central Amazonia, Brazil. Acta Amazonica 49: 122–130.

1128 https://doi.org/10.1590/1809-4392201800284.

1129 Mühlenhaupt, Max, James Baxter-Gilbert, Buyisile G Makhubo, Julia L Riley, and

- John Measey. 2021. Growing up in a new world: trait divergence between
- rural, urban, and invasive populations of an amphibian urban invader.
- 1132 *NeoBiota* 69. Pensoft Publishers: 103–132.
- 1133 Mühlenhaupt, Max, James Baxter-Gilbert, Buyisile G Makhubo, Julia L Riley, and

John Measey. 2022. No evidence for innate differences in tadpole behavior

between natural, urbanized, and invasive populations. *Behavioral Ecology* 

and Sociobiology 76: 11. https://doi.org/10.1007/s00265-021-03121-1.

1137 Ofori, Benjamin Yeboah, John Bosu Mensah, Roger Sigismund Anderson, and

- Daniel Korley Attuquayefio. 2021. Diet composition, body condition and
- sexual size dimorphism of the common African toad *Amietophrynus regularis*)
- in urban and agricultural landscape. *bioRxiv*: 2021.01.25.428067.
- 1141 https://doi.org/10.1101/2021.01.25.428067.
- 1142 Oliveira-Souza, Aline Emanuele, Maria Madalena Salviano Santana, Maria
- Jeovana Lima Martins, Jessica Stefany Costa Anaissi, Patrick Ribeiro
- 1144 Sanches, and Carlos Eduardo Costa-Campos. 2022. Diversity of ants in the
- diet of *Rhinella major* (Anura: Bufonidae) in an urban area in North Brazil.

1146 *Herpetology Notes* **15**: 663–670.

1147 Perevra, Laura C, M S Akmentins, M J Salica, M F Quiroga, C E Moreno, and M Vaira. 2021. Tolerant and avoiders in an urban landscape: anuran species 1148 richness and functional groups responses in the Yungas' forest of NW 1149 Argentina. Urban Ecosystems 24: 141–152. https://doi.org/10.1007/s11252-1150 020-01025-y. 1151 Petrozzi, F, G C Akani, E A Eniang, S N Ajong, S M Funk, J E Fa, N Amadi, D 1152 Dendi, and L Luiselli. 2021. Generalist, selective or 'mixed' foragers? Feeding 1153 strategies of two tropical toads across suburban habitats. Journal of Zoology 1154 1155 315. John Wiley & Sons, Ltd: 288–300. https://doi.org/10.1111/jzo.12925. Santana, Diego José, Vanessa Gonçalves Ferreira, Gabriel Nassif Crestani, and 1156 Matheus Oliveira Neves. 2019. Diet of the Rufous Frog Leptodactylus fuscus 1157 (Anura, Leptodactylidae) from two contrasting environments. Herpetozoa 32. 1158 Pensoft Publishers: 1-6. https://doi.org/10.3897/herpetozoa.32.e35623. 1159 Wei, Xu, Meiling Huang, Qu Yue, Shuo Ma, Ben Li, Zhiqiang Mu, Chuan Peng, et 1160 al. 2021. Long-term urbanization impacts the eastern golden frog (*Pelophylax*) 1161 plancyi) in Shanghai City: Demographic history, genetic structure, and 1162 1163 implications for amphibian conservation in intensively urbanizing environments. Evolutionary Applications 14. John Wiley & Sons, Ltd: 117-1164 135. https://doi.org/10.1111/eva.13156. 1165 1166 Zhang, Wei, Ben Li, Xiaoxiao Shu, Enle Pei, Xiao Yuan, Yujie Sun, Tianhou Wang, and Zhenghuan Wang. 2016. Responses of anuran communities to rapid 1167 urban growth in Shanghai, China. Urban Forestry & Urban Greening 20: 365-1168 374. https://doi.org/10.1016/j.ufug.2016.10.005. 1169

62

1	CAPÍTULO 1
2	
3	Manuscrito em revisão na <i>Ecology</i> (ISSN:1939-9170)
4	
5	The effect of urbanisation and local environmental heterogeneity on phenotypic
б	variability of a tropical treefrog
7	
8	Marcos R. Severgnini <sup>1</sup> , Diogo B. Provete <sup>2,3,4*</sup>
9	
10	<sup>1</sup> Graduate Program in Ecology and Conservation, Federal University of Mato Grosso
11	do Sul, Campo Grande, 79002-900, Brazil.
12	<sup>2</sup> Institute of Biosciences, Federal University of Mato Grosso do Sul, Campo Grande,
13	79002-900, Mato Grosso do Sul, Brazil.
14	<sup>3</sup> Martin-Luther-Universität Halle-Wittenberg, Halle, Germany.
15	<sup>4</sup> German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig,
16	Leipzig, Germany.
17	* Corresponding author: diogo.provete@ufms.br
18	
19	Open research statement
20	Data are published in Dryad: <u>https://doi.org/10.5061/dryad.3xsj3txp2</u> .
21	Associated code are published in Zenodo: <u>https://doi.org/10.5281/zenodo.10327989</u> .
22	
23	
24	

- 25 Abstract
- 26

Urbanisation reduces species richness and changes community composition. 27 28 However, little is known on how the phenotype of organisms with low dispersal ability responds to environmental changes associated with urbanisation in fast urbanizing 29 centres. Here, we tested how urbanisation rate, local environmental heterogeneity, 30 land surface temperature, and spatial gradients affect phenotypic traits of the Dwarf 31 Treefrog (Dendropsophus nanus). We measured 768 individuals in 21 ponds along an 32 urban gradient, along with local environmental variables, which were summarized with 33 a PCA. The spatial arrangement of ponds was described using Moran Eigenvector 34 Maps. Variables were entered into a Structural Equation Model to test their effects on 35 the mean and coefficient of variation (CV) of traits. Body size decreased from the 36 periphery to the urban centre, whereas CV of body size and head shape had the 37 opposite pattern. Body size increased, whereas CV of body size and head shape 38 decreased in man-made ponds. The CV of leg length decreased with increasing land 39 surface temperature. None of the traits were spatially autocorrelated. Our results 40 suggest that the lack of a clear spatial variation in phenotypic traits can be the result 41 of a weak selection, due to a recent, although intense, urbanisation process. 42

43

Key-words: Global South; Adaptive evolution; Eco-Evolutionary dynamics; Trait environment relationships; Urban ecosystems.

- 46
- 47
- 48
- 49

50 Introduction

51

Novel ecosystems, such as cities can be challenging to resident species 52 because of their altered environment intended to benefit humans. Urbanisation has 53 negatively been impacting biodiversity and altering the pace of natural selection 54 around the globe (Alberti, 2015; Hendry et al., 2008). Built environments in cities 55 promote habitat fragmentation and isolation, reduce gene flow, and create small 56 populations more prone to genetic drift (Miles et al., 2019; Rivkin et al., 2019). Species 57 58 may respond to these novel ecological conditions by changing either their morphology, physiology, behaviour, and others (McDonnell & Hahs, 2015). For example, the urban 59 heat island imposes strong metabolic costs to organisms, favouring a decrease in 60 body size (Merckx et al. 2018). Indeed, some studies (e.g., Jennette et al., 2019) have 61 found smaller body sized frogs in urban populations after controlling for age. However, 62 larger body sizes are usually associated with increased fitness (Smith & Belk, 2018). 63 Consequently, maladapted phenotypes may be fixed in urban populations, decreasing 64 survival and reproduction (Urban, 2011; Brady et al., 2019a). 65

Recent studies found that urbanisation changes morphological traits of birds. 66 reptiles, and mammals (Alberti et al., 2017; Fugère & Hendry, 2018). However, 67 organisms with high dispersal ability (e.g., birds) can still maintain moderate gene flow 68 (Miles et al., 2019) and are more likely to adapt to urban environments (Schmidt et al., 69 2020). Conversely, the effects of urbanisation on organisms with low dispersal ability 70 are expected to be more pronounced (Khimoun et al., 2020), because dispersal 71 limitation reduces gene flow and population size. Dispersal can also mediate how 72 individuals assess habitats that maximize their performance (Edelaar et al., 2008), 73 resulting in higher phenotypic variation in more heterogeneous habitats (Thompson et 74

al. 2022). Yet, little is known about how phenotype-environment relationships of
strongly dispersal-limited organism respond to urbanisation, especially in the Global
South (Severgnini et al. 2025), which has been experiencing a fast and recent
urbanisation process (Myers, 2021; Shackleton et al., 2021).

Although several studies have evaluated how phenotypic traits are affected by 79 urban ecosystems (Callaghan et al., 2021; Jennette et al., 2019; Komine et al., 2022), 80 81 most of them focused on trait mean and did not incorporate phenotypic variance (but see Thompson et al., 2022). While the phenotypic mean can reveal if a trait has 82 83 already been or will be shaped by natural selection, phenotypic variation can help identify if some trait values in a population are better fit to the environment (Edelaar et 84 al. 2008; Sanderson et al. 2023), and why they evolve more rapidly (Des Roches et 85 al., 2018). Intraspecific variation modulates ecological and evolutionary processes 86 (Moran et al., 2016, Sanderson et al. 2023). It can be caused by different processes 87 acting at same time, such as genetic drift, mutation, phenotypic plasticity, or relaxed 88 natural selection (Sanderson et al. 2023). For example, the phenotypes of two bird 89 species increased in variance, but had lower means in urban sites (Thompson et al., 90 2022) compared to their forest counterparts. However, dispersal limited groups, whose 91 distribution is constrained to the surroundings of water bodies, might experience a 92 decrease in phenotypic variation due to lower population sizes as a result of habitat 93 connectivity loss (Thompson et al., 2022). Also, this phenotypic variation can result in 94 variation in fitness and can be an opportunity for selection to act upon these 95 populations. In fact, a previous study across two urbanised landscapes found gene 96 flow up to 6 km in a frog (Homola et al. 2019). 97

98 Here, we tested how urbanisation rate, local environmental heterogeneity, and 99 spatial gradients affect phenotypic traits associated with dispersal, resource acquisition, and jumping performance in the Dwarf Treefrog (*Dendropsophus nanus*).
 We assume frogs living in ponds with high urbanisation rates have been under a strong
 directional selective pressure for longer than those in areas that have been urbanized
 recently. We expect that: (i) both the mean and coefficient of variation (CV) of body
 size, leg length, and head shape decrease with increasing urbanisation and increase
 with local heterogeneity; (ii) mean and CV of body size, leg length, and head shape
 decrease with land surface temperature (Figure S1).

107

#### 108 Methods

109

# 110 Study site and sampling design

We conducted fieldwork in Campo Grande, Mato Grosso do Sul, central Brazil (Figure S2). Campo Grande is a tropical, young city founded 126 years ago (Arruda 2006; PLANURB 2019). The city has about 898,100 people (IBGE, 2023), and its urban area has about 35,903 ha with most human population living in the urban centre (PLANURB, 2019). Although recent, urbanisation has been fast, with the city gaining ~ 13,000 ha of urban infrastructure from the centre to boundaries between 1985 and 2021 (MRS, pers. obs. based on data from MapBiomas, 2023).

We sampled adult males of the hylid frog *Dendropsophus nanus* in 21 ponds, on average 12.4 km apart from one another (range 0.55–31.2 km; Figure S2, S3, S4) along an urban gradient through surveys at breeding sites (Scott Jr. et al., 1994). Field work was conducted between 17:30 and midnight from November 2021 to April 2022 and from November 2022 to January 2023 (i.e., nine months) visiting two ponds per day. We did not visit all ponds in all months. Instead, each pond was visited until we obtained 30 individuals to guarantee equal sampling effort.

# 126 Phenotypic traits

We took linear measurements in the field using a digital calliper (MTX 150 mm) 127 to the nearest 0.01 mm of the following traits: Snout-Vent Length, head width, head 128 length, and leg length, following Watters et al. (2016). Relative leg length is related to 129 vulnerability to predation (Emerson, 1985a), jumping performance, and dispersal 130 131 ability (Phillips et al., 2006), while head length and width is related to size and variety of feeding resources (Emerson, 1985b; Parmelee, 1999). Body size is related to 132 133 thermoregulation, dispersal, and desiccation resistance (Hillman et al. 2008). After measurements, every frog was tagged with visible implant elastomer (VIE) and 134 released back. Further details on morphometric data processing are in the Supporting 135 information. 136

137

# 138 Environmental and spatial predictor variables

We measured urbanisation as the percentage of buildings and road area (Szulkin et al., 2020) in a 500-m buffer around each pond (Figure S4). We also extracted this same variable between 1985 and 2021 (MapBiomas 2023). To obtain urbanisation rate, we subtracted the current (2021) urban area from that of 1985 and divided it by 36. Urbanisation rate varied from 0% to 2.11% per year.

To quantify pond environmental heterogeneity, we measured pond area (m<sup>2</sup>; using Google Earth®), depth (in m), hydroperiod, cattle presence, aquatic predators, margin profile (categorical variables), water temperature (in °C), and percentage of floating vegetation (visual estimation). Ponds with excavated margins were artificial, man-made ponds. Likewise, ponds with vertebrate predators and cattle were associated with rural environments (Figures S5, S6). Margin type limits the amount

and kinds of perches available for frogs, influencing their microhabitat use 150 (Vasconcelos et al. 2009), likely constraining the range of phenotypic traits. All 151 variables (Table S1) were later summarized using a Hill-Smith Principal Component 152 Analysis (Hill & Smith, 1976). To describe the spatial arrangement of ponds at multiple 153 scales, we used Moran's Eigenvector Maps (MEMs). This analysis allowed us to 154 detect if there was some spatial pattern in the arrangement of ponds, which could 155 156 indicate a possible local adaptation (see Urban, 2011). Lastly, we calculated land surface temperature (LST) and urban heat island (UHI; Figure S7) from satellite 157 158 images. Further details on environmental and spatial data processing are available in the Supporting Information. 159

160

#### 161 Data analysis

Finally, the PC1 (pond environmental heterogeneity), MEM3 (spatial 162 arrangement), urbanisation rate, and LST were entered into a Structural Equation 163 Model to test for their direct and indirect effects on the mean and CV of each 164 phenotypic trait. We calculated the mean as a sum of each phenotypic trait divided by 165 the number of individuals in each pond. We calculated the Kvålseth coefficient of 166 variation (see Kvålseth, 2017) for each trait, following Lobry et al., (2023). Path 167 analysis was performed in lavaan (Rosseel, 2012). The data and associated R code 168 are available in Dryad (Severgnini & Provete 2024). 169

170

#### 171 **Results**

172

Urbanisation rate and the spatial structure were directly and positively related
to local heterogeneity in all models (Figure 1, Table S2). Mean body size was positively

related to PC1, which means it increased in ponds with excavated margins and 175 decreased in those with temporary hydroperiod, invertebrate predators, and cattle 176 (Table S1; Figures 1, S6, S9). Mean body size was negatively associated with MEM3, 177 which means it decreased from the periphery to the urban centre (contrast Figure S6 178 with Figure S8). Mean head shape was negatively associated with local environmental 179 heterogeneity and positively related to MEM3 (Figures 1, S9). This means that frogs 180 181 in Northeast-Southwest distributed ponds had short and narrow heads, while frogs with long and wide heads were found in ponds with excavated banks (Figures S6, S9, 182 183 S10).

The coefficient of variation (CV) of body size was negatively related to 184 environmental heterogeneity and positively with MEM3 (Figure 1, S9, Table S2). This 185 means that the body size of frogs varied less in ponds with excavated margins, but 186 varied more in temporary ponds with invertebrate predators and cattle. Ponds in 187 Northeast-Southwest regions had higher variation in body size than those in the 188 Northwest-Southeast region. The CV of leg length was negatively affected by land 189 surface temperature (Table S2; Figure 1, S7, S8). The remaining traits were not 190 directly affected by any predictor variable (Figure 1). Finally, neither trait nor predictor 191 variable were spatially autocorrelated (Figure S11, S12). Interestingly, we found no 192 direct effect of urbanisation rate on any trait, except for a small negative effect on mean 193 body size. Instead, its effect on the mean and variance body size and mean head 194 shape seems to be indirect, via pond environmental variables. 195

196

### 197 Discussion

Our results showed that mean body size, head shape, and the variation in body size were directly influenced, but in opposite ways, by the spatial arrangement of ponds and local environmental heterogeneity. The variation in leg length decreased with increasing land surface temperature. Contrarily to our initial hypotheses, we did not find a direct effect of urbanisation rate on traits, except for a small effect on mean body size. Instead, urbanisation influenced phenotypes indirectly via pond heterogeneity.

Mean body size increased in ponds with excavated margins and decreased in 206 207 those with temporary hydroperiod, cattle, and invertebrate predators. Urbanisation rate was also positively related to local heterogeneity. These results suggest that 208 urbanisation is indirectly influencing mean body size via changes in pond environment. 209 Dendropsophus nanus is a small treefrog that calls perched on tree branches or leaves 210 on pond margins (Menin et al., 2005). Man-made ponds in highly urbanized areas 211 might have less appropriate microhabitats (Hutto & Barrett, 2021) and calling site 212 availability (Hamer & McDonnell, 2008). Thus, frog populations may be responding to 213 these environmental filters by altering their phenotypic traits. Most previous studies 214 found smaller frogs in urban environments compared to rural or undisturbed sites (e.g., 215 Jennette et al., 2019; Komine et al., 2022; Liu et al., 2021; Matías-Ferrer & Escalante, 216 2015). Also, Liu et al., (2021) found that larger frog species were more tolerant to 217 anthropogenic habitat modification, but this pattern was reversed after controlling for 218 phylogenetic relationships. The fact that the mean body size of frogs is responding 219 more strongly to environmental changes driven by urbanisation, instead of 220 temperature-related variables like urban heat island suggests that physiological 221 constraints seem less important than phenotype-habitat matching (Edelaar et al. 2008) 222 in young cities. This is somewhat unexpected, since frogs have a narrow thermal 223

performance (see Hillman et al., 2008). However, the relatively recent urbanisation
 process might not yet be strong enough to induce local adaptation.

In contrast, the phenotypic variation in body size was negatively related to 226 environmental heterogeneity, decreasing in man-made ponds. Ponds in Northeast-227 Southwest regions (i.e. most of the urbanised ponds) tend to have frogs with more 228 variable body sizes, but this spatial pattern was less strong. This pattern is similar to 229 230 what previous studies found for mean body size (e.g., Jennette et al., 2019; Komine et al., 2022). Artificial ponds are usually simplified environments (Hutto & Barrett, 231 232 2021), which can constrain the variance of body size around an optimal value. This result can be a consequence of genetic assimilation process, which canalize an 233 optimal phenotype reducing plastic responses environmentally induced (Levis and 234 Pfennig 2016, 2021), consequently reducing phenotypic variation. Moreover, it 235 suggests that the phenotype of this frog population might be experiencing ongoing, 236 stabilizing natural selection. Alternatively, other processes as developmental plasticity 237 and growth rate changes can be acting over phenotypic variation, since that 238 developmental disruption modulated by environmental factors during ontogeny can 239 promote shifts in phenotype of the post-metamorphic frogs (Tejedo et al. 2010; 240 Gomez-mestre et al. 2010). For instance, some species delaying their time of 241 developing when exposed to predators (Relyea 2001), whereas others accelerating 242 (Van Buskirk and Saxer 2001). Overall, predation risk decreases developmental time 243 and growth rate affecting whole-body development, yielding smaller sizes at 244 metamorphosis or shorter hindlimbs (see Tejedo et al. 2010). As such, as our urban 245 ponds had fewer aquatic predators compared to rural ones, frogs in urban ponds might 246 face less accelerated development and consequently reduced developmental 247 disruptions decreasing phenotypic variation via developmental canalization 248
(Thompson et al. 2022). Therefore, body size seems to have idiosyncratic responses
to urbanisation (Langerhans & Kern, 2020) and differ between tropical and temperate
cities. Our study adds new evidence indicating reduced phenotypic variation in urban
areas.

Treefrogs had narrow and short heads in mean in man-made ponds. This 253 phenotypic response might be due to the reduced size and low prey diversity in urban 254 255 ponds (e.g., Santana et al., 2019). Mouth width and head size are the main traits influencing the size, amount, and diversity of prey consumed by frogs (Parmelee, 256 257 1999). Therefore, narrower heads in frog populations might be a phenotypic response to the decrease in body size of their prey in urban sites (e.g., Ishitani et al., 2003; 258 Merckx et al., 2018; Piano et al., 2020; Ulrich et al., 2008) or correlated response to 259 changes in growth rate due to environmental conditions (e.g., low food availabitiy; 260 Gomez-mestre et al. 2010). Therefore, mean head shape showed a different pattern 261 compared to body size, increasing from Northeast-Southwest to Northwest-Southeast 262 ponds, suggesting that different phenotypic traits can be under selective pressures at 263 fine spatial scales (e.g., pond level). 264

The CV of leg length decreased with increasing land surface temperature. 265 Environmental stressors, such as increased surface temperature, can promote 266 developmental disruptions, which can increase variance by breaking developmental 267 canalization (Thompson et al. 2022). Conversely, a meta-analysis found that 268 temperature variation in the larval stage had a small, yet significant effect on the leg 269 length of adult frogs (i.e., carry-over effects) due to disruptions in developmental, but 270 not growth rate (Tejedo et al. 2010). Differences in the timing of developmental events 271 can produce significant differences in relative leg length (Emerson 1986). In this 272 context, our results can be explained by divergent reaction norms hidden under certain 273

thermic regimes, which can promote morphological variation via developmental 274 plasticity (Mariotto et al. 2025). As such, some ponds might have specific thermic 275 regimes what can amplify the amount of trait variation (i.e., plasticity) via divergent 276 reaction norms, while others can reduce this variation. Increasing temperature usually 277 decreases relative humidity, which poses a further challenge to frogs in dealing with 278 evaporative water loss. Frogs can avoid water loss by adopting a water-conserving 279 280 posture during diurnal sleeping (Mitchell & Bergmann, 2016), in which they hide their limbs against their body to reduce their surface area. Habitat selection and behaviours 281 282 to avoid desiccation seem not to affect their jumping performance (Mitchell & Bergmann, 2016). Therefore, lower variance of leg length in ponds with higher 283 temperatures are likely due to changes in developmental time (Tejedo et al. 2010) 284 associated to divergent reaction norms (Mariotto et al. 2025) and can constrain the 285 evolution of this phenotype, with potential consequences for thermoregulatory 286 physiology and performance. 287

Neither trait nor environmental variable exhibited a clear spatial 288 autocorrelation pattern. These results suggest that we analysed a pure environmental 289 gradient and the trait-environment relationships found are not confused with spatial 290 autocorrelation (Urban, 2011). Furthermore, it provides evidence that there is no local 291 adaptation in any phenotypic trait, suggesting that populations still maintain a 292 moderate-to-high gene flow (see Urban, 2011). Some amphibian populations can 293 maintain gene flow in urbanized areas up to 6 km, preserving genetic diversity (e.g., 294 Fusco et al., 2020), but preventing local adaptation (Miles et al., 2019; Rivkin et al., 295 2019). Our study tested how phenotypic traits differentiate along rural-urban sites 296 while accounting for their environmental characteristics and spatial arrangement. The 297 absence of a clear spatial autocorrelation in environmental variables also allows 298

separating adaptive from neutral responses of phenotypes, akin of the sampling
design used in Gilbert & Lechowicz (2004) for testing neutral versus niche-based
processes in plant communities. Thus, our sampling design allowed us to disentangle
the isolated effects of space and environment on the variation of phenotypic traits.

In summary, most results did not agree with our initial hypotheses, except for 303 CV of body size that decreased with urbanisation, CV of leg length that decreased with 304 land surface temperature. Our results suggest that the lack of a clear spatial variation 305 in phenotypic traits can be due to weak selection as a result of a recent, although 306 307 intense, urbanisation process. As such, populations might be experiencing moderateto-high gene flow that prevents local adaptation. However, as the natural selection 308 erase its traces (Haller and Hendry 2013), the populations can already be adapted to 309 this novel environment, and therefore, detect net-selection over generations can be 310 challenging. Thus, eco-evolutionary dynamics in tropical cities seem to have a different 311 pace compared to temperate ones, producing less significant phenotypic changes 312 between rural and urban frog populations. Our results can contribute to building urban 313 ecological theory that explicitly includes city age, their development, growth rate, and 314 history. 315

316

# 317 Acknowledgements

Mauricio Vancine kindly adapted an R code used to draw buffers in raster files. We thank landowners for allowing access to their properties. Heloísa M. Rodrigues, Marciane R. Severgnini, Bruna C. Yoshida, Philip T. Soares, Adriana C. Acero-Murcia, Nicolle Prado, Klysman Fernandes, Bruno Fines, Letícia A. da Cruz, Eduardo Morel, and Ana Torres helped with field work. Megan J. Thompson kindly discussed preliminary results. We dedicate this study to Prof. Marcelo Menin, who passed away
 due to COVID-19 in 2021.

325

# 326 **Conflict of interest**

327 The authors declare no conflict of interest.

328

## 329 Funding

DBP is supported by a grant from the Brazilian National Council on Research and 330 331 Technological development – CNPg (#407318/2021-6). This study was funded in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil 332 (CAPES) - Finance Code 001 to MRS and DBP. DBP received a fellowship for 333 experienced researchers from the Humboldt Foundation during the final stages of 334 writing. DBP has been supported by a productivity grant from FUNDECT (Proc # 335 83//027.032/2024). ICMBio provided collecting permits (#80075-1). This study was 336 approved by the ethics committee of our university (#1.203/2021). 337

338

#### 339 **References**

|--|

- 342 *Ecology & Evolution*, 30(2), 114–126.
- 343 https://doi.org/10.1016/j.tree.2014.11.007
- Alberti, M., Correa, C., Marzluff, J. M., Hendry, A. P., Palkovacs, E. P., Gotanda, K.
- M., Hunt, V. M., Apgar, T. M., & Zhou, Y. (2017). Global urban signatures of phenotypic change in animal and plant populations. *Proceedings of the*
- 347 National Academy of Sciences, 114(34), 8951 8956.

- Arruda, A. M. V. 2006. Campo Grande: Arquitetura, Urbanismo E Memoria. Editora
   UFMS, Campo Grande, Mato Grosso do Sul.
- Brady, S. P., Bolnick, D. I., Angert, A. L., Gonzalez, A., Barrett, R. D. H., Crispo, E.,
- 351 Derry, A. M., Eckert, C. G., Fraser, D. J., Fussmann, G. F., Guichard, F.,
- Lamy, T., McAdam, A. G., Newman, A. E. M., Paccard, A., Rolshausen, G.,
- 353 Simons, A. M., & Hendry, A. P. (2019). Causes of maladaptation.
- 354 *Evolutionary Applications*, 12(7), 1229–1242.
- 355 Callaghan, C. T., Liu, G., Mitchell, B. A., Poore, A. G. B., & Rowley, J. (2021).
- Urbanisation negatively impacts frog diversity at continental, regional, and
   local scales. *Basic and Applied Ecology*, *54*, 64–74.
- 358 Des Roches, S., Post, D. M., Turley, N. E., Bailey, J. K., Hendry, A. P., Kinnison, M.
- T., Schweitzer, J. A., & Palkovacs, E. P. (2018). The ecological importance
   of intraspecific variation. *Nature Ecology & Evolution*, 2(1), 57–64.
- 361 Edelaar, P., Siepielski, A. M., & Clobert, J. (2008). Matching habitat choice causes
- directed gene flow: A neglected dimension in evolution and ecology.
- 363 *Evolution*, 62(10), 2462–2472.
- Emerson, S. B. (1985a). Jumping and leaping. In M. Hildebrand, D. M. Bramble, K.
- F. Liem, & D. B. Wake (Eds.), *Functional vertebrate morphology* (pp. 58–72).
   Harvard University Press.
- 367 Emerson, S. B. (1985b). Skull Shape in Frogs: Correlations with Diet. *Herpetologica*,
   368 *41*(2), 177–188.
- Emerson, S. B. (1986). Heterochrony and frogs: The relationship of a life history trait
   to morphological form. *American Naturalist*, 127(2), 167–183.
- Fugère, V., & Hendry, A. P. (2018). Human influences on the strength of phenotypic
   selection. *Proceedings of the National Academy of Sciences*, *115*(40), 10070

- 10075.
- Fusco, N. A., Pehek, E., & Munshi-South, J. (2020). Urbanisation reduces gene flow
   but not genetic diversity of stream salamander populations in the New York
   City metropolitan area. *Evolutionary Applications*, 14: 99-116.
- Gilbert, B., & Lechowicz, M. J. (2004). Neutrality, niches, and dispersal in a
   temperate forest understory. *Proceedings of the National Academy of Sciences*, 101(20), 7651–7656.
- 380 Gomez-mestre I, Saccoccio VL, lijima T, et al (2010) The shape of things to come:
- 381 linking developmental plasticity to post-metamorphic morphology in anurans.
- 382 J Evol Biol 23:1364–1373. https://doi.org/10.1111/j.1420-9101.2010.02016.x
- Haller BC, Hendry AP (2014) Solving the paradox of stasis: Squashed stabilizing
   selection and the limits of detection. Evolution (NY) 68:483–500.
   https://doi.org/10.1111/evo.12275
- Hamer, A. J., & McDonnell, M. J. (2008). Amphibian ecology and conservation in the
   urbanising world: A review. *Biological Conservation*, 141(10), 2432–2449.
- Hendry, A. P., Farrugia, T. J., & Kinnison, M. T. (2008). Human influences on rates
- of phenotypic change in wild animal populations. *Molecular Ecology*, *17*(1),
  20–29.
- Hill, M. O., & Smith, A. J. E. (1976). Principal Component Analysis of Taxonomic
   Data with Multi-State Discrete Characters. *Taxon*, *25*(2/3), 249–255.
- Hillman, S. S., Withers, P. C., Drewes, R. C., & Hillyard, S. D. (2008). *Ecological and environmental physiology of amphibians*. Oxford University Press.
- Hutto, D., & Barrett, K. (2021). Do urban open spaces provide refugia for frogs in
  urban environments? *Plos One*, *16*(1), e0244932.
- <sup>397</sup> IBGE. (2023). "Instituto Brasileiro de Geografia e Estatística" (Brazilian Institute of

398	Geography and Statistics), Censo Brasileiro de 2023.
399	Ishitani, M., Kotze, D. J., & Niemelä, J. (2003). Changes in carabid beetle
400	assemblages across an urban-rural gradient in Japan. Ecography, 26(4),
401	481–489.
402	Jennette, M. A., Snodgrass, J. W., & Forester, D. C. (2019). Variation in age, body
403	size, and reproductive traits among urban and rural amphibian populations.
404	Urban Ecosystems, 22(1), 137–147.
405	Khimoun, A., Doums, C., Molet, M., Kaufmann, B., Peronnet, R., Eyer, P. A., &
406	Mona, S. (2020). Urbanisation without isolation: the absence of genetic
407	structure among cities and forests in the tiny acorn ant Temnothorax
408	nylanderi. Biology Letters, 16(1), 20190741.
409	Komine, H., Yasumiba, K., & Schwarzkopf, L. (2022). The country toad and the city
410	toad: comparing morphology of invasive cane toads (Rhinella marina) from
411	rural and urban environments. Biological Journal of the Linnean Society,
412	blac100.
413	Kvålseth, T. O. (2017). Coefficient of variation: the second-order alternative. Journal
414	of Applied Statistics, 44(3), 402–415.
415	https://doi.org/10.1080/02664763.2016.1174195
416	Langerhans, R. B., & Kern, E. M. A. (2020). Urbanisation and Evolution in Aquatic
417	Environments. In M. Szulkin, J. Munshi-South, & A. Charmantier (Eds.),
418	Urban Evolutionary Biology (p. 352). Oxford Univeristy press.
419	Levis NA, Pfennig DW (2016) Evaluating 'Plasticity-First' Evolution in Nature: Key
420	Criteria and Empirical Approaches. Trends Ecol Evol 31:563-574.
421	https://doi.org/https://doi.org/10.1016/j.tree.2016.03.012
422	Levis NA, Pfennig DW (2021) Innovation and Diversification Via Plasticity-Led

- 423 Evolution. In: Levis NA, Pfennig DW (eds) Phenotypic Plasticity & Evolution,
  424 1st edn. CRC Press, pp 211–240
- 425 Li, G., Fang, C., Li, Y., Wang, Z., Sun, S., He, S., Qi, W., Bao, C., Ma, H., Fan, Y.,
- 426 Feng, Y., & Liu, X. (2022). Global impacts of future urban expansion on 427 terrestrial vertebrate diversity. *Nature Communications* 13(1), 1–12.
- Liu, G., Rowley, J. J. L., Kingsford, R. T., & Callaghan, C. T. (2021). Species' traits
- drive amphibian tolerance to anthropogenic habitat modification. *Global Change Biology*, *27*(13), 3120–3132.
- 431 https://doi.org/https://doi.org/10.1111/gcb.15623
- Lobry, J. R., Bel-Venner, M.-C., Bogdziewicz, M., Hacket-Pain, A., & Venner, S.
- 433 (2023). The CV is dead, long live the CV! *Methods in Ecology and Evolution*,
  434 14(11), 2780–2786.
- 435 MapBiomas, P. (2023). Projeto MapBiomas Coleção [v.7.1] da Série Anual de

436 Mapas de Cobertura e Uso do Solo do Brasil. https://mapbiomas.org/

- 437 Mariotto LF, Lofeu L, Kohlsdorf T (2025) Developmental Plasticity in Growth and
- 438 Performance Blur Taxonomic Boundaries in South American True Toads
- 439 (*Rhinella*). J Exp Zool Part B Mol Dev Evol 344:80–93.

440 https://doi.org/https://doi.org/10.1002/jez.b.23283

441 Matías-Ferrer, N., & Escalante, P. (2015). Size, body condition, and limb asymmetry

- in two hylid frogs at different habitat disturbance levels in Veracruz, México.
- 443 *Herpetological Journal*, 25(July), 169–176.
- McDonnell, M.J., & Hahs, A.K. (2015) Adaptation and Adaptedness of Organisms to
  Urban Environments. Annu Rev Ecol Evol Syst 46:261–280.
- 446 Menin, M., Rossa-Feres, D. de C., & Giaretta, A. A. (2005). Resource use and
- 447 coexistence of two syntopic hylid frogs (Anura, Hylidae). *Revista Brasileira*

448 *de Zoologia* 22:1.

Merckx, T., Souffreau, C., Kaiser, A., Baardsen, L. F., Backeljau, T., Bonte, D., 449 Brans, K. I., Cours, M., Dahirel, M., Debortoli, N., De Wolf, K., Engelen, J. M. 450 T., Fontaneto, D., Gianuca, A. T., Govaert, L., Hendrickx, F., Higuti, J., Lens, 451 L., Martens, K., ... Van Dyck, H. (2018). Body-size shifts in aquatic and 452 terrestrial urban communities. Nature, 558(7708), 113-116. 453 Miles, L. S., Rivkin, L. R., Johnson, M. T. J., Munshi-South, J., & Verrelli, B. C. 454 (2019). Gene flow and genetic drift in urban environments. Molecular 455 Ecology, 28(18), 4138-4151. 456 Mitchell, A., & Bergmann, P. J. (2016). Thermal and moisture habitat preferences do 457 not maximize jumping performance in frogs. Functional Ecology, 30(5), 733-458 742. 459 Moran, E. V, Hartig, F., & Bell, D. M. (2016). Intraspecific trait variation across 460 scales: implications for understanding global change responses. Global 461 Change Biology, 22(1), 137-150. 462 Myers, G. (2021). Urbanisation in the Global South. In C. M. Shackleton, S. S. 463 Cilliers, E. Davoren, & M. J. du Toit (Eds.), Urban Ecology in the Global 464 South (pp. 27–49). Springer International Publishing. 465 Parmelee, J. R. (1999). Trophic Ecology of a Tropical Anuran Assemblage. Natural 466 History Museum The University of Kansas, 11, 1–59. 467 Phillips, B. L., Brown, G. P., Webb, J. K., & Shine, R. (2006). Invasion and the 468 evolution of speed in toads. Nature, 439(7078), 803. 469 Piano, E., Giuliano, D., & Isaia, M. (2020). Islands in cities: Urbanisation and 470 fragmentation drive taxonomic and functional variation in ground arthropods. 471 Basic and Applied Ecology, 43, 86–98. 472

473	PLANURB. (2019). Agência Municipal de Meio Ambiente e Planejamento Urbano.
474	Perfil Sócio Econômico de Campo Grande. In Campo Grande (26th ed.).
475	Relyea RA (2001) The Lasting Effects of Adaptive Plasticity : Predator-Induced
476	Tadpoles Become Long-Legged Frogs. Ecology 82:1947–1955.
477	https://doi.org/10.1890/0012-9658(2001)082[1947:TLEOAP]2.0.CO;2
478	Rivkin, L. R., Santangelo, J. S., Alberti, M., Aronson, M. F. J., de Keyzer, C. W.,
479	Diamond, S. E., Fortin, M. J., Frazee, L. J., Gorton, A. J., Hendry, A. P., Liu,
480	Y., Losos, J. B., MacIvor, J. S., Martin, R. A., McDonnell, M. J., Miles, L. S.,
481	Munshi-South, J., Ness, R. W., Newman, A. E. M., … Johnson, M. T. J.
482	(2019). A roadmap for urban evolutionary ecology. Evolutionary Applications,
483	12(3), 384–398.
484	Rosseel, Y. (2012). lavaan: An R Package for Structural Equation Modeling. Journal
485	of Statistical Software; 1(2) .
486	Sanderson, S., Bolnick, D.I., Kinnison, M.T., O'Dea, R.E., Gorne, L.D., Hendry, A.P.
487	& Gotanda, K.M. (2023) Contemporary changes in phenotypic variation, and
488	the potential consequences for eco-evolutionary dynamics. Ecology Letters,
489	26 Suppl 1, S127-S139.
490	Santana, D. J., Ferreira, V. G., Crestani, G. N., & Neves, M. O. (2019). Diet of the
491	Rufous Frog Leptodactylus fuscus (Anura, Leptodactylidae) from two
492	contrasting environments. Herpetozoa, 32, 1–6.
493	Schmidt, C., Domaratzki, M., Kinnunen, R. P., Bowman, J., & Garroway, C. J.
494	(2020). Continent-wide effects of urbanisation on bird and mammal genetic
495	diversity. Proceedings of the Royal Society B, 287(1920), 20192497.
496	Scott Jr., N. J., Woodward, B. D., Scott, N. J., & Woodward, B. D. (1994). Surveys at
497	breeding sites. In W Ronald Heyer, M. A. Donnelly, R. W. McDiarmid, LA.

498	C. Hayek, & M. S. Foster (Eds.), Measuring and monitoring biological
499	diversity: Standard methods for amphibians (pp. 118–125). Smithsonian
500	Institution Press.
501	Severgnini, M. R., Ganci, C. C., Souza, F. L., Almeida-Gomes, M., Angeoletto F. H.
502	S., & Provete, D. B. (2025). Ecological dynamics of frogs in tropical cities:
503	Uncovering bias using a systematic literature review. F. Angeoletto, P.
504	Tryjanowski, & M. Fellowes (Eds.), Ecology of Tropical Cities. Springer
505	Nature.
506	Severgnini, M. R. & Provete, D. B. (2024). The effect of urbanisation and local
507	environmental heterogeneity on phenotypic variability of a tropical treefrog
508	[Dataset]. Dryad. https://doi.org/10.5061/dryad.3xsj3txp2
509	Shackleton, C. M., Cilliers, S. S., du Toit, M. J., & Davoren, E. (2021). The Need for
510	an Urban Ecology of the Global South. In Urban Ecology in the Global South
511	(pp. 1–26). Springer, Cham.
512	Smith, A. N., & Belk, M. C. (2018). Does body size affect fitness the same way in
513	males and females? A test of multiple fitness components. Biological Journal
514	of the Linnean Society, 124(1), 47–55.
515	Szulkin, M., Garroway, C. J., Corsini, M., Kotarba, A. Z., & Dominoni, D. (2020). How
516	to Quantify Urbanisation When Testing for Urban Evolution? In M. Szulkin, J.
517	Munshi-South, & A. Charmantier (Eds.), Urban Evolutionary Biology. Oxford
518	University Press.
519	Tejedo, M., Marangoni, F., Pertoldi, C., Richter-Boix, A., Laurila, A., Orizaola, G.,
520	Nicieza, A. G., Álvarez, D., & Gomez-Mestre, I. (2010). Contrasting effects of
521	environmental factors during larval stage on morphological plasticity in post-
522	metamorphic frogs. Climate Research, 43(1–2), 31–39.

523	Thompson, M. J., Capilla-Lasheras, P., Dominoni, D. M., Réale, D., & Charmantier,
524	A. (2022). Phenotypic variation in urban environments: mechanisms and
525	implications. Trends in Ecology & Evolution, 32(2), 171–182.
526	Ulrich, W., Komosiński, K., & Zalewski, M. (2008). Body size and biomass
527	distributions of carrion visiting beetles: do cities host smaller species?
528	Ecological Research, 23(2), 241–248.
529	Urban, M. C. (2011). The evolution of species interactions across natural
530	landscapes. Ecology Letters, 14(7), 723–732.
531	Van Buskirk J, Saxer G (2001) Delayed Costs of an Induced Defense in Tadpoles?
532	Morphology, Hopping, and Development Rate at Metamorphosis. Evolution
533	(NY) 55:821–829.
534	Vasconcelos, T.S., Santos, T.G., Rossa-Feres, D.C. & Haddad, C.F.B. (2009)
535	Influence of the environmental heterogeneity of breeding ponds on anuran
536	assemblages from southeastern Brazil. Canadian journal of Zoology, 87,
537	699-707.
538	Watters, J. L., Cummings, S. T., Flanagan, R. L., & Siler, C. D. (2016). Review of
539	morphometric measurements used in anuran species descriptions and
540	recommendations for a standardized approach. Zootaxa, 4072(4), 477–495.

## 541 Figures and Table legends



**Figure 1.** Path diagrams showing the standardized coefficients (β) of models testing the influence of predictor variables: Urbanization rate (urRa); Moran eigenvector maps (MEM3); Land Surface Temperature – Urban heat island (uhi), and Local heterogeneity (hetr) - (PC1 of a Hill-Smith PCA) on mean and coefficient of variation of body size (svl); leg length (llen); and head width (hwid), shape (hshp), and length (hlen). Goodness fit:  $\chi^2$ = 1.115; DF=2; P = 0.573. Frogs, cow, fish, and dragonfly silhouettes are CC-BY from

548 by Ircham; thermometer created by Andi Nur Abdillah, pond depth created by Alessandro Suraci, urban heat island created by

- 549 Softscape, and urbanization rate created by WiStudio).

- - -

Supporting Information

556

- 557 Supplementary Methods
- 558
- 559 Morphometric data processing

Prior to analysis, we transformed head width, head length, and leg length to 560 remove the effect of body size by applying a linear regression on each trait as a 561 function of body size and retaining the residuals. To obtain head shape, we took the 562 log-shape ratio (Mosimann, 1970) by computing the geometric mean of head length 563 and width to obtain a measurement of size. Then, we divided each value by their 564 size and log-transformed the result (Claude, 2013). Finally, we performed a Principal 565 Component Analysis onto this matrix and took the first axis to represent head shape. 566 PC1 retained 92.5% of the variance and was negatively correlated with head length 567 (-0.88) and width (-0.47). Negative scores along PC1 represent individuals with 568 wider and longer heads, while positive ones represent those with shorter and 569 narrower heads. Analysis was performed in R v. 4.2.3 (R Core Team, 2023). 570

571

#### 572 Further details on environmental data processing

To quantify pond environmental heterogeneity, we measured the following variables: pond area ( $m^2$ ; using Google Earth®), pond depth (mean of five points per pond; in m), pond hydroperiod (permanent or temporary – binary variable), presence or absence of cattle (binary variable), aquatic predators (vertebrate or both invertebrate and invertebrate – categorical variable), margin profile (excavated, flat or sloping – categorical variable), water temperature (using a thermometer submerged at 1 m for 1 min; in °C), and percentage of floating vegetation (visual estimation). All variables were later summarized using a Hill-Smith Principal Component Analysis (Hill & Smith, 1976), which allows combining discrete and quantitative variables. The PC1 retained ~ 63% of the variance in the data. The variables that most contributed to PC1 (correlation > 0.6) were: excavated margin (positive), temporary hydroperiod (negative), invertebrate predators (negative), and cattle presence (negative; Table S1, Figure S5 and S6). Analysis was conducted in the ade4 R package (Dray & Dufour, 2007).

587

## 588 Further details on spatial data processing

The buffer size of 500-m was chosen as it is related to maximum movement distances of small treefrogs (Wells, 2007, Almeida-Gomes et al., 2016) and has often been used in urban landscape ecology (Ganci et al., 2021). We repeated all analyses using a 1-km buffer and results did not differ (Severgnini & Provete 2024). Spatial data handling was conducted in the R packages terra (Hijmans, 2023) and landscapemetrics (Hesselbarth et al., 2019).

Ponds in the core urban had high urbanisation rates and had already 595 undergone a moderate building process before 1985, providing evidence that ponds 596 in urban core areas experienced a long-lasting change (see Severgnini & Provete 597 2024). Thus, we assume frogs living in highly urbanised ponds have been 598 experiencing the effects of urbanisation for more time than those in peri-urban or 599 rural sites. Provided small treefrogs live for approximately 12 years in the wild (de 600 Magalhães & Costa 2009). Also, some treefrogs can reach at sexual maturity in two 601 years (e.g., Baraquet et al., 2021). Thus, we assume that at least 18 generations 602 may have passed since 1985.. 603

To calculate land surface temperature (LST) and urban heat island (UHI; 604 Figure S7) we used satellite images from LANDSAT 8 thermal band 10 for 2022 (7) 605 November 2022). All images were obtained from Earth Explorer (United States 606 Geological Survey – USGS: https://earthexplorer.usgs.gov/). The pixels of these 607 images contain solar light variables, such as reflectance and radiance. To extract 608 land surface temperature, we used the equation proposed by Coelho & Correa 609 (2013), which converts digital numbers to solar radiance, radiance to brightness 610 temperature, and then to temperature in Kelvin or Celsius. Then, we used land 611 surface temperature to calculate urban heat island (UHI) (see Ahmed et al., 2013; 612 613 Faisal et al., 2021) for the whole urban area, as follows:

$$UHI = \frac{Ts - tm}{SD}$$

615 where, *Ts* is land surface temperature; *tm* is the mean of land surface 616 temperature, and SD is its standard deviation. Finally, we built an urban heat island 617 map and calculated maximum, minimum, variance, and mean temperature in 500-618 m buffers around each pond. Mean land surface temperature was later used as 619 predictor variable in the path analysis. Data handling and extraction were done in 620 QGIS v. 3.22.1 (QGIS.org, 2020).

621

# 622 Building spatial predictors and quantifying spatial autocorrelation

To describe the spatial arrangement of ponds at multiple scales, we used Moran's Eigenvector Maps (MEMs). Firstly, we used the geographical coordinates of ponds to build a spatial neighbourhood network. We tested different types of networks that represent alternative hypotheses of connections between ponds (i.e., Delaunay triangulation, Gabriel graphs, Minimum spanning trees, and a custombuilt connectivity network). Then, we built a spatial weighting matrix assigning a

linear function of the inverse of distance. Afterwards, we generated a set of MEMs 629 for each spatial neighbourhood network. Finally, we performed a selection of spatial 630 matrices (Bauman et al. 2018) in the adespatial R package (Dray et al., 2023). We 631 retained the best subset of MEMs that represents the autocorrelation of body size, 632 built from the best spatial neighbourhood, which was selected based on the Akaike's 633 Information Criterion corrected for small sample sizes (AICc). Then, we computed 634 the corresponding Moran's I statistic of each MEM and calculated its significance 635 using a Monte Carlo permutation test to retain only eigenvectors with positive and 636 significant autocorrelation. 637

The best spatial model was a custom-build neighbour network that did not 638 connect sites through the urban core, with six positive and significant MEMs (Figure 639 S8). MEM3 had the lowest AICc and was used to represent pond spatial 640 arrangement in the path analysis. Positive scores along MEM3 were arranged in a 641 Northeast-Southwest direction and comprised most of the urbanized ponds (Figure 642 S8). Finally, to test for spatial autocorrelation in traits and environmental variables, 643 we used Moran's / correlograms (Legendre & Legendre, 2012) using the same 644 custom-built spatial neighbourhood network. 645

Before entering all variables in the Structural Equation Model, we checked for multicollinearity of the predictors variables using Variance Inflation Factor (VIF) in the R package usdm (Naimi et al., 2014). All variables had VIF < 3 (Dormann et al., 2013) and were retained for further analysis.

650

#### 651 References for extended Methods

Ahmed, B., Kamruzzaman, M., Zhu, X., Rahman, M. S., & Choi, K. (2013).

653 Simulating Land Cover Changes and Their Impacts on Land Surface

Temperature in Dhaka, Bangladesh. Remote Sensing 5(11):5969–5998. 654 https://doi.org/10.3390/rs5115969 655 Almeida-Gomes, M., Rocha, C.F.D. and Vieira, M.V. (2016), Local and Landscape 656 Factors Driving the Structure of Tropical Anuran Communities: Do 657 Ephemeral Ponds have a Nested Pattern? *Biotropica*, 48: 365-372. 658 https://doi.org/10.1111/btp.12285 659 Bauman, D., Drouet, T., Fortin, M.J. and Dray, S. (2018), Optimizing the choice of 660 a spatial weighting matrix in eigenvector-based methods. Ecology, 99: 661 2159-2166. https://doi.org/10.1002/ecy.2469 662 Baraquet, M., Pollo, F. E., Otero, M. A., Grenat, P. R., Salas, N. E., and Martino, 663 A. L. (2021). Body size, age and growth in males populations of Boana 664 pulchella (Anura, Hylidae). Anais da Academia Brasileira de Ciências, 93: 665 e20200991. https://doi.org/10.1590/0001-3765202120200991 666 Claude, J. (2013). Log-Shape Ratios, Procrustes Superimposition, Elliptic Fourier 667 Analysis: Three Worked Examples in R. Hystrix, the Italian Journal of 668 Mammalogy, 24(1), 94-102. https://doi.org/10.4404/hystrix-24.1-6316 669 Coelho, A. L. N., and Correa, W. de S. C. (2013). Temperatura de Superfície 670 671 Celcius do Sensor TIRS/ Landsat – 8. Revista Geográfica Acadêmica, 7(1), 32-45. https://doi.org/10.18227/1678-7226rga.v7i1.2996 672 De Magalhães, J. P., and J. Costa. 2009. A database of vertebrate longevity 673 records and their relation to other life-history traits. Journal of Evolutionary 674 Biology 22:1770–1774. 675 Dormann, C. F., J. Elith, S. Bacher, C. Buchmann, G. Carl, G. Carré, J. R. G. 676 Marquéz, B. Gruber, B. Lafourcade, P. J. Leitão, T. Münkemüller, C. 677 McClean, P. E. Osborne, B. Reineking, B. Schröder, A. K. Skidmore, D. 678

- Zurell, and S. Lautenbach. 2013. Collinearity: a review of methods to deal
  with it and a simulation study evaluating their performance. Ecography
  36:27–46.
- Dray, S., & Dufour, A.-B. (2007). The ade4 Package: Implementing the Duality
  Diagram for Ecologists. *Journal of Statistical Software*, *22*(4 SE-Articles),
  1–20. https://doi.org/10.18637/jss.v022.i04
- Dray, S., Bauman, D., Blanchet, G., Borcard, D., Clappe, S., Guénard, G.,

Jombart, T., Larocque, G., Legendre, P., Madi, N., & Wagner, H. H.

- (2023). {adespatial}: Multivariate Multiscale Spatial Analysis. https://cran.r project.org/package=adespatial
- Epskamp, S. (2022). semPlot: Path Diagrams and Visual Analysis of Various SEM
   Packages' Output. R package version 1.1.6, <a href="https://CRAN.R-">https://CRAN.R-</a>
- 691 project.org/package=semPlot>.
- Faisal, A.-A.-, Kafy, A.-A., Al Rakib, A., Akter, K. S., Jahir, D. M. A., Sikdar, M. S.,
- Ashrafi, T. J., Mallik, S., & Rahman, M. M. (2021). Assessing and
- 694 predicting land use/land cover, land surface temperature and urban
- 695 thermal field variance index using Landsat imagery for Dhaka Metropolitan
- area. Environmental Challenges, 4, 100192.
- 697 https://doi.org/https://doi.org/10.1016/j.envc.2021.100192
- Ganci, C.C., Provete, D.B., Püttker, T. *et al.* 2022. High species turnover shapes
   anuran community composition in ponds along an urban-rural gradient.
- 700 Urban Ecosystems **25**, 633–642. https://doi.org/10.1007/s11252-021-
- 701 **01174-8**
- Hesselbarth, M. H. K., Sciaini, M., With, K. A., Wiegand, K., & Nowosad, J. (2019).
- <sup>703</sup> landscapemetrics: an open-source R tool to calculate landscape metrics.

704	Ecography, 42, 1648–1657. https://doi.org/10.1111/ecog.04617
705	Hijmans, R. J. (2023). terra: Spatial Data Analysis. R package version 1.7-18.
706	https://cran.r-project.org/package=terra
707	Legendre, P. (2018). Imodel2: Model II Regression. R package version 1.7-3.
708	https://cran.r-project.org/package=Imodel2
709	Legendre, P., & Legendre, L. (2012). Numerical ecology (3rd. Engli). Elsevier
710	Academic Press.
711	Mosimann, J. E. (1970). Size Allometry: Size and Shape Variables with
712	Characterizations of the Lognormal and Generalized Gamma
713	Distributions. Journal of the American Statistical Association, 65(330),
714	930–945. https://doi.org/10.1080/01621459.1970.10481136
715	Naimi, B., Hamm, N. A. S., Groen, T. A., Skidmore, A. K., & Toxopeus, A. G.
716	(2014). Where is positional uncertainty a problem for species distribution
717	modelling? <i>Ecography</i> , <i>37</i> (2), 191–203.
718	https://doi.org/https://doi.org/10.1111/j.1600-0587.2013.00205.x
719	R Core Team. (2023). R: A language and environment for statistical computing. R
720	Foundation for Statistical Computing, Vienna, Austria, URL https://www.R-
721	project.org/.
722	QGIS.org. (2020). QGIS Geographic Information System. Open Source Geospatial
723	Foundation Project. http://qgis.org (3.4.1).
724	Wells, K. D. (2007). The ecology and behavior of amphibians. University of
725	Chicago Press.
726	
727	Supplementary tables

**Table S1.** Local environmental variables used to compute Hill-Smith Principal
Component Analysis and their loadings along the first two axes. The first axis was
used to represent local heterogeneity.

Variables	CS1	CS2
Hydroperiod permanent	0.146	0.058
Hydroperiod temporary	-0.878	-0.347
Margin profile excavated	0.634	-0.217
Margin profile flat	-0.330	0.613
Margin profile sloping	-0.304	-0.396
Predator both (vertebrate and invertebrate)	0.298	-0.065
Predator invertebrate	-0.596	0.131
Cattle absent	0.196	0.165
Cattle present	-0.628	-0.528
Floating vegetation (%)	-0.341	0.381
Temperature (°C)	-0.319	-0.431
Area (m²)	0.012	0.577
Pond depth (mean, cm)	0.380	-0.160

732

Table S2. Results of Structural Equation Models showing models results about the
influence of predictor variables: Local heterogeneity (hetr) – (first axis of Hill-Smith
Principal Component Analysis); Urbanisation rate (urRa); Urban heat island – Land
Surface Temperature (uhi); and Moran's Eigenvector Maps (MEM3) on mean body
size; mean leg length; mean head width; mean head length; and mean head shape.
We also tested the effects of the same predictors on the Kvålseth coefficient of
variation (KCV) of phenotypic traits. The last subheading shows paths present in all

models with the same results. Goodness of fit:  $\chi^2 = 1.115$ , DF = 2, P = 0.573. Std.

 $\beta$  = Standardized regression coefficients; z-value = z statistics; P(|z|) = probability

value; Est. ( $\beta$ ) = regression coefficients; Std. Err = Standard error. Significant P

values are in bold.

	Est. (β)	Std. Err	z-value	<i>P</i> (> z )	Std. β
Mean					
Body size	~	-			
Local heterogeneity	0.300	0.127	2.363	0.018	0.658
Urbanisation rate	-0.205	0.114	-1.803	0.071	-0.449
Urban heat Island	0.076	0.083	0.911	0.362	0.166
MEM3	-0.269	0.096	-2.792	0.005	-0.589
Leg length	~				
Local heterogeneity	-0.027	0.096	-0.278	0.781	-0.091
Urbanisation rate	0.017	0.086	0.196	0.844	0.058
Urban heat Island	0.026	0.063	0.408	0.683	0.088
MEM3	-0.052	0.073	-0.709	0.478	-0.177
Head width	~				
Local heterogeneity	0.014	0.020	0.680	0.496	0.192
Urbanisation rate	0.022	0.018	1.247	0.213	0.315
uhi	0.018	0.013	1.358	0.175	0.251
MEM3	-0.005	0.015	-0.323	0.747	-0.069
Head length	~				
Local heterogeneity	0.023	0.022	1.006	0.314	0.317
Urbanisation rate	0.00	0.020	0.015	0.988	0.004
Urban heat Island	0.009	0.015	0.601	0.548	0.124
MEM3	0.001	0.017	0.065	0.948	0.016
Head shape	~				
Local heterogeneity	-0.015	0.006	-2.626	0.009	-0.714
Urbanisation rate	0.007	0.005	1.312	0.19	0.319
Urban heat Island	-0.005	0.004	-1.387	0.165	-0.247
MEM3	0.011	0.004	2.646	0.008	0.545
Coefficient of variation		-			
cv Body size	~				
Local heterogeneity	-0.007	0.003	-2.89	0.004	-0.751
Urbanisation rate	0.00	0.002	0.194	0.846	0.045
Urban heat Island	0.001	0.002	0.720	0.471	0.122
MEM3	0.005	0.002	2.486	0.013	0.489
cv Leg length	~				
Local heterogeneity	0.003	0.004	0.778	0.437	0.209
Urbanisation rate	-0.002	0.003	-0.523	0.601	-0.126
Urban heat Island	-0.008	0.002	-3.263	0.001	-0.574
MEM3	-0.003	0.003	-1.031	0.303	-0.210
cv Head width	~				_
Local heterogeneity	0.006	0.006	0.988	0.323	0.311
Urbanisation rate	-0.005	0.006	-0.986	0.324	-0.278

Urban heat Island	-0.005	0.004	-1.324	0.185	-0.273
MEM3	-0.003	0.005	-0.704	0.482	-0.168
cv Head length	~				
Local heterogeneity	0.002	0.005	0.314	0.753	0.101
Urbanisation rate	-0.001	0.005	-0.164	0.870	-0.047
Urban heat Island	-0.004	0.003	-1.227	0.220	-0.257
MEM3	0.003	0.004	0.658	0.510	0.160
cv Head shape	~				
Local heterogeneity	-0.111	0.067	-1.651	0.099	-0.493
Urbanisation rate	-0.012	0.06	-0.192	0.848	-0.051
Urban heat Island	0.001	0.044	0.013	0.990	0.003
MEM3	0.068	0.051	1.330	0.184	0.301
Predictor variables					
Urban Heat Island	~				
Urbanisation rate	0.172	0.215	0.800	0.423	0.172
Local heterogeneity	~				
Urbanisation rate	0.600	0.143	4.202	0.000	0.600
MEM3	0.382	0.143	2.676	0.007	0.382

746 Supplementary figures



Figure S1. Hypothesis of this study. Green arrows represent positive and red arrows 749 represent negative effects. Dashed arrows represent indirect and continuous arrows 750 represent direct effects. Urbanisation was measured as rate of change in building 751 and roads area from 1985 to 2021. Space is the spatial arrangement of ponds 752 quantified by Moran's Eigenvector Maps. The environment was represented by the 753 first axis of PCA summarizing local environmental variables. Temperature is land 754 755 surface temperature/ urban heat island. Phenotype represents mean or coefficient of variation of phenotypic traits. Frog silhouette is CC-BY from PhyloPic. 756 Environment, urbanization, and temperature silhouettes are CC-BY 3.0 from Noun 757

Project (ponds created by Ircham; urban heat island created by Softscape, and
 urbanization rate created by WiStudio).

760



Figure S2. Map of land use and land cover change (1985–2021) showing ponds
sampled (black spots) along the rural–urban gradient in Campo Grande, Mato
Grosso do Sul, Brazil. DATUM=SIRGAS/2000. Map features extracted from Instituto
Brasileiro de Geografia e Estatística (IBGE) and MapBiomas data base 2023; and
prepared on QGIS v. 3.22.1.



**Figure S3.** Ponds sampled along the rural–urban gradient in Campo Grande, Mato

Grosso do Sul, Brazil. P11 and P21 are examples of ponds with excavated margins,

P23 had a flat margin, while P04 and P05 had slopping margins.



**Figure S3**. Continued.



Figure S4. Percentage of impervious surface (roads and buildings) as of 2021 within



a 500-m buffer around the 21 sampled ponds along the urbanization gradient.

Figure S5. Ordination diagram of the first two axes of the Hill-Smith Principal
Component Analysis showing the ponds (numbers in squares) and local
environmental variables (arrows).



**Figure S6.** Scores of the local environmental variables along the first axis of the Hill-Smith Principal Component Analysis. For the quantitative variables  $r^2$  = squared regression coefficients, and for discrete variables cr = correlation ratio are showed.



**Figure S7**. Map of land surface temperature/urban heat islands of Campo Grande, Mato Grosso do Sul, Brazil. White circles represent sampling sites (ponds). Map features LANDSAT 8 thermal band 10 extracted from United States Geological Survey (USGS). Black line delimits the urban perimeter extracted from https://sisgran.campogrande.ms.gov.br/; and prepared on QGIS v. 3.22.1.

786



792

**Figure S8**. The six Moran Eigenvector Maps (MEMs) generated with the custom spatial neighbourhood network that had positive (Moran's l > 0) and significant spatial autocorrelation. Each dot represents a sampled pond. Black dots represent positive and white dots represent negative scores along each eigenvector. Each eigenvector describes the spatial arrangement of ponds at a given spatial scale, ranging from broad to fine scale.



800

Figure S9. Scatter plots showing the relationship between significant variables in the structural equation model. MEM3: Moran Eigenvector Maps (MEMs); cv: coefficient of variation; UHI: urban heat island – land surface temperature (next panel).



**Figure S10**. Mean and coefficient of variation of body size (svl); leg length (llen); head length (hlen); head width (hwid); and head shape (hshp) along the urban

- gradient of Campo Grande. Circles represent trait values in grey gradient ranging
  - cvllen ° cvsvl 0 cvhlen 0 • 0 
     [○ -0.02] ○ -0.01] ○ 0] ○ 0.01] ○ 0.02] ● 0.03] ● 0.04] ● 0.05] ●
     [○ 0.04] ○ 0.045] ◎ 0.055] ◎ 0.055] ● 0.06] ● 0.065] ● 0.07] ● 0.075] ● ○ -0.01] ○ 0] ● 0.01] ● 0.02] ● 0.03] ● cvhwid ® cvhshp • 0 0 ○ -0.02] ◎ 0] ● 0.02] ● 0.04] ● ○ -0.5] ○ 0] ● 0.5] ● 1] ●
- 811 from min to max.

813 **Figure S10**. Continued.

814



815

○ 16] ○ 17] ● 18] ● 19] ● 20] ●

Figure S11. Local heterogeneity (hetr); Urbanisation rate (urRa); Urban heat island (uhi); and Moran's Eigenvector Maps (MEM3) across urban gradient of Campo Grande. Circles represents variable values that are presented in grey gradient ranging from min to max values of each variable.

○ -1.5] ○ -1] ○ -0.5] ● 0] ● 0.5] ● 1] ● 1.5] ●


**Figure S12**. Spatial correlogram using a Moran's *I* for the mean (this panel) and coefficient of variation (next panel) of phenotypic traits used in the Structural Equation Models. Lags are distance classes.

821



Figure S12. Continued.



**Figure S13**. Spatial correlograms using a Moran's / for local and landscape predictor

variables used in the Structural Equation Models. Lags are distance classes.

1	CAPÍTULO 2
2	
3	Target journal: Urban Ecosystems (ISSN: 1573-1642)
4	
5	Frog call selection surface along an urbanisation gradient
6	
7	Marcos R. Severgnini <sup>1</sup> , Diogo B. Provete <sup>2,3,4*</sup>
8	
9	<sup>1</sup> Graduate Program in Ecology and Conservation, Federal University of Mato
10	Grosso do Sul, Campo Grande, 79002-900, Brazil.
11	<sup>2</sup> Institute of Biosciences, Federal University of Mato Grosso do Sul, Campo
12	Grande, 79002-900, Brazil.
13	<sup>3</sup> Gothenburg Global Biodiversity Centre, PO Box 100, SE-413 19, Göteborg,
14	Sweden.
15	<sup>4</sup> German Centre for Integrative Biodiversity Research-iDiv, Halle-Jena-Leipzig,
16	Leipzig, Germany.
17	
18	* Corresponding author: diogo.provete@ufms.br
19	
20	Abstract
21	
22	The acoustic adaption hypothesis posits that species in open habitats should
23	increase their call frequency and use repeated signals to avoid information loss.
24	However, there is little consensus about how the way frog call frequency evolves

in tropical urban landscapes. Here, we estimated the adaptive landscape for the 25 frequency of a treefrog advertisement call under distinct urbanisation levels in a 26 Tropical city. We hypothesize that frogs will call at higher frequencies and have 27 lower body size due to negative body size - call frequency allometric relationship 28 in core urban than peri-urban areas to overcome urban noise. We quantified 29 urbanisation using a multivariate approach, considering both environmental and 30 socioeconomic variables for 21 ponds. To estimate the selection surfaces, we 31 used a modification of Lande-Arnold's approach using the Scaled Mass Index 32 (SMI) as a fitness proxy. We found no differences in dominant frequency among 33 areas, but it was less variable in urban areas. Individuals calling at high dominant 34 35 frequencies in both rural and urban area had lower fitness. Our study contributes to understanding how natural selection processes are shaped by urbanisation in 36 a tropical young city in the Global South. 37

38

39 **Key-words**: anuran call; selective gradients; acoustic communication.

40

### 41 Introduction

42

Urbanisation has increased across the globe following human population growth (UN-Habitat 2022; Mainwaring et al. 2024). Several factors associated with urbanisation, such as habitat split, habitat loss, artificial light at night, and noise pollution are impacting biodiversity (Szulkin et al. 2020) by changing both phenotypic (i.e., physiological, morphological, and behaviour; Alberti et al. 2017) and genetic traits (e.g., genetic diversity; Miles et al. 2019). One of the documented consequences of urbanisation is the decrease in phenotypic and genetic variation, promoting biodiversity homogenization (Piano et al. 2020). An important behavioural trait affected by urbanisation, specifically by anthropogenic noise (i.e., traffic, engines, airplanes), is acoustic communication, since noise pollution impairs hearing and acoustic signals of several species, such as insects, fish, frogs, birds, and mammals (Parris 2015; Shannon et al. 2016). Also, as urban density and urban noise are highly correlated (Salomons and Berghauser Pont 2012), in highly urbanized areas these effects could be even more intense.

Species that use acoustic signals for communication and reproduction 57 can experience negative consequences in urban ecosystems due to increased 58 levels of anthropogenic noise (Zaffaroni-Caorsi et al. 2023), since these 59 environments have high sound pressure levels that can mask acoustic signals 60 (Simmons and Narins 2018). For instance, frogs highly depend on acoustic 61 communication for sexual and social behaviour (Wells 2007), since acoustic 62 communication transmits information about their body size and location as well 63 as is involved in sexual selection (Erdtmann and Lima 2013). Also, acoustic 64 signals and auditory systems play a crucial role in mate attraction and mate-65 choice (Gerhardt 1994). In frogs, advertisement call is related to sexual behaviour 66 and has two components: spectral (static - less variable) and temporal 67 parameters (dynamic - more variable), which are used for species recognition 68 and transmit information on mate quality (Gerhardt 1991). Although spectral traits 69 have been classified as static (Gerhardt 1991, 1994; Köhler et al. 2017), a recent 70 review on the effect of anthropogenic noise on dominant frequency in anurans 71 found contrasting patterns. Some species increased their dominant frequency in 72 noisy environments, while others decreased or did not show any response (see 73 table 1 in Zaffaroni-Caorsi et al. 2023). The same lack of change in dominant 74

frequency following anthropogenic noise in urban environments was found in tropical cities (Severgnini et al. 2025). Species that decrease their call frequencies vocalized at lower-frequencies (i.e., < 2 kHz) near anthropogenic noise frequency (Zaffaroni-Caorsi et al. 2017), while species with no changes called at high frequencies. However, this seems highly variable and depends on species and anthropogenic noise intensity.

The acoustic adaptation hypothesis (AAH) poses that the emission and 81 receival of acoustic signal by organisms are impacted by habitat structural 82 complexity (Hardt and Benedict 2021). As a result, natural selection can favours 83 individuals that change call parameters depending on environmental 84 85 characteristics (i.e., plasticity) in order to preserve signal quality. For example, call frequency should be lower and longer in denser environments (e.g., forests) 86 for a better signal propagation, while the opposite should happen in open sites 87 (Erdtmann and Lima 2013; Hardt and Benedict 2021). However, a recent meta-88 analysis did not support this hypothesis, since different patterns can be found 89 across several taxa and organisms (e.g., anurans, birds, insects) likely because 90 of plastic responses to the environment, but this work did not examine differences 91 92 between rural and urban areas (Freitas et al. 2024). So far, while some studies focused more on changes in temporal than spectral call parameters between rural 93 and urban environments (e.g., Halfwerk et al. 2019), few have accessed how 94 spectral traits and its allometry (i.e., body size - dominant frequency relationship) 95 change across rural-urban gradients. Also, no study has estimated how selection 96 gradients for this trait changes in rural, peri-urban, and urban areas. Thus, the 97 relationship between changes in calling behaviour and changes in fitness still 98 remains unclear. 99

One strategy to resolve this apparent ambiguity in the acoustic response 100 101 to the environment is to explicitly estimate selection gradients for call parameters known to have evolved under natural and sexual selection (Ryan 1988; Gerhardt 102 1994; Wells 2007; Köhler et al. 2017), such as call dominant frequency. Thus, 103 estimate selection gradient allows detect if a trait is under selection pressure and 104 what direction of this selection. Dominant frequency is the portion of the call 105 106 frequency that concentrates the highest energy (Köhler et al. 2017). The evolution of dominant frequency is constrained by a trade-off (Tonini et al. 2020) due to 107 allometric relationships between an organism body size and its pharyngeal 108 109 apparatus, so that bigger frogs will call at lower dominant frequencies and smaller ones will call at higher dominant frequencies. Higher frequencies are produced 110 by small cords, and can change by altering tension on the vocal cords (Gerhardt 111 1994; Wells 2007) or by morphological changes in the arytenoid cartilages 112 (Duellman and Trueb 1986; Gerhardt 1994). Thus, mass and tension of vocal 113 cords play an important role in dominant frequency in frogs, in which an 114 increasing mass of vocal cords decrease dominant frequency (Wells 2007). Frogs 115 calling frequency is also expected to be positively related to mating and 116 117 reproductive success in some species, what affect directly fitness (e.g., Lesbarrères et al. 2008; Zhang et al. 2020). For instance, in noisy environments 118 (e.g., streams) dominant frequency might be under high selective pressure, since 119 120 some females prefer males calling at high frequencies (see Zhang et al. 2020). Therefore, analysing how natural selection acts on dominant frequency across 121 putative noise gradients can help understanding why previous studies have 122 observed such a variation in responses of call parameters to urbanisation. The 123 selection surface can be understood as a metaphor (Olson et al. 2019) that 124

relates the average fitness of a population  $\overline{W}$  as a function of average trait values 125  $\bar{z}$  (Lande and Arnold 1983; Arnold 2023). However, this relationship allows 126 estimating the mode of selection and its intensity (i.e., selection gradients  $\beta$  and 127 y) in a population, because changes in phenotype mean, variances, and 128 covariances are expressed by slopes inclination and curvature (Arnold 2003). 129 Selection gradients can be defined as linear deviations (directional -  $\beta$ ) and 130 131 quadratic deviations (non-linear - y) from the optimum (Arnold 2023). These selection gradients allowing us to predict evolutionary changes (Arnold 2003). 132 133 Thus, as urban areas can affect acoustic traits and consequently fitness, we expect different optima for populations inhabiting contrasting environments (e.g., 134 rural, peri-urban, urban areas). Frogs are ideal organisms to investigate this 135 question, since they have calls modulated by the environment (Hardt and 136 Benedict 2021), their call pitch is constrained by body size, and thus sexual and 137 138 natural selection might impose contrasting selective pressures on acoustic and morphophysiological traits, making frogs having to deal with a complex trade-off 139 in urbanised areas. Also, frogs are supposed to have less variation in acoustic 140 traits in urbanised areas (i.e., high noise pollution), since plastic responses might 141 be reduced and canalized to an optimum trait to deal with high anthropogenic 142 noise level that masks low-frequency signal (Schwartz and Bee 2013). 143

Herein, we estimate the selective gradients of frog call for populations inhabiting three distinct degrees of urbanisation. We also tested how spectral parameters (i.e., dominant, high and low frequencies) vary across rural, periurban and urban areas. We hypothesized that: (i) frogs will call at higher frequencies in core than peri-urban and rural areas as a way to overcome anthropogenic noise, while species in peri-urban and urban areas will decrease their dominant frequencies as a response to a lower sound pressure levels; (ii) Consequently, these phenotypic traits would have divergent selection surfaces in each population, with phenotypes showing lower fitness in core urban than periurban and rural areas; Finally (iii) frogs from urban areas will have less intraspecific variation in acoustic parameters than those from peri-urban and rural areas, due to strong selection cities impose due to anthropogenic noise.

156

- 157 Material and Methods
- 158

## 159 Study site and sampling design

We conducted the study at the urban perimeter of Campo Grande, Mato 160 Grosso do Sul, central Brazil (20° 26' 37" S, 54° 38' 52" W; Datum – WGS84). 161 Campo Grande has an Equatorial Savanna climate or Köppen's Aw (Kottek et al. 162 2006). The city has about 225.9 km<sup>2</sup> of urbanised areas (MRS, pers. obs. based 163 on data from MapBiomas 2024) and over 630,000 buildings, with a building 164 density ranging from 10 to 50 % per neighbourhood (MRS, pers. obs. based on 165 data from OpenStreetMaps 2024). The population is approximately 898,100 166 people (demographic census IBGE 2022). The urban core has a high degree of 167 noise pollution, ranging from 65 to 80 dB, with average traffic about 13,668 168 vehicles per hour (Souza Filho 2012). Artificial light at night (ALAN) ranging from 169 µcd/m<sup>2</sup> in rural areas to 5,220 µcd/m<sup>2</sup> at the 170 283 urban core (www.lightpollutionmap.info/). 171

We sampled populations of *Dendropsophus nanus* in 21 ponds along rural-to-urban transition (see more details in Severgnini and Provete, 2025). We choose this species due to its wide occurrence along the urbanisation gradient (see Ganci et al. 2022). This species has a diphasic call, with two note types (Fig.
1), and calls at high frequencies with a dominant frequency ranging from 3,937
to 4,593 Hz (Teixeira et al. 2016) or 2,950 and 4,850 Hz with fundamental
frequency around 3,900 Hz (Martins and Jim 2003). Field work was conducted
between 17:30 and midnight, between November 2021 and April 2022 and
between November 2022 and January 2023, visiting two ponds per night (approx.
3 h per pond).

182

### 183 Bioacoustics and morphometric measurements

184 We recorded calls of ten adult male frogs per pond using a digital recorder (Tascam DR-40X) with a sampling rate of 44 kHz and 16-bit resolution 185 coupled to a semi-directional microphone (Sennheiser MKE 600) approximately 186 1 m away from the calling individual for 5 min. Air and water temperature during 187 call recordings were 26.1 ± 2°C and 30.6 ± 2°C, respectively. We analysed 10 188 calls of each individual, totalling 2,100 calls (individuals\*calls\*ponds) and 1,050 189 minutes recorded. We randomly selected ten calls to be analysed within the 5 190 min recorded, but avoided the beginnings and endings of recordings. We 191 192 quantified three spectral parameters: dominant, minimum, and maximum frequency. Analysis was performed in Raven Pro v. 1.6.1 (Bioacoustics 2019). 193

For each frog, we measured body size (Snout-Vent Length) using a digital calliper (MTX 150 mm), following Watters et al. (2016). To remove the effect of body size on dominant frequency (see Tonini et al. 2020), we built a linear model for body size (predictor variable) and dominant frequency (response variable) in R v. 4.2.3 (R Core Team 2023) and took the residuals. Then, we calculated the mean of the 10 calls per individual, to obtain a mean call frequency for each individual. We also weighted each individual using an analogic hanging
 scale (Pesola Lightline 10 g) and tagged them with visible implant elastomer (VIE)
 before releasing them back to the same site.

203

### 204 Sociodemographic and environmental variables

We built a 500-m buffer around each sampling site and quantified several environmental and sociodemographic variables (Fig. S1). We obtained spatialized data for the year 2019 on the number of residents and average household income per capita from (Pereira et al. 2022). We accessed, extracted, and processed the data using QGIS v. 3.22.1 (QGIS.org 2020).

We obtained data for the number and density of buildings for 20 march 210 2024 from Open Street Maps (OpenStreetMap contributors 2024) using Google 211 212 Earth Engine and R v. 4.2.3 (R Core Team 2023). We calculated the percentage of urbanisation, forest, and pasture for 1985 and 2021 inside each buffer using 213 data from MapBiomas (MapBiomas 2024). Then, we calculated a rate of change 214 for these land use types by subtracting 2021 from 1985 and dividing it by 36. We 215 also obtained artificial light at night (ALAN) data from 2012 to 2023 from 216 217 www.lightpollutionmap.info v.2.8.26. This project uses the VIIRS satellite Day Night Band (DNB) to quantify light pollution over the world. We calculated the 218 mean and rate of change of ALAN by subtracting the value of 2023 from that of 219 2012 and dividing by 11. 220

We obtained LANDSAT 8 satellite images for 07 November 2022 from Earth Explorer (United States Geological Survey – USGS: https://earthexplorer.usgs.gov/) and used the thermal band 10 to calculate land surface temperature (LST) for the urban perimeter of Campo Grande. We used an equation proposed by Coelho and Correa (2013) to convert solar radiance into
temperature in Celsius. Then, we mapped this variable on the study area with
QGIS v. 3.22.1 (QGIS.org 2020) and extracted LST inside a 500-m buffer around
each pond. For more details see Severgnini and Provete (2025).

We used Google Earth® tools to measure the area (m<sup>2</sup>) and elevation for each pond with images from 2022. Finally, we measured sound pollution directly by quantifying sound pressure level (SPL) at each pond using a sound level meter (Extech EN300). We directed the equipment to the ponds and recorded noise and measured it for 1 min. We also used building density (Fig. S1) as a proxy for noise pollution, due to its correlation with traffic noise (see Salomons and Berghauser Pont 2012).

236

### 237 Delimiting populations along urban-rural transition

We entered all sociodemographic and environmental variables into a cluster analysis to find three groups of ponds along the urbanisation gradient: rural (group 1), peri-urban (group 2), and urban area (group 3). This procedure was meant to delimit different frog populations that would be under distinct putative selection gradients based on environmental variables. It also served to establish the populations to which we will estimate the adaptive landscape (see below).

First, we standardized all variables to zero mean and unit variation in the R package vegan (Oksanen et al. 2018). We used a non-hierarchical cluster approach (k-means) that partitions the data into pre-chosen k groups (three groups in our case) that maximizes the sum of squares inside groups and minimize it between them. This configuration retained a total sum of squares to

45.5%. As a cross-validation, we used the cascadeKM function from the vegan 250 251 R package (Oksanen et al. 2018) to find the best number of groups using the simple structure index criterion ("ssi"), whose maximum value represents the 252 optimal numbers of clusters. This analysis returned four groups with ssi equal to 253 1.01. However, one of those groups had only two ponds. Therefore, we decided 254 to keep three groups (ssi = 0.85), which provided a more balanced number of 255 ponds in each group (Fig. S2). Urbanisation ranged from 0 % to 18.1% in ponds 256 assigned to the rural group; 33.6% to 62.9% for peri-urban; 70.8% to 94.6% for 257 urban. The variables that most contributed to cluster definition were current 258 259 urbanisation percentage, building density (proxy for noise pollution), mean of artificial light, number of residents, and average household income per capita 260 (correlation > 0.33 to PC1). 261

262

### 263 Fitness proxy

We initially used mark-recapture methods to estimate apparent survival. 264 However, despite intensively sampling for nine months over two breeding 265 seasons, and recording 210 frogs along the urban gradient, we had a recapture 266 rate around 9.5%. This is very low and does not allow us to fit common Cormack-267 Jolly-Seber models to estimate survival. As an alternative, we used body weight 268 and body size to calculate the Scaled Mass Index (SMI) to estimate fitness. Body 269 270 condition indices have often been used as a fitness proxy in amphibians (e.g., MacCracken and Stebbings 2012), since it provides information about body lipidic 271 content. In anurans, previous studies found a positive relationship between SMI 272 and protein and lipids (MacCracken and Stebbings 2012; Brodeur et al. 2020). 273 Fat reserves and body condition are expected to positively affect jumping 274

performance and locomotor capacities in frogs (Drakulić et al. 2016; but see 275 276 Álvarez and Nicieza 2002 and Nicieza et al. 2006). Also, lipid content in turn is correlated with survival in amphibians (Scott et al. 2007), while body condition 277 also seems directly correlated with survival in a wild toad population (Reading 278 2007). Estimating survival for open populations in the field is challenging, 279 especially for vertebrates with noticeably low recapture rates (Klocke et al. 2023: 280 Stock et al. 2023), such as adult frogs in tropical environments. We acknowledge 281 that using SMI as a fitness proxy can be problematic (Wilder et al. 2016). 282 However, evidence exists linking lipidic storage to energetic reserves and 283 284 ultimately to physiological status in frogs. In this context, SMI can be considered a performance index (but see Franklin and Morrissey 2017), since animals with 285 good body condition that have energetic reserves, provided by a past successful 286 feeding, are more likely to survive (Jakob et al. 1996; Wells 2007). 287

First, we modelled log body weight as a function of log body size using 288 standardized major axis (SMA) regression in the smart R package (Warton et al. 289 2012) and ordinary least squares (OLS). Then, we used the slopes from each 290 method separately to calculate SMI, following Peig and Green (2009) and Peig 291 292 and Green (2010). These authors suggest using standardized major axis regression to estimate the *b* exponent of the SMI formula. However, both body 293 size and weight had a limited range of variation and the relationship between 294 them was not as strong as previous studies (e.g., Brodeur et al. 2020). Therefore, 295 using the slope of an OLS regression instead of SMA in the calculation of SMI 296 efficiently removed the effect of body size (Fig. S3, S4). 297

298

299 Data analysis

For allometric relationship between dominant frequency (continuous response) and body size (continuous response) and its interaction among sites (categorical with three levels – rural, peri-urban, urban) we performed an Analysis of Covariance (ANCOVA) by running a Linear Mixed-effects Model (LMM) in the R package *Ime4* (Bates et al. 2015). Ponds were included in the model as a sampling site replication. Residuals had normality, homogeneity of variance (Fig. S5), no overdispersion and no spatial autocorrelation.

We model dominant and minimum frequency (continuous response 307 variables) separately on each dataset as a function of area (categorical predictor 308 309 variable with three levels), using a Linear Mixed-effects Model (LMM) including 310 pond as replication in the R package Ime4 (Bates et al. 2015). Hypothesis testing was conducted using an Analysis of Deviance table, with Type II Wald's  $\chi^2$  test. 311 Models were diagnosed in the R package DHARMa (Hartig 2024). Residuals 312 313 showed normality, homogeneity of variance (Fig. S4), no overdispersion and no 314 spatial autocorrelation.

To model maximum frequency (continuous response variable) in function 315 of area (categorical with three levels), we built four models with different 316 distribution probabilities using a Generalized Linear Mixed Model (GLMM), in 317 which pond was included as a replication in the model. This analysis was 318 performed in the glmmTMB R package (Brooks et al. 2017). Then, we used 319 Akaike information criterion (AIC) to choose the best model based on lowest 320 values of AIC. The best model was a lognormal GLMM with log link. Hypothesis 321 testing was conducted using an Analysis of Deviance table, with Type II Wald's 322  $\chi^2$  test. Residuals showed normality, no overdispersion, but exhibited spatial 323

autocorrelation and a small deviance from homogeneity of variance, probably due
to unbalanced data in rural area (Fig. S5 D).

To estimate the selection surface, we modelled Scaled Mass Index 326 (continuous response variable, that vary between 0 and 1) as a function of size-327 free dominant frequency (continuous predictor variable) separately for each 328 group of ponds recovered from the k-means clustering. We used a non-329 parametric regression in the R package gsg (Morrissey 2014). This analysis, 330 based on the classical Lande and Arnold (1983) approach, use a projection-331 pursuit regression (see Morrissey and Sakrejda 2013; Morrissey 2014) to 332 estimate linear and guadratic selection gradients allowing us not only to estimate 333 334 the strength, but also the shape of the selection gradient for a given phenotype in a population (Morrissey and Sakrejda 2013). 335

336

### 337 **Results**

338

We recorded call spectral parameters from about 100 frogs in the rural 339 area, 60 in peri-urban, and 50 in the urban area; and the call frequencies among 340 areas showed similar values in terms of mean, but the variation was subtly higher 341 in the rural area despite of similar coefficient of variation values (Table 1). 342 343 Allometric relationship between dominant frequency and body size did not differ among areas (Table 2, Fig. 2). Size-free dominant, maximum, and minimum 344 frequency varied more in rural areas than others (Table 1, Fig. 3, Fig. S6). 345 However, we found no significant difference in either size-free dominant (Wald's 346  $\chi^2$  type II test = 5.17; DF = 2; P > 0.07), max (0.66; DF = 2; P > 0.72), or min 347 frequency (0.68; DF = 2; P > 0.71) among areas (see Table 3; Fig. 3), except for 348

significant difference in size-free dominant frequency between rural and peri-urban areas (Table 3).

The combined selection surface for size-free dominant frequency 351 showed negative and significant directional selection (Table 4). Size-free 352 dominant frequency showed a negative and significant directional selection in the 353 rural and urban area (Table 4). The linear selection gradient was negative but 354 non-significant in the peri-urban area (Table 4). The quadratic selection 355 coefficient was not significant in either area. However, we can see a slight curve 356 in the adaptive landscape for both peri-urban and urban areas that suggests a 357 weak, stabilizing selection in the peri-urban and disruptive selection in the urban 358 359 area (Fig. 4).

360

### 361 **Discussion**

362

We found that spectral parameters of the advertisement call of the 363 treefrog Dendropsophus nanus did not vary significantly along the urbanisation 364 gradient. Also, dominant frequency varies more within rural ponds than peri-365 urban and urban ones, suggesting that rural populations might be under relaxed 366 selection (Thompson et al. 2022; Charmantier et al. 2024), while selection is 367 stronger in urban areas. This agrees with our estimation of the linear selection 368 gradient, which despite being weak, was twice as high for the urban than the rural 369 population. 370

We found no difference in frog call parameters along the urbanisation gradient. This is consistent with some anuran species that showed no changes in dominant frequency facing anthropogenic noise (see Zaffaroni-Caorsi et al.

2023), and agrees with the fact that dominant frequency is more "static" in many 374 375 species or shows small variations (Köhler et al. 2017). However, there is no clear pattern, since there are species increasing (e.g., Kruger and Du Preez 2016; 376 Higham et al. 2021) while others decrease (e.g., Zaffaroni-Caorsi et al. 2017; 377 Azevedo Cezila et al. 2024) their dominant frequencies in response to 378 anthropogenic noise. However, this lack of response to urban noise might be due 379 to the already high call frequencies in which *D. nanus* calls (i.e., around 3,900 380 Hz; Martins and Jim 2003) compared to other species (e.g., Leptodactylus 381 podicipinus – 1,156 Hz; Marquez et al. 1995); Rhinella diptycha – 700 Hz; Köhler 382 383 et al. 1997), which might be sufficient to overcome low-frequency anthropogenic noise in cities (< 2,000 Hz; see Lowry et al. 2019), which could be a 384 "preadaptation" to noisy environments (McDonnell and Hahs 2015). Higher 385 frequency calls are less susceptible to masking, but these louder signals can 386 impact acoustic communication by altering natural range of calling spectrum 387 affecting recognition and detection by receivers (Schwartz and Bee 2013). 388 Nonetheless, the consequences of urban noise in call frequencies still remains 389 unclear, since some species respond to noise in different ways. 390

391 Allometric relationship between dominant frequency and body size among areas was very similar. Call frequency is constrained by an allometric 392 relationship with body size (Tonini et al. 2020), which means that selection for 393 394 increasing call frequency would imply a reduction in body size (Roff 1977; Arnold 2023). However, a decrease in body size in urban environments can be 395 maladaptive, because smaller body sizes are more prone to desiccation than 396 larger ones (Hillman et al. 2008; Murphy and Boone 2022), especially in urban 397 heat islands with high temperatures and reduced air humidity (Landsberg 1981). 398

Conversely, smaller bodies are favoured with increasing of warming due to 399 400 reduction in metabolic costs and facility to dissipate heat, which favour thermoregulation, especially in ectotherms (Caruso et al. 2014; Scheffers et al. 401 2016). In addition, an increase in calling frequency can directly impact sexual 402 selection, since females prefer frogs calling at low-frequencies because their 403 auditory system is better able to recognize low-frequencies over high ones (see 404 Gerhardt 1994). Also, females seem to choose low-quality calls in noisy 405 environments than in non-noisy ones (Schou et al. 2021), which might affect mate 406 attraction (Tennessen et al. 2014) and induce errors in mate-choice, causing 407 408 negative evolutionary consequences (e.g., deleterious effects), but see Cunnington and Fahrig (2013). However, some species as Odorrana tormota in 409 noisy environments (e.g., stream) prefer smaller males that calling at higher 410 411 frequencies (Zhang et al. 2020). Thus, at the same time that increasing call frequency allows frogs to overcome anthropogenic noise, its allometric constrain 412 might restrict morphophysiological responses. As such, natural and sexual 413 selection seem acting in opposite ways in urban environments. Therefore, our 414 results might suggest a plastic response to noise instead of adaptive. 415

416 Although we no found difference in mean call frequencies, the range of variation in rural areas was higher than in peri- and urban ones. Similar results 417 were found by Lima et al. (2022), who found no difference in the mean, but 418 419 decreased variation in dominant frequency near anthropogenic noise source. Rural populations face a complex soundscape, with increased competition for 420 acoustic space (Silva et al. 2008; Sugai et al. 2021) due to higher species 421 richness (see Ganci et al. 2022; Menezes et al. in prep.). As a response, male 422 frogs can display plastic responses in dominant frequency to minimize overlap in 423

acoustic niche (Forrest 1994; Schwartz and Bee 2013). Conversely, as 424 urbanisation increases, the number of species decreases (Piano et al. 2020), 425 reducing competition in acoustic space. Nonetheless, intra-specific competition 426 for mates increased in urban areas in male Túngara frogs, despite reduced 427 predation risk (Halfwerk et al. 2019). Thus, frogs are facing different selective 428 pressures in both areas. Urban noise can act as a selective agent on calling 429 behaviour, given it masks acoustic signals and increases intra-specific 430 competition, which might select for a narrower range of call frequencies. In 431 contrast, frogs in rural areas face higher environmental variance, free from noise 432 pressure level imposed by anthropogenic noise, allowing fluctuations in dominant 433 frequencies, despite acoustic competition with other species. 434

Individuals calling at high dominant frequencies in both rural and urban 435 area had lower fitness, but this relationship was weak. Also, in contrast with our 436 initial hypothesis, we found a single peak with a weak linear selection. Previous 437 studies found that dominant frequency evolves under stabilizing or weak 438 directional selection, since females prefer low-frequency calls near the population 439 mean (Gerhardt 1994). Thus, when additive genetic variance is low, it has less 440 441 effects on evolutionary changes (Gerhardt 1994). Low frequency call might be indeed more efficient in rural ponds since the environments we sampled are 442 open, typical of the Cerrado. These environments, according to the AAH (Ey and 443 Fischer 2009) can favour low call frequency, because the signal would reach 444 more distant areas (but see Freitas et al. 2024). This would help explain why 445 individuals with lower pitch have higher fitness in those areas. However, this 446 might represent a maladaptation in urban environments, since they require higher 447 frequencies to overcome traffic and other anthropogenic noises, but there are 448

severe morphophysiological costs and implications for sexual selection. The city 449 450 in which we developed our study is a relatively young one, with 126 years (Arruda 2006) and an urbanisation rate around 3.6% per year from 1985 to 2021 451 (Severgnini and Provete 2025). This might be not enough time for populations to 452 adapt to urban environments. Consequently, urban frog populations might be 453 facing maladaptation that can lead to local extinction. As the advertisement call 454 is related to reproductive successful in anurans (Littlejohn 1977; Wells 2007; 455 Köhler et al. 2017; Guerra et al. 2018), changes in the acoustic communication 456 driven by the environment can directly affect the reproductive behaviour by 457 458 altering the course of sexual selection across urban landscapes.

459

### 460 **Conclusion and Caveats**

461

Our study provides for the first time an estimation of selection coefficients 462 for a key call parameter in frogs in an urbanised landscape. Our results can help 463 not only understand how frog call evolves in tropical cities, but also shed light on 464 the contradictory results previously reported in the literature. However, one key 465 limitation of our study is that our fitness proxy is a body condition index. We 466 acknowledge that using SMI as a fitness proxy can be problematic (Wilder et al. 467 2016; Franklin and Morrissey 2017). However, evidence exists linking lipidic 468 storage to energetic reserves and ultimately to physiological status in frogs 469 (Reading 2007; Scott et al. 2007). 470

Other limitation, is that we assessed only spectral traits and as the advertisement call is composed of spectral and temporal traits (Erdtmann and Lima 2013), temporal parameters might be more affect by urban noises than 474 spectral ones. In addition, future studies should focus beyond the transmission of 475 the acoustic signal and analyse the evolution of female auditory systems to 476 recognize altered signals in cities. Such insights of evolution of auditory systems 477 in anurans and efficiency of signal transmission under noisy environments can 478 elucidate underlying mechanisms of evolutionary changes in cities.

479

#### 480 **References**

481

Alberti, M., C. Correa, J. M. Marzluff, A. P. Hendry, E. P. Palkovacs, K. M.
Gotanda, V. M. Hunt, et al. 2017. Global urban signatures of phenotypic
change in animal and plant populations. Proceedings of the National
Academy of Sciences 114:8951 LP – 8956.

Arnold, S. J. 2003. Performance Surfaces and Adaptive Landscapes. Integrative
 and Comparative Biology 43:367–375.

488 ——. 2023. Evolutionary quantitative genetics. Oxford University Press.

Arruda, A. M. V. 2006. Campo Grande: Arquitetura, Urbanismo E Memoria.
 Editora UFMS, Campo Grande, Mato Grosso do Sul.

Álvarez D, Nicieza AG (2002) Effects of induced variation in anuran larval
 development on postmetamorphic energy reserves and locomotion.
 Oecologia 131:186–195. https://doi.org/10.1007/s00442-002-0876-x

494 Azevedo Cezila, B., R. Rebouças, and C. Sabino Lisboa. 2024. Effects of traffic

- 495 noise on calling activity of *Aplastodiscus leucopygius* (Anura, Hylidae).
  496 Acta Herpetologica 19:29–39.
- Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting Linear MixedEffects Models Using Ime4. Journal of Statistical Software 67:1–48.

- Bioacoustics, C. for C. 2019. Raven Pro: Interactive Sound Analysis Software
  (Version 1.6.1). Ithaca, NY: The Cornell Lab of Ornithology.
- Brodeur, J. C., M. J. Damonte, J. Vera Candioti, M. B. Poliserpi, M. F. D'Andrea,
   and M. F. Bahl. 2020. Frog body condition: Basic assumptions,
   comparison of methods and characterization of natural variability with
   field data from *Leptodactvlus latrans*. Ecological Indicators 112:106098.
- Caruso, N. M., M. W. Sears, D. C. Adams, and K. R. Lips. 2014. Widespread
   rapid reductions in body size of adult salamanders in response to climate
   change. Global Change Biology 20:1751–1759.
- Charmantier, A., T. Burkhard, L. Gervais, C. Perrier, A. I. Schulte-Hostedde, and
   M. J. Thompson. 2024. How does urbanization affect natural selection?
   Functional Ecology 38:2522–2536.
- Coelho, A. L. N., and W. de S. C. Correa. 2013. Temperatura de Superfície
   Celcius do Sensor TIRS/ Landsat 8. Revista Geográfica Acadêmica
   7:32–45.
- 514 Cunnington, G. M., and L. Fahrig. 2013. Mate attraction by male anurans in the 515 presence of traffic noise. Animal Conservation 16:275–285.
- 516 Duellman, W. E., and L. Trueb. 1986. Biology of amphibians. McGraw-Hill, New 517 York.
- Drakulić S, Feldhaar H, Lisičić D, et al (2016) Population-specific effects of
  developmental temperature on body condition and jumping performance
  of a widespread European frog. Ecol Evol 6:3115–3128.
  https://doi.org/https://doi.org/10.1002/ece3.2113
- 522 Erdtmann, L. K., and A. P. Lima. 2013. Environmental effects on anuran call 523 design: What we know and what we need to know. Ethology Ecology and

524 Evolution 25:1–11.

- Ey, E., and J. Fischer. 2009. The "Acoustic Adaptation Hyphotesis"—A review of
  the evidence from birds, anurans and mammals. Bioacoustics 19:21–48.
  Fick, S. E., and R. J. Hijmans. 2017. WorldClim 2: new 1-km spatial resolution
  climate surfaces for global land areas. International Journal of
  Climatology 37:4302–4315.
- Forrest, T. G. 1994. From Sender to Receiver: Propagation and Environmental
   Effects on Acoustic Signals. American Zoologist 34:644–654.
- Franklin, O. D., and M. B. Morrissey. 2017. Inference of selection gradients using
   performance measures as fitness proxies. Methods in Ecology and
   Evolution 8:663–677.
- 535 Freitas, B., P. B. D'Amelio, B. Milá, C. Thébaud, and T. Janicke. 2024. Meta-536 analysis of the acoustic adaptation hypothesis reveals no support for the 537 effect of vegetation structure on acoustic signalling across terrestrial 538 vertebrates. Biological Reviews n/a.
- Ganci, C. C., D. B. Provete, T. Püttker, D. Lindenmayer, and M. Almeida-Gomes.
  2022. High species turnover shapes anuran community composition in
  ponds along an urban-rural gradient. Urban Ecosystems 25:633–642.
- Gerhardt, H. C. 1991. Female mate choice in treefrogs: static and dynamic
  acoustic criteria. Animal Behaviour 42:615–635.
- 544 ——. 1994. The Evolution of Vocalization in Frogs and Toads. Annual Review
   545 of Ecology and Systematics 25:293–324.
- Guerra, V., D. Llusia, P. G. Gambale, A. R. de Morais, R. Márquez, and R. P.
   Bastos. 2018. The advertisement calls of Brazilian anurans: Historical
   review, current knowledge and future directions. PLOS ONE

- 549 **13:e0191691**.
- Halfwerk, W., M. Blaas, L. Kramer, N. Hijner, P. A. Trillo, X. E. Bernal, R. A. Page,
  et al. 2019. Adaptive changes in sexual signalling in response to
  urbanization. Nature Ecology & Evolution 3:374–380.
- Hardt, B., and L. Benedict. 2021. Can you hear me now? A review of signal
   transmission and experimental evidence for the acoustic adaptation
   hypothesis. Bioacoustics 30:716–742.
- Hartig, F. 2024. DHARMa: Residual Diagnostics for Hierarchical (Multi-Level /
   Mixed) Regression Models. R package version 0.4.7. https://cran.r project.org/web/packages/DHARMa/index.html/DHARMa/. R package
   version 0.4.7.
- Higham, V., N. D. S. Deal, Y. K. Chan, C. Chanin, E. Davine, G. Gibbings, R.
  Keating, et al. 2021. Traffic noise drives an immediate increase in call
  pitch in an urban frog. Journal of Zoology 313:307–315.
- Hillman, S. S., P. C. Withers, R. C. Drewes, and S. D. Hillyard. 2008. Ecological
   and environmental physiology of amphibians. Oxford University Press,
   New York.
- IBGE. 2022. Censo Demográfico 2022: população e domicílios. Instituto
   Brasileiro de Geografia e Estatística (IBGE), Coordenação Técnica do
   Censo Demográfico.
- Jakob, E. M., S. D. Marshall, and G. W. Uetz. 1996. Estimating Fitness: A
   Comparison of Body Condition Indices. Oikos 77:61–67.
- Klocke, B., A. Estrada, M. Mataya, D. Medina, E. Baitchman, L. Belden, J.
   Guerrel, et al. 2023. Movement and survival of captive-bred Limosa
   harlequin frogs (*Atelopus limosus*) released into the wild. Frontiers in

- 574 Amphibian and Reptile Science 1.
- Köhler, J., M. Jansen, A. Rodríguez, P. J. R. Kok, L. F. Toledo, M. Emmrich, F.
  Glaw, et al. 2017. The use of bioacoustics in anuran taxonomy: theory,
  terminology, methods and recommendations for best practice. Zootaxa
  4251:1–124.
- Köhler, J., S. Reichle, and G. Peters. 1997. Advertisement calls of three species
  of Bufo (amphibia: anura: Bufonidae) from lowland Bolivia. Stuttgarter
  Beitraege zur naturkunde 562:1–8.
- Kottek, M., J. Grieser, C. Beck, B. Rudolf, and F. Rubel. 2006. World Map of the
   Köppen-Geiger climate classification updated. Meteorologische
   Zeitschrift 15:259–263.
- Kruger, D. J. D., and L. H. Du Preez. 2016. The effect of airplane noise on frogs:
   a case study on the Critically Endangered Pickersgill's reed frog
   (*Hyperolius pickersgilli*). Ecological Research 31:393–405.
- Lande, R., and S. J. Arnold. 1983. The Measurement of Selection on Correlated
   Characters. Evolution 37:1210–1226.
- Landsberg, H. 1981. The Urban Climate. The Urban Climate 275.
- Lesbarrères D, Merilä J, Lodé T (2008) Male breeding success is predicted by
   call frequency in a territorial species, the agile frog (*Rana dalmatina*). Can
   J Zool 86:1273–1279. https://doi.org/10.1139/Z08-121
- Lima, N. de A. P., R. Rebouças, L. F. Toledo, and C. S. Lisboa. 2022. Influence
   of urban noise in call traits of the Atlantic Forest treefrog *Bokermannohyla hylax*. Zoologischer Anzeiger 300:41–46.
- <sup>597</sup> Littlejohn, M. J. 1977. Long-Range Acoustic Communication in Anurans: An <sup>598</sup> Integrated and Evolutionary Approach. Pages 263–294 *in* D. H. Taylor

- and S. I. Guttman, eds. The Reproductive Biology of Amphibians.
  Springer US, Boston, MA.
- Lowry, H., A. Lill, and B. B. M. Wong. 2019. Do the Calls of a Bird, the Noisy
   Miner (*Manorina melanocephala*), Need Adjustment for Efficient
   Communication in Urban Anthropogenic Noise? Animals.
- MacCracken, J. G., and J. L. Stebbings. 2012. Test of a Body Condition Index
   with Amphibians. Journal of Herpetology 46:346–350.
- Mainwaring, M. C., G. Song, and S. Zhang. 2024. Urban biodiversity in the Anthropocene. Scientific Reports 14:27851.
- MapBiomas, P. 2024. Projeto MapBiomas Coleção [v.8] da Série Anual de
   Mapas de Cobertura e Uso do Solo do Brasil.
- Marquez, R., I. de la Riva, and J. Bosch. 1995. Advertisement calls of Bolivian
   Leptodactylidae (Amphibia, Anura). Journal of Zoology 237:313–336.
- Martins, I. A., and J. Jim. 2003. Bioacoustic analysis of advertisement call in *Hyla nana* and *Hyla sanborni* (Anura, Hylidae) in Botucatu, São Paulo, Brazil.
   Brazilian journal of biology 63:507–516.
- McDonnell, M. J., and A. K. Hahs. 2015. Adaptation and Adaptedness of
   Organisms to Urban Environments. Annual Review of Ecology,
   Evolution, and Systematics 46:261–280.
- Miles, L. S., L. R. Rivkin, M. T. J. Johnson, J. Munshi-South, and B. C. Verrelli.
  2019. Gene flow and genetic drift in urban environments. Molecular
  Ecology 28:4138–4151.
- Morrissey, M. B. 2014. In search of the best methods for multivariate selection analysis. Methods in Ecology and Evolution 5:1095–1109.
- Morrissey, M. B., and K. Sakrejda. 2013. Unification Of Regression-Based

- 624 Methods For The Analysis Of Natural Selection. Evolution 67:2094– 625 2100.
- Murphy, M., and M. Boone. 2022. Evaluating the role of body size and habitat type in movement behavior in human-dominated systems: A frog's eye view. Ecology and Evolution 12:e9022.
- Nicieza AG, Álvarez D, Atienza EMS (2006) Delayed effects of larval predation
   risk and food quality on anuran juvenile performance. J Evol Biol
   19:1092–1103. https://doi.org/https://doi.org/10.1111/j.1420-
- 632 9101.2006.01100.x
- Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P.
   R. Minchin, et al. 2018. vegan: Community Ecology Package. R package
   version 2.5-3 URL https://CRAN.R-project.org/package=vegan.
- Olson, M. E., A. Arroyo-Santos, and F. Vergara-Silva. 2019. A User's Guide to
   Metaphors In Ecology and Evolution. Trends in Ecology & Evolution
   34:605–615.
- OpenStreetMap contributors. 2024. Planet dump retrieved from
   https://planet.osm.org.
- Parris, K. M. 2015. Ecological Impacts of Road Noise and Options for Mitigation.
   Pages 151–158 *in*Handbook of Road Ecology.
- Peig, J., and A. J. Green. 2009. New perspectives for estimating body condition
  from mass/length data: the scaled mass index as an alternative method.
  Oikos 118:1883–1891.
- 646 . 2010. The paradigm of body condition: a critical reappraisal of current
  647 methods based on mass and length. Functional Ecology 24:1323–1332.
  648 Pereira, R. H. M., D. Herszenhut, C. K. V. Braga, J. P. Bazzo, J. L. A. Oliveira, J.

- P. Parga, M. Saraiva, et al. 2022. Distribuição espacial de características
  sociodemográficas e localização de empregos e serviços públicos das
  vinte maiores cidades do Brasil. Ipea Instituto de Pesquisa Econômica
  Aplicada.
- Piano, E., C. Souffreau, T. Merckx, L. F. Baardsen, T. Backeljau, D. Bonte, K. I.
  Brans, et al. 2020. Urbanization drives cross-taxon declines in
  abundance and diversity at multiple spatial scales. Global Change
  Biology 26:1196–1211.
- QGIS.org. 2020. QGIS Geographic Information System. Open Source Geospatial
   Foundation Project. http://qgis.org.
- R Core Team. 2023. R: A language and environment for statistical computing. R
   Foundation for Statistical Computing, Vienna, Austria URL
   https://www.R-project.org/.

## Reading, C. J. 2007. Linking global warming to amphibian declines through its effects on female body condition and survivorship. Oecologia 151:125– 131.

- Roff, D. A. 1977. Evolutionary quantitative genetics. Springer Science & Business
   Media, New York.
- Ryan, M. J. 1988. Constraints and Patterns in the Evolution of Anuran Acoustic
  Communication. Pages 637–677 *in* B. Fritzsch, T. Hetherington, M. J.
  Ryan, W. Walkowiad, and W. Wilczynski, eds. The Evolution of the
  Amphibian Auditory System. Wiley, John & Sons, New York.
- Salomons, E. M., and M. Berghauser Pont. 2012. Urban traffic noise and the
  relation to urban density, form, and traffic elasticity. Landscape and
  Urban Planning 108:2–16.

- Scheffers, B. R., L. De Meester, T. C. L. Bridge, A. A. Hoffmann, J. M. Pandolfi,
  R. T. Corlett, S. H. M. Butchart, et al. 2016. The broad footprint of climate
  change from genes to biomes to people. Science 354:aaf7671.
- Schou, C. P. E., A. L. Levengood, and D. A. Potvin. 2021. Limited effects of traffic
   noise on behavioural responses to conspecific mating calls in the eastern
   sedge frog *Litoria fallax*. acta ethologica 24:217–226.
- Schwartz, J. J., and M. A. Bee. 2013. Anuran Acoustic Signal Production in Noisy
   Environments. Pages 91–132 *in* H. Brumm, ed. Animal Communication
   and Noise. Springer Berlin Heidelberg, Berlin, Heidelberg.
- Scott, D. E., E. D. Casey, M. F. Donovan, and T. K. Lynch. 2007. Amphibian lipid
   levels at metamorphosis correlate to post-metamorphic terrestrial
   survival. Oecologia 153:521–532.
- Shannon, G., M. F. McKenna, L. M. Angeloni, K. R. Crooks, K. M. Fristrup, E.
  Brown, K. A. Warner, et al. 2016. A synthesis of two decades of research
  documenting the effects of noise on wildlife. Biological Reviews 91:982–
  1005.
- Silva, R. A., I. A. Martins, and D. de C. Rossa-Feres. 2008. Bioacoustics and
   calling site in anuran assemblages of open area in the northwest of São
   Paulo State, Brazil. Biota Neotropica 8.
- Simmons, A. M., and P. M. Narins. 2018. Effects of Anthropogenic Noise on
   Amphibians and Reptiles. Pages 179–208 *in* Effects of Anthropogenic
   Noise on Animals. Springer, New York, NY.
- Souza Filho, J. J. de. 2012. Avaliação do ruído urbano da cidade de Campo
   Grande/MS. 150 f. Dissertação (Mestrado em Tecnologias Ambientais) Universidade Federal de Mato Grosso do Sul, Campo Grande, Mato

- 699 Grosso do Sul.
- Stock, S. E., K. Klop-Toker, S. Wallace, O. Kelly, A. Callen, R. Seeto, S. V
   Mahony, et al. 2023. Uncovering inbreeding, small populations, and
   strong genetic isolation in an Australian threatened frog, *Litoria littlejohni*.
   Conservation Genetics 24:575–588.
- Sugai, L. S. M., D. Llusia, T. Siqueira, and T. S. F. Silva. 2021. Revisiting the
   drivers of acoustic similarities in tropical anuran assemblages. Ecology
   n/a:e03380.
- Szulkin, M., J. Munshi-South, and A. Charmantier. 2020. Urban Evolutionary
   Biology. (M. Szulkin, J. Munshi-South, & A. Charmantier, eds.). Oxford
   University Press.
- Teixeira, B. F. da V., V. H. Zaracho, and A. A. Giaretta. 2016. Advertisement and
  courtship calls of *Dendropsophus nanus* (Boulenger, 1889) (Anura:
  Hylidae) from its type locality (Resistencia, Argentina). Biota Neotropica
  16.

# Tennessen, J. B., S. E. Parks, and T. Langkilde. 2014. Traffic noise causes physiological stress and impairs breeding migration behaviour in frogs. Conservation physiology 2:cou032.

- Thompson, M. J., P. Capilla-Lasheras, D. M. Dominoni, D. Réale, and A.
  Charmantier. 2022. Phenotypic variation in urban environments:
  mechanisms and implications. Trends in Ecology & Evolution 37:171–
  182.
- Tonini, J. F. R., D. B. Provete, N. M. Maciel, A. R. Morais, S. Goutte, L. F. Toledo,
   and R. A. Pyron. 2020. Allometric escape from acoustic constraints is
   rare for frog calls. Ecology and Evolution 10:3686–3695.

- UN-Habitat, U. N. H. S. P. 2022. World Cities Report 2022: Envisaging the Future
   of Cities.
- Warton, D. I., R. A. Duursma, D. S. Falster, and S. Taskinen. 2012. smatr 3– an
   R package for estimation and inference about allometric lines. Methods
   in Ecology and Evolution 3:257–259.
- Wells, K. D. 2007. The ecology and behavior of amphibians. University of
   Chicago Press, Chicago.
- Wilder, S. M., D. Raubenheimer, and S. J. Simpson. 2016. Moving beyond body
   condition indices as an estimate of fitness in ecological and evolutionary
   studies. Functional Ecology 30:108–115.
- Zaffaroni-Caorsi, V., C. Both, S. Cechin, R. Antunes, and M. Borges-Martins.
- 2017. Effects of traffic noise on the calling behavior of two Neotropical
  hylid frogs. PLOS ONE 12:e0183342.
- 737 Zaffaroni-Caorsi, V., C. Both, R. Márquez, D. Llusia, P. Narins, M. Debon, and M.
- Borges-Martins. 2023. Effects of anthropogenic noise on anuran
  amphibians. Bioacoustics 32:90–120.
- Zhang F, Yuan C, Feng AS (2020) Female concave-eared torrent frogs prefer
- smaller males. J Zool 311:239–245.
- 742 https://doi.org/https://doi.org/10.1111/jzo.12775
- 743

## 744 Author Contributions

- 745 Marcos R. Severgnini: Writing–Original Draft (Lead), Methodology (Equal), Data
- 746 Curation (Lead), Formal Analysis (Lead).
- 747 Diogo B. Provete: Methodology (Equal), Writing, Reviewing and Editing,
- 748 Conceptualization (Lead), Supervision.

All authors contributed critically to the drafts and gave final approval for publication.

751

## 752 Acknowledgements

<sup>753</sup> We are grateful to the owners of properties for allowing access the study areas.

Yasmin R. Zavelinski helped us with data curation. Higor Cirilo provided sociodemographic data. Maurício Vancine provided R code to calculate the number and density of buildings. Heloísa M. Rodrigues, Marciane R. Severgnini, Bruna C. Yoshida, Philip T. Soares, Adriana C. Acero-Murcia, Nicolle Prado, Klysman Fernandes, Bruno Fines, Letícia A. da Cruz, Eduardo Morel, and Ana Torres helped with data collection in the field.

760

### 761 **Conflict of interest**

The authors declare no conflict of interest.

763

### 764 **Data availability**

All data and associated R code used to run the analysis will be available atFigShare.

767

## 768 **Funding**

DBP is supported by a CNPq grant (#407318/2021-6) and receives a fellowship
from FUNDECT (#83//027.032/2024). This study was funded in part by the
Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil
(CAPES) – Finance Code 001 to MRS and DBP. During the final stages of the

writing, DBP was supported by a fellowship for experienced researchers from theAlexander von Humboldt Foundation.

775

776

### 777 Ethics approval

ICMBio provided the collecting permit (#80075-1). Field procedures were
 approved by the Institutional Animal Care and Use Committee of our university
 (CEUA-UFMS #1.203/2021).

781

## 782 Figures and Table legends

783

**Table 1.** Mean and standard deviation of dominant, maximum, and minimum frequency, along with body size and weight of frogs collected in each set of ponds along the urbanisation gradient. Data for body size are in millimetres (mm); in grams (g) for body weight; and in hertz (Hz) for call frequencies. All data are presented as mean  $\pm$  SD | CV (range). SS: Sample size; SD: Standard deviation; CV: Coefficient of variation.

Measurements	Rural (SS: 100)		Peri-urban (SS:		Urban (SS: 50)	
			60)			
Dominant	4,523 ±	253.7	4,372	± 238.5	4,417 ±	212.5
frequency	0.055	(4,091–	0.054	(3,729–	0.047	(3,944–
	5,254)		4,814)		4,823)	

Max Frequency	21,540 ± 331.6	21,447 ± 466.4	21,601 ± 203.1	
	0.015 (20,291–	0.021 (20,329–	0.009 (20,860–	
	21,937)	21,933)	21,920)	
Min Frequency	2,982 ± 649.9	2,746 ± 451.1	2,936 ± 562.0	
	0.212 (1,632–	0.161 (1,919–	0.186 (1,801–	
	4,223)	3,952)	3,981)	
Body size	21.15 ± 1.40	21.62 ± 1.08	21.51 ± 1.09	
	0.065 (16.45–	0.049 (18.83–	0.050 (18.21–	
	23.89)	24.21)	23.40)	
Body weight	0.43 ± 0.10  0.242	0.47 ± 0.10  0.220	0.53 ± 0.12  0.230	
	(0.10–0.75)	(0.25–0.75)	(0.35–0.75)	

790

Table 2. Results of linear mixed model for allometric relationship between
 dominant frequency and body size among rural, peri- and urban areas.

Parameters:  $\sigma^2$  = variance;  $\tau 00$  = variance between ponds; N = sample size; ICC = intraclass correlation coefficient; CI = confidence interval; df = degrees of freedom. Significant *P*-values are in bold.

Dominant frequency/10		
Estimates	CI	р
652.71	589.09 - 716.34	<0.001
-9.47	-12.476.48	<0.001
-12.07	-133.89 - 109.74	0.845
-35.56	-162.65 - 91.54	0.582
0.06	-5.59 - 5.71	0.982
1.32	-4.60 - 7.23	0.661
	D <u>Estimates</u> 652.71 -9.47 -12.07 -35.56 0.06 1.32	Estimates         CI           652.71         589.09 - 716.34           -9.47         -12.476.48           -12.07         -133.89 - 109.74           -35.56         -162.65 - 91.54           0.06         -5.59 - 5.71           1.32         -4.60 - 7.23

Random Effects
$\sigma^2$	383.99
$\tau_{00 \text{ Pond}}$	49.40
ICC	0.11
N Pond	21
Observations	210
Marginal $\mathbb{R}^2$ / Conditional $\mathbb{R}^2$	0.289 / 0.370

**Table 3.** Results of linear mixed-effect model of size-free dominant frequency and min frequency among rural, peri- and urban areas, using sampling sites (ponds) as a replication; and result of generalized linear mixed model (GLMM) using Template Model Builder (TMB) with lognormal distribution family for max frequency. Parameters:  $\sigma^2$  = variance;  $\tau 00$  = variance between ponds; N = sample size; ICC = intraclass correlation coefficient; CI = confidence interval; df = degrees of freedom. Significant *P*-values are in bold.

	Siz	e-free dominan frequency/10	t	I	Min frequency/10		Max	frequency/10	)^5
Predictors	Estimates	Cl	р	Estimates	Cl	р	Estimates	Cl	р
(Intercept)	4.52	-1.47 – 10.52	0.130	298.21	260.97 – 335.44	<0.001	-1.54	-1.54 – - 1.53	<0.001
Area [Peri-urban]	-10.15	-19.94 – -0.36	0.043	-23.56	-84.38 – 37.25	0.426	-0.00	-0.02 - 0.01	0.536
Area [Urban]	-6.81	-17.20 – 3.57	0.185	-4.57	-69.07 – 59.93	0.883	0.00	-0.01 - 0.02	0.703
Random Effects									
$\sigma^2$	384.17			674.68			0.00		
T00	43.02 Pond			3074.37	Pond		0.00 Pond		
ICC	0.10			0.82			0.98		
Ν	21 Pond			21 Pond			21 Pond		
Observations	210			210			210		

0.038 / 0.979

**Table 4.** Results of the selection surface for all ponds combined; and for rural, peri- and urban areas showing linear selection coefficients ( $\beta$ ) and quadratic coefficients ( $\gamma$ ). SE – Standard error; Estimates – Slope. Significant *P* values are in bold.

Trait	Selection	estimates	SE	Р
	coefficient			value
All ponds	Beta	-0.0002605347	0.00007262568	0.001
combined	Gamma	0.00000079984	0.00000856076	0.808
Rural	Beta	-0.0002284968	0.00009746219	0.012
	Gamma	0.000001234037	0.000000996407	0.776
Peri-urban	Beta	-0.00009506533	0.0001226017	0.430
	Gamma	-0.000001628401	0.000001005207	0.538
Urban	Beta	-0.0004925823	0.0002087136	0.016
	Gamma	0.000006751917	0.00000305194	0.110

806



Figure 1. Spectrogram (top) and oscillogram (bottom) of the advertisement call
of *Dendropsophus nanus* showing two notes recorded in a rural pond (P06) in
Campo Grande.



Figure 2. Scatter plot showing the allometric relationship between dominant frequency and body size for rural (green), peri-urban (grey), and urban areas (red). Colours represent groups to which each pond was assigned based on a kmeans clustering (see Fig. S1).



Figure 3. Boxplots of the size-free dominant frequency, minimum and maximum frequency in Hertz of frogs recorded in rural, peri-,
 and urban areas. Colours represent the three groups to which each pond was assigned based on the k-means multivariate clustering
 procedure (see Fig. S1).



Figure 4. Selection surfaces of the dominant frequency for all ponds combined; and for rural, peri- and urban areas. Fitness proxy
was estimated using Scaled Mass Index (SMI) represented as s(covariate name, edf – degrees of freedom of the smooth) – y axis.
Colours represent the three groups to which each pond was assigned based on the k-means multivariate clustering procedure (see
Fig. S1).



833

Figure S1. Correlogram of environmental variables used into clustering 834 procedure. All variables were extracted from a 500-m buffer around each 835 sampling site. Area\_m2 = Pond area in squared meters; elev\_mean\_m = average 836 elevation in meters; Urb 1985 500 = urbanisation percentage for year 1985; 837 Urb\_2021\_500 = urbanisation percentage for year 2021; Urb\_rate\_500 = 838 urbanization rate; rate forest = rate of forested area; rate pasture = rate of pasture 839 area; t\_mean1985uhi\_500 and t\_mean2022uhi\_500 = average land surface 840 temperature for year 1985 and 2022; density\_build\_percen = percentage of built 841 area in square meters; build\_pocas = number of houses; rate\_light\_2012\_2023 842 = rate of light pollution from 2012 to 2023; mean\_light\_2012\_2023 = average of 843

light pollution from 2012 to 2023; populacao\_n = number of residents; renda =
house income per capita; t\_mean\_air\_1981\_2010 and t\_varian\_air\_1981\_2010
average and variance of air temperature from year 1981 to 2010.



847

Component 1 These two components explain 57.89 % of the point variability.

**Figure S2.** Ordination diagram showing the result of the k-means multivariate clustering procedure based on environmental variables: current urbanisation percentage, building density, mean of artificial light, number of residents and average household income per capita (correlation > 0.33), that recovered three groups of sampling sites (ponds). Red triangles = Urban area; Grey pluses = Periurban; Green circles = Rural. Numbers represent pond IDs.





**Figure S3.** Residual diagnostic of the ordinary least squares regression used to

calculate the slope for entering in the Scaled Mass Index.



Figure S4. Scatter plots showing the relationship between body weight and body size (left) and Scaled Mass Index (SMI) and body size (right). The left plot shows that there is no relationship between the two variables, demonstrating that SMI is independent of body size. The right plot shows the positive allometric relationship between body size and weight in log scale, whose slope was used to calculate the SMI. Fitted lines of both plots are from OLS model. Colours represent the three groups to which each pond was assigned based on the k-means multivariate clustering procedure (see Fig. S1). Green dots: rural area; grey: peri-urban; red: urban. 





**Figure S5.** Residuals of linear mixed-effects model (Imer) for relationship between dominant frequency and body size among areas (A); size-free dominant frequency among areas (B); minimum frequency (C); and residuals of generalized linear mixed model (GLMM) using Template Model Builder (TMB) for maximum frequency with lognormal distribution family (D).



**Figure S6.** Raincloud plot showing the boxplot and frequency distribution of sizefree dominant frequency along the gradient of urbanisation rate. Values on the x axis were the ones recorded in each pond. Colours represent the three groups to which each pond was assigned based on the k-means multivariate clustering procedure (see Fig. S1).

1	CAPÍTULO 3
2	
3	Target journal: Functional ecology (ISSN: 1365-2435)
4	
5	How the Pace-of-Life Syndrome across multiple morphophysiological and
6	behavioral traits in a tropical frog is affected by urban environments
7	
8	Marcos R. Severgnini <sup>1</sup> , Sabrina F. Gonçalves <sup>2</sup> , Carlos E. Fernandes <sup>3</sup> , Rafael P.
9	Bovo <sup>4</sup> , Lilian Franco-Belussi <sup>3</sup> , Diogo B. Provete <sup>3,5,6,*</sup>
10	
11	<sup>1</sup> Graduate Program in Ecology and Conservation, Federal University of Mato
12	Grosso do Sul, Campo Grande, 79002-900, Brazil.
13	<sup>2</sup> Graduate Program in Animal Biology. Federal University of Mato Grosso do Sul,
14	Campo Grande, Mato Grosso do Sul, Brazil.
15	<sup>3</sup> Institute of Biosciences, Federal University of Mato Grosso do Sul, Campo
16	Grande, 79002-900, Brazil.
17	<sup>4</sup> Department of Evolution, Ecology, and Organismal Biology, University of
18	California Riverside, Riverside, CA, USA
19	<sup>5</sup> Gothenburg Global Biodiversity Centre, PO Box 100, SE-413 19, Göteborg,
20	Sweden.
21	<sup>6</sup> German Centre for Integrative Biodiversity Research-iDiv, Halle-Jena-Leipzig,
22	Leipzig, Germany.
23	
24	* Corresponding author: diogo.provete@ufms.br
25	

26 Abstract

27

Urban ecosystems pose new challenges for biodiversity by altering life-history 28 strategies. However, the impact of urban areas on multiple life-history traits of 29 amphibians in tropical regions remains unclear. Here, we tested how behavioral 30 and morphophysiological traits change in response to urbanization in frog 31 populations. We quantified these changes through lab experiments and tested 32 our hypotheses using uni- and multivariate approaches. We found that urban 33 frogs displayed a faster pace of life compared to their rural counterparts. For 34 35 example, urban frogs showed a shorter flight initiation distance, indicating 36 habituation to human presence. They had a higher hepatosomatic index, and elevated glucose levels. Urban frogs had thicker epidermis and EK skin layers, 37 and their skin was darker. Also, urban and rural populations had different red and 38 white blood cell counts depending on age. We did not find any differences in 39 hepatic melanin and sperm count between populations. These findings support 40 the hypothesis that urbanization imposes selective pressures that lead to 41 changes in behavior, metabolism, hydric balance, and immunity in frogs, resulting 42 43 in a faster pace of life. Also, our results provide a first step towards understanding adaptation to urbanization. However, long-term studies across species and cities 44 are needed to fully understand the effects of urbanization on amphibian health. 45

46

47 **Key-words**: FEAR; body darkness; tropical; cities; Global South.

48

49

Urbanization is a main driver of global biodiversity loss (Piano et al. 53 2020). Environmental stressors associated with urbanization include pollutants, 54 exposure to novel pathogens, and elevated temperatures as a consequence of 55 urban heat islands (Diamond and Martin 2020). These factors negatively impact 56 genetic diversity and phenotypic traits, such as morphology, physiology, behavior 57 (Alberti et al. 2017), as well as organismal health status (Murray et al. 2019). The 58 environmental changes associated with urbanization may induce changes in life 59 history strategies because of novel selective pressures. The hypothesis of the 60 pace-of-life syndrome - PoLS (Ricklefs and Wikelski 2002) predicts that 61 populations living under stressful conditions should change their behavior, 62 physiology, and immune system to achieve a faster pace of life (but see Stott et 63 al. 2024). Therefore, we can expect urban organisms to display alternative life 64 history strategies as an adaption or simply phenotypic plasticity to cope with 65 modified city environments (Diamond et al. 2022), which include changes in 66 behavior (Møller et al. 2015), coloration (Leveau 2021), reproductive traits 67 (Minias 2016; Jennette et al. 2019), and immune responses (Minias 2023; 68 Franco-Belussi et al. 2024). A faster pace of life in cities have already been 69 reported for several taxa (Charmantier et al. 2017; Brans and De Meester 2018; 70 Franco-Belussi et al. 2024). However, the majority of studies focused on one or 71 two life-history traits, and few assessed changes across multiple 72 morphophysiological traits (but see Franco-Belussi et al. 2024). Nevertheless, 73 studies assessing multiple life-history traits of urban amphibians remain scarce, 74

especially in megadiverse tropical regions that experience a recent, but rapid
 urbanization process (Myers 2021).

Anti-predatory defenses are an innate mechanism of survival in several 77 taxa, including frogs. Anti-predatory mechanisms in adult frogs can be passive, 78 such as camouflage or poison, and active as escape behavior (Wells 2007). 79 Escape behavior is highly related to jumping performance in frogs (Gomes et al. 80 2009). Then, maximizing jumping distance increase survival. However, instead 81 of investing all energy in escaping, the "Flush Early and Avoid the Rush" (FEAR) 82 hypothesis predicts that prey should escape earlier to avoid the costs of 83 84 monitoring predators (Blumstein 2010). Evidence for this behavior exists for birds, 85 mammals, lizards (Samia et al. 2013), and frogs (Nishiumi and Mori 2020) by measuring alert distance (AD) and flight initiation distance (FID). FID is the 86 distance between prey and predator when escape occurs (Blumstein 2010). 87

This distance can be modulated by risk assessment or "fear" (Stankowich and 88 Blumstein 2005), and a habituation process to potential predator can occur under 89 repeated exposures to a specific stimulus (Blumstein 2014). Humans can be 90 perceived as predators (Stankowich and Blumstein 2005), such that urban 91 92 animals should experience a high exposure to human presence than those living in natural areas. Previous studies with birds and skinks support this hypothesis 93 (e.g., Fernández-Juricic et al. 2001; McGowan et al. 2014). Furthermore, 94 changes in the shy-bold axis are also predicted by PoLS to occur in environments 95 in which natural selection favors a faster pace of life. However, no study has yet 96 evaluated the interplay between escape behavior and FEAR in divergent life-97 history environments for frogs. 98

Chronic stress, pollution, and novel pathogens are the main drivers of 99 100 immunosuppression and immunostimulation in urban areas (Minias 2023). Although the effect of stress induced by human disturbance on immunity is 101 controversial (see Iglesias-Carrasco et al. 2017, 2020), recent reviews and meta-102 analysis suggest that novel environmental disturbances and stressors in cities, 103 such as artificial light at night (ALAN), anthropogenic noise, high temperatures 104 (heat stress), and chemical pollution activate the hypothalamic-pituitary-interrenal 105 (HPI) axis to increase glucocorticoid hormone levels, which suppresses the 106 immune system (Minias 2023; Pérez-Ortega and Hendry 2023), while novel 107 108 pathogens (e.g., bacteria and fungi) can induce immunostimulation (Murray et al. 2019; Minias 2023). One way to assess immune responses in vertebrates is using 109 white blood cell count and leukocyte differentiation, since these cells promptly 110 respond to stress (Davis et al. 2008). Previous studies found altered leukocyte 111 profiles in urban frog populations compared to rural areas (Zhelev and 112 Popgeorgie 2021; Franco-Belussi et al. 2024). Likewise, erythrocytes also can 113 respond to environmental factors and participate in innate immunity in fish and 114 amphibians (Anderson et al. 2018). Moreover, oxidative stress driven by 115 116 environmental alterations can damage erythrocytes, impairing oxygen delivery to tissues (Mohanty et al. 2014) and negatively impacting metabolism (Zhelev and 117 Popgeorgie 2021). Thus, both white and red blood cells can provide information 118 119 about the immune system and metabolism. Consequently, divergent responses in these traits should be expected along the slow-fast continuum (Réale et al. 120 2010). 121

Organosomatic indexes can provide information about health status, reproduction, and metabolism. For example, the ratio between body mass and

liver weight, or hepatosomatic index (HSI) has been used as a biomarker to 124 125 xenobiotic exposure (Bruslé and Anadon 1996). It also provides information about stored energy and metabolism (Chaves et al. 2017). Likewise, glucose 126 levels can be related to stored glycogen and metabolism (de Amaral et al. 2022). 127 The gonadosomatic index (GSI) gives information about reproductive output 128 (Chaves et al. 2017), while sperm and gonadal size can be altered at different 129 phases of spermatogenesis (Méndez-Tepepa et al. 2023). As the reproductive 130 cycle of nocturnal animals is affect by photoperiod (Canavero and Arim 2009). 131 ALAN can disrupt reproductive behavior affecting dial and seasonal phenology 132 133 (Dias et al. 2019). Previous studies also found that anthropogenic noise decreased sperm count and viability (Kaiser et al. 2015), while others found no 134 difference between reproductive investment in females between rural and urban 135 areas (Jennette et al. 2019). Also, Franco-Belussi et al. (2024) found that urban 136 frogs reach reproductive maturation earlier than rural ones. Nevertheless, studies 137 examining somatic and reproductive traits changes in urban areas remain scarce 138 in the tropics. 139

Amphibian integument play important roles, such as in respiration, 140 141 osmoregulation, thermoregulation (Duellman and Trueb 1994), mechanical protection (Greven et al. 1995), and chemical defense (Varga et al. 2019). The 142 epidermis in amphibians has been associated with protection, thermoregulation, 143 and immune response (Haslam et al. 2014), while the Eberth-Kastschenko (EK) 144 layer in the dermis has crucial role in controlling evaporative water loss due to its 145 biochemical properties (Toledo and Jared 1993; Ponssa et al. 2017). Likewise, 146 melanocytes in amphibian's skin are also associated with thermal tolerance 147 (Vences et al. 2002; Laumeier et al. 2023), UV exposure (Franco-Belussi et al. 148

2016) and protection against pathogens (Laumeier et al. 2023). The melanin of 149 150 internal organs (e.g., liver) is expected to respond to thermal stress (De Souza Santos et al. 2014). Studies examining coloration changes in urban areas are 151 classical textbook examples in evolutionary biology, such as the Biston betularia 152 melanism associated with urban centers (Kettlewell 1959). However, no study 153 assessed so far how amphibian internal and external melanism nor how skin 154 morphometric is affected in urban areas. Cities can be used as testbeds to study 155 climate change (Diamond and Martin 2021). Therefore, understanding how the 156 skin of frogs change to deal with thermal stress can shed light on primary 157 158 compensatory mechanisms to overcome global warming, since it is the main driver of global amphibian decline (Luedtke et al. 2023). 159

Franco-Belussi et al. 2024 found that frogs living in urban sites had an 160 early reproduction, high metabolic rate, and high red cell abnormalities and 161 altered leukocyte profile. However, this study focusing on urban and rural sites 162 separated by 270 km. Herein, we build upon their findings expanding to urban 163 and rural sites at the same city and including new phenotypic axes to further 164 investigate how pace of life syndrome is affected in divergent life history 165 166 environments. Specifically, we tested how behavioral and morphophysiological traits in frogs change in urban vs. rural areas. We expect that: (i) frogs in urban 167 areas will be more tolerant to human presence than those from rural areas, 168 consequently flight initiation distance will be shorter in urban areas; (ii) the 169 distance of first jump will be longer in rural than urban areas as a response to 170 maximize escape, due to less habituation to human presence. We also expect 171 that: (i) the amount of sperm and testes locule area will be higher in urban frogs, 172 as a strategy to maximize reproductive output at early ages: (ii) hepatic melanin 173

as well as body darkness will be different in frogs living urban and rural area, 174 175 since this pigment is important for thermoregulation and change in response to ultraviolet radiation; (iii) total thickness, epidermis, and EK-layer are important 176 against evaporative water loss, then urban frogs will have thicker skins to prevent 177 dehydration; (iv) erythrocytes, leukocytes, and glucose levels will be higher in 178 urban frogs due to anthropogenic stressors, such as high temperatures, novel 179 pathogens and pollutants. Likewise, somatic indices and leukocyte count will be 180 different in rural and urban areas, as a result of distinct life history strategies that 181 better fit their respective environment. This is the first study to concomitantly test 182 183 how antipredator behavior and multiple morphophysiological traits related to 184 reproduction, thermoregulation, metabolism, and immune response change in a tropical frog in response to urbanization. 185

186

#### 187 Material and Methods

188

### 189 Study site and animal model

Animals used in this study were collected in three sites in Campo Grande, 190 Mato Grosso do Sul, central Brazil (20° 26' 37" S, 54° 38' 52" W; Datum: WGS84; 191 Fig. 1). These sampling sites are, in mean, 22.05 km apart from each other (range 192 193 14.18–31.20 km). The urban site has 98% of buildings and streets area in a 500m buffer (MRS, pers. obs. based on data from MapBiomas 2024). Also, this pond 194 is highly polluted (MRS, pers. obs.) and are in the Bandeira hydrographic basin, 195 196 which has a highly polluted stream as well (Diniz et al. 2021). We collected 34 adult males of *Leptodactylus podicipinus* for morphophysiological analyses by 197 searching in breeding sites at night (Scott Jr. et al. 1994), of which 18 were from 198

a rural (P20) and 16 from an urban pond (P26). This species was the only to 199 occur at both ends of the urbanization gradient in this city. Field work was 200 conducted between 6:00 p.m. and 9:00 p.m. on 22 and 29 January 2024. We also 201 collected 28 frogs that were used in the jumping performance experiment (see 202 below), of which 13 were from a rural (P08; see Fig. 1) and 15 from an urban 203 pond (P26), totaling 63 individuals collected. Frogs for experiment were collected 204 on 22 and 23 February 2024. Animals were brought to the laboratory the following 205 day after collection. 206

207

## 208 Acclimation and experimental design

We first placed frogs in clean containers in the laboratory for 1–2 days to guarantee they started the acclimation process at the same time. We started the acclimation on 24 February 2024 by allocating frogs to two containers (41 x 27 x 28 cm) with soil (approx. 2 cm deep) and a Petri dish with tap water, replaced every 2–3 days. Soil was replaced once a week. We also removed the lid of each container and used a fly net. We placed a beaker with 600 mL water next to each container to maintain air humidity, which was filled every 2–3 days.

Then, we started the acclimation for 20 days under room temperature, light, and humidity conditions. We used a thermohygrometer (Extech EN300) to monitor temperature and humidity near the containers twice a day (around 1 p.m and 5 p.m). Mean temperature and humidity recorded were, respectively, 28.9 °C (range 26.9–31.3) and 55.69 % (range 46.5–67.6). Frogs were fed every 2–3 days *ad libitum* with ants, fly larvae, and small earthworms that were uniformly distributed in each container.

After acclimation, we started the behavioral experiments. Before starting, 223 224 we filled containers with water (approx. 1 cm height) to guarantee continuous hydration (following Bovo et al. 2023). To conduct the experiment, we built an 225 enclosed arena without refugia (3.60 x 2.60 x 1.00 m) with two red light bulbs. 226 We also sticked graph paper on the ground (20 mm x 20 mm) as a reference 227 scale. At the center of arena, we set up a cellphone (iPhone 11 Pro) to record 228 videos on an aerial view and wide mode (approx. 2.5 m height). The experiment 229 was conducted between 6:00 p.m. and 8:00 p.m., and consisted of (Fig. 2): (i) 230 placing each frog at the center of arena and covering it with a box for 231 232 approximately 1 min; (ii) removing the box and start recording the video, while one person walked towards the frog in a straight line always starting on the same 233 point (i.e., 1.5 m away from the frog) and maintaining the same speed; (iii) after 234 the first jump, the person stop walking. We recorded the movement of frogs only 235 once. 236

After the experiment, we measured body size (Snout-Vent Length) and 237 leg length (i.e., thigh + tibiofibula + foot) of each frog, following Watters et al. 238 (2016). We also weighted all frogs using an analogic hanging scale (Pesola 239 240 Lightline 10 g and 30 g). Then, we anesthetized each frog with 2% lidocaine and removed toe IV to be used in skeletochronology. The following day, we released 241 frogs back to the same site they were collected. We used Tracker v. 6.1.2 242 software (Brown et al. 2024) to take linear measures from recorded videos of frog 243 trajectory to obtain flight initiation distance (FID) and distance of first jump 244 (D1stJ). 245

246

247 Body coloration (Darkness)

Rural and urban animals were photographed before anesthesia under the same light conditions. To measure body darkness, we used two images per animal, in which we used the delimitation tool in Adobe Photoshop® (Adobe Systems Inc., Mountain View, CA, USA) to standardize the area measured, specifically 2cm<sup>2</sup> of the dorsal region below the insertion of forelimbs. We used landmarks to standardize measurements in each animal. We calculated the mean darkness for each animal following (Franco-Belussi et al. 2016).

255

## 256 Biometrics data and somatic indices

Animals were anesthetized with 2% lidocaine before taking blood sample and then euthanatized by lethal dose of lidocaine. Afterwards, they were photographed with a scale bar (1 mm) and weighed on a precision scale (to the nearest 0.001 g). Then, we mensurated Snout-Vent Length of each frog from images using Image-Pro Plus 6.0 (Media Cybernetics, Silver Spring, USA). Liver, spleen, heart, and testes were removed and weighed to calculate somatic indices (SI) by: SI= weight of the organ/weight of animal.

Liver, testes, and skin were taken to routine histological processing for inclusion in Paraplast® (Leica). Blood glucose (mg/dL<sup>-1</sup>) was estimated using a portable digital glucometer (Accu-Chek Active).

267

## 268 Skin layer morphometry

Skin sections of 3 µm were stained with Hematoxylin-Eosin (HE) and 10 images per animal are taken at 400× magnification. We used Image-Pro Plus 6.0 for measuring the thickness of skin layers (i.e., epidermis, stratum spongiosum (SS), stratum compactum (SC), and Eberth-Kastschenko (EK)) and the total thickness of the skin, following Franco-Belussi et al. (2023). In each image, five
random measurements are taken of each stratum, totaling 25 measurements per
image.

276

277 Testes morphometry and spermatozoa analyses

Testes sections of 3µm were stained with HE and 10 images per animal are taken at 400× magnification. For estimating locular area, we analyzed 5 locules per image, totalizing 50 locules per animal, following Franco-Belussi et al. (2023). For spermatozoa quantification, we estimated the percentage of spermatozoa using structural volumetric density analyses, following Franco-Belussi et al. (2024).

284

### 285 Estimating age with skeletochronology

To estimate the age of frogs, we followed standard and adapted protocols of skeletochronology as described in Franco-Belussi et al. (2024). We sliced the phalanges of the fourth toe and count the Lines of Arrested Growth (LAGs), in which each LAG represents one year of age.

290

# 291 Blood cell analyses

Blood was collected by cardiac puncture in syringes and needles with 3% Ethylenediaminetetraacetic acid - EDTA (Ranzani-Paiva et al. 2013; Franco-Belussi et al. 2022). We counted red blood cells and total leukocytes in a Neubauer chamber (number of cells x 106  $\mu$ L<sup>-1</sup>) after blood dilution (1:200) using a Natt and Herrick (1952) solution. A blood smear was made immediately after blood collection and stained with May-Grunwald-Giemsa-Wrigth (MGGW) by the modified Rosenfeld method (Tavares-Dias and Moraes 2006) for differential
 leukocyte count.

300

301 Data analysis

We modeled separately flight initiation distance (FID) (continuous response variable) and distance of first jump (D1stJ) (continuous response variable) as a function of body size or size-free leg length (continuous predictor variables) and their interaction with sites (categorical predictor variable with two levels) using a linear model in the R package *stats*.

307 To model the percentage of sperm (continuous response variable, normalized to vary between 0 and 1) as a function of site and LAGs (categorical 308 variable with three levels) and their interaction, we used an ordered beta 309 310 Generalized Linear Mixed-effects Model (GLMM) with logit link. Animal was included as random intercept to account for multiple measurements within the 311 same animal. Analysis considered 30 animals, as we could not determine the 312 LAGs of all specimens. Analysis was conducted in the R package glmmTMB 313 (Brooks et al. 2017). Residual diagnosis was conducted in the R packages 314 315 DHARMa (Hartig 2024) and performance (Lüdecke et al. 2021). Hypothesis testing was conducted using an Analysis of Deviance table, with Type II Wald's 316  $\chi^2$  test. Model residuals had normality, no outlier, but exhibited a small deviance 317 from homogeneity of variance (Fig. S1). 318

To model locule area (continuous response variable), we used a lognormal GLMM as described above with a log link. Model residuals had normality, no outlier, but exhibited slight deviance from homogeneity of variance. To model the area of hepatic melanin (continuous response variable), we used a Linear Mixed-effects Model (LMM) in the R package Ime4 (Bates et al. 2015). Model residuals had normality, no outlier, but exhibited slight deviance from homogeneity of variance.

To model the total number of circulating red cells (count response variable), we used a Negative Binomial GLM with quadratic parametrization in the R package glmmTMB. Similarly, to model the number of white cells (continuous response variable, log-transformed), we used a simple linear model. Residuals of both models showed normality and homogeneity of variance.

We modelled the EK layer and the Total depth (continuous response variables, log-transformed) using a LMM in which photomicrographies were included as a random intercept, nested within Animal. Analysis was conducted in the R package Ime4. For the epidermis, we used a lognormal GLMM with log link in the glmmTMB R package.

To model the percentage of darkness on the skin dorsum of live specimens, we used a beta GLMM including Animal as a random intercept, as we took two pictures of the same specimen. Residuals showed normality and homogeneity of variance.

We run a phenotypic trajectory analysis (Collyer and Adams 2013) separately for somatic indices and the differential white cell counts. Phenotypic trajectory analysis describes the change in a multivariate phenotype as a function of length, shape, and angle and are especially useful to visualize patterns when one predictor variable is an ordered factor, such as age (Collyer and Adams 2013). We first conducted a Principal Component Analysis (PCA) using a correlation matrix in R. HSI, ESI, and Glucose were positively related to PC1,

while RVM was negatively related to PC2. Lymphocyte and Monocyte were 347 positively related to PC1, while Eosinophil was negatively, and Neutrophil was 348 positively related to PC2. Then, we used the first three PCs as response variables 349 in a 2x3 Multivariate Analysis of Variance (MANOVA), including site and LAGs 350 (ordered categorical variable, with three levels) as predictor variables, along with 351 their interaction. Analysis was conducted in the R package RRPP (Collver and 352 Adams 2018). Residuals showed normality and homogeneity of variance. 353 Hypothesis testing was conducted using Type III Sum of Squares and 354 Randomized Residual Permutation. Afterwards, we used this model to calculate 355 356 three trajectory statistics: magnitude differences, trajectory correlations, and shape differences. Therefore, this analysis allowed us to understand how the 357 leukocyte profile and somatic indices variables varied between urban and rural 358 frog populations throughout their adult life span. 359

360

# 361 **Results**

362

There was no difference in the distance of first jump between urban and rural areas, but flight initiation distance was shorter in urban areas (Fig. 3). However, there was a difference between areas in FID after accounting for sizefree leg length (Table 1), in which area alone explained about 25% of the variation in FID.

Neither area nor or age (i.e., lines of arrested growth – LAGs) influenced the percentage of sperm, locule area, or hepatic melanin (Table 2; Fig. 4). However, the interaction between area and LAGs was significant for the total number of red (Wald's  $\chi^2$  = 8.376, DF = 2, *P* = 0.015) and white cells (F<sub>2,31</sub> = 3.33,

P = 0.048), as the difference between areas in these two variables became 372 relevant only in older frogs. EK-layer (Wald's  $\chi^2 = 79.895$ , DF = 1, P < 0.001) and 373 epidermis (Wald's  $\chi^2$  = 13.272, DF = 1, *P* < 0.001) were different between areas 374 (Table 2). Darkness was different between areas (Wald's  $\chi^2$  = 17.595, DF = 1, P 375 < 0.001; Table 2). Similarly, rural and urban populations had a difference in mean 376 somatic indices (Table 3), which explained about 33% of their variation. There 377 were no differences in either length or shape of trajectories, but the correlation 378 between them was different (Table 4; Fig. 5). Neither area nor LAGs influenced 379 leukocyte profile, with these variables explained less than 6% of its variation. This 380 means that there is no difference in mean leukocyte counts between areas or 381 ages. The patterns in leukocyte trajectory were similar to the somatic indices, with 382 383 only the correlation of angles between areas significant.

384

# 385 **Discussion**

386

We found that frogs in the rural and urban site differed in behavioral traits. 387 as well as hematological variables, somatic indices, dorsal color, and skin 388 morphometry. Frogs living in the urban site were less sensitive to human 389 presence, had a higher hepatosomatic index and glucose levels, their 390 hematological parameters varied depending on age, and their skin was thicker 391 and darker than rural ones, which might be associated to thermoregulation, 392 response to ultraviolet radiation (Franco-Belussi et al. 2016) and protection 393 against pathogens. In contrast to our initial hypothesis, urban frogs did not show 394 any differences in reproductive traits, as found by Jennette et al. (2019) or in 395 hepatic melanin. However, changes over different phenotypic axes, such as 396

behavior and physiology suggest that these populations exhibit divergent lifehistory strategies, supporting the PoLS (Réale et al. 2010), meaning urban frogs
display a faster pace of life.

Flight initiation distance (FID) was smaller in urban frogs, suggesting they 400 are more tolerant to human presence. This is the first study to test the FEAR 401 hypothesis for frogs in urban environments. This hypothesis state that prev 402 should escape after detecting a potential predator to reduce energetic costs 403 (Blumstein 2010). As humans can be recognized as a potential predator by 404 animals (Stankowich and Blumstein 2005), risk assessment of human presence 405 406 can be altered in human-modified habitats, resulting in a lower FID distance (Stankowich and Blumstein 2005). Previous studies assessing anti-predatory 407 behaviors to human presence in lizards and birds (McGowan et al. 2014; 408 Blumstein 2014) (Fernández-Juricic et al. 2001; Møller et al. 2015) found that 409 habituation to human disturbance is the main driver of this behavioral change. 410 Thus, as habituation process can impact directly the trade-off between 411 minimization of risk and energy maximization in urban areas and human 412 presence does not offer direct risk for urban frogs, they might reduce energy 413 414 investment on escape behavior and allocating in optimal foraging behavior and reproduction (see Lima and Dill 1990). 415

Hepatosomatic index (HSI) and glucose levels were higher in urban frogs. HSI can provide a rough estimation of exposure to xenobiotics (Bruslé and Anadon 1996). Also, the relative size of the liver is highly associated with energy storage in frogs (Hillman et al. 2008). As frogs use water bodies for reproduction and development (Duellman and Trueb 1994), they are more exposed to chemical pollutants common in cities (Hamer and McDonnell 2008), like those

from sewage contamination. As a consequence, the liver needs to metabolize 422 423 these substances to detoxify the organism, increasing its size and number of hepatocytes (Laurén and Wails 1990). A recent study found a positive correlation 424 between frog hepatocyte area and volume with HSI (Leão et al. 2021). Also, high 425 HSI values were found in fish exposed to pollutants (e.g., Carrola et al. 2009; 426 Khan 2010; Morado et al. 2017; da Silva et al. 2025). As our urban sampling site 427 is highly polluted (MRS, pers. obs.; Diniz et al. 2021), this fact could explain the 428 hepatosomatic alterations we found. Likewise, chronic stress in cities, such as 429 high temperature, noise pollution, and ALAN can increase blood corticosterone 430 431 levels, which through gluconeogenesis can enhance glucose levels (Tindal 1956; Feder and Burggren 1992). Moreover, as higher temperatures in cities can 432 increase evaporative water loss, a hyperglycemic response is expected to avoid 433 dehydration, since glucose can act on cells as osmoprotectants (Churchill and 434 Storey 1994). High glucose levels have already been reported for urban frogs in 435 a recent study and it might be associate with high metabolic demand in urban 436 areas (Franco-Belussi et al. 2024). However, in our case the urban and rural site 437 had similar temperatures. Therefore, induced stress experiments are necessary 438 439 to improve our knowledge about underlying processes involved in physiological responses to urbanization. 440

Frogs in urban environments had fewer red blood cells and elevated white cells depending on the age. Erythrocytes are responsible for regulating oxygen delivery to tissues through hemoglobin and are highly associated to metabolic rate (Hillman et al. 2008). As most respiration in frogs is cutaneous (Hillman et al. 2008), lower red blood cells count can negatively impact oxygen exchange in tissues. Therefore, older urban frogs might experience a faster

decrease of their metabolic rate in comparison to rural frogs. Furthermore, higher 447 temperatures like those of urban areas (i.e., urban heat island effect; Diamond 448 and Martin 2020) can cause oxidative stress, decreasing the viability and 449 promoting apoptosis of red blood cells (Chaiyasing et al. 2024). Oxidative stress 450 also can damage red blood cells, which are removed from circulation, further 451 impairing oxygen delivery to tissues (Mohanty et al. 2014). Moreover, red blood 452 cells also participate in frog innate immunity (Anderson et al. 2018), then a 453 decreasing of these cells in older frogs can directly impact the immune system. 454 The total number of white cells slight increased young urban frogs, but decreased 455 456 in older ones. This is consistent with a recent study that assessed immune system 457 for the same species in a urban area (Franco-Belussi et al. 2024). A decrease in leukocyte count over five years was also found by Romanova and Egorikhina 458 (2006) in an urban environment. Although increasing white cells in young frogs 459 suggests certain resistance over tolerance (Schoenle et al. 2018) to 460 anthropogenic stressors, they might be more susceptible to infectious diseases 461 due to an immature immune system (Rollins-Smith 2017). Likewise, several 462 stressors in cities (e.g., poluttants, parasites; Murray et al. 2019) can act as 463 immunomodulators, suppressing immune functions in amphibians (Minias 2023; 464 Assis et al. 2023), which can explain lower white cell counts in older frogs (but 465 see Davis et al. 2008). Therefore, these abrupt decreases in the total number of 466 red blood cells and white cells in older urban frogs might be associated with a 467 faster immunosenescence (Torroba and Zapata 2003) in comparison with natural 468 areas, which is predicted by the pace of life syndrome (Réale et al. 2010). 469

470 The epidermis and EK-layer were thicker in urban frogs. The primarily 471 functions of the epidermis are water exchange, protection to mechanical damage,

and protection against parasites (Lindemann and Voûte 1976). Also, a thicker 472 epidermis can reduce water loss in dryer environments (Kosmala et al. 2020). 473 Likewise, EK-layer provide protection against desiccation in frogs, due to its 474 hydrophilic glycosaminoglycans (Toledo and Jared 1993). Then, a thickening of 475 this layer in urban frogs can be an adaptation or simply a physiological adjustment 476 to reduce evaporative water loss (EWL) (Hillman et al. 2008), since these 477 environments usually show an increased temperature (see Fig. S7; Severgnini 478 and Provete in review). Also, this layer is more common in terrestrial amphibians 479 (Toledo and Jared 1993), making them more tolerant to dehydration than aquatic 480 481 species (Hillman et al. 2008). However, a recent study with Leptodactylus species (Ponssa et al. 2017) found no differences in the EK-layer when considering 482 different habitats. Conversely, a thickening of the calcified layer was associated 483 with increased temperatures and decreased humidity in frogs from different 484 habitats (Cao et al. 2024). Additionally, as the frog skin also participates in innate 485 immune defenses against pathogens (Varga et al. 2019), a thickening of skin in 486 urban populations might eventually suggest an adaptation to avoid water loss and 487 pathogens or pollutants. Our study provides for the first time an integument 488 comparison of frogs between rural and urban environments and is the first step 489 towards understanding the mechanisms of thermal tolerance of frogs to 490 urbanization (Diamond and Martin 2021). 491

Urban frogs had a more darkened skin than rural ones. Body darkness is
modulated by the amount of melanocytes over the skin, which absorb ultraviolet
radiation through melanin, developing a photo-protective barrier that help in heat
dissipation (Ortonne 2002). Several factors can change color patterns in frogs.
For instance, body darkness increased in frogs exposed to UV radiation (Franco-

Belussi et al. 2016), or to continuous ionizing radiation (Burraco and Orizaola 497 2022). Also, other studies found melanism in urban areas across several taxa 498 (Leveau 2021; Cosentino and Gibbs 2022). Likewise, the Túngara frogs in urban 499 areas had a higher frequency of the black striped pattern compared to rural ones 500 (Anderson et al. 2019). UV radiation in large cities should be low, due to high 501 levels of aerial chemical pollutants (Landsberg 1981). Additionally, low reflecting 502 surfaces, such as buildings and pavements, contribute to the so-called urban heat 503 islands (Landsberg 1981). Then, the skin in urban frogs should be lighter to avoid 504 overheating (Rudh and Qvarnström 2013). Here, we found the reverse. Besides 505 506 protection against UV radiation, darker individuals can have fitness advantages 507 in warm and wet environments, since melanin can be an immunoprotector against pathogens (Laumeier et al. 2023). Thus, pleiotropic effects may be involved in 508 melanogenic genes. Darker individuals also can lose camouflage ability in the 509 environment, but a recent review across several taxa found that darker colors can 510 help camouflage in impervious surfaces (i.e., dullness; Leveau 2021). Although 511 there is a complex trade-off between thermoregulation and immune protection, a 512 darker skin in urban frogs in our study might suggest an adaptation to urban 513 514 conditions driven by the protection against pathogens than UV radiation. However, this is the first study comparing frog body darkness between urban and 515 rural areas, and underlying mechanisms acting over color in urban areas 516 517 associated with UV, thermoregulation, and pathogens remain unclear.

518

519 **Conclusion** 

Our study adds consistent results about pace of life syndrome for 521 522 amphibians in urban environments, suggesting urban frogs display a faster pace of life with consequences for several morphophysiological and behavioral traits. 523 Following our initial hypotheses, frogs in urban areas are habituated to human 524 presence, but human-associated activities (e.g., chemical, noise, and light 525 pollution) affect directly their health status by altering their immune system and 526 metabolism. As human beings are ecosystems engineers, understanding our 527 impact on environment is the first step to build cities that are biodiversity friendly. 528 529

#### 530 **References**

- Alberti M, Correa C, Marzluff JM, et al (2017) Global urban signatures of phenotypic change in animal and plant populations. Proc Natl Acad Sci
- 533 114:8951 LP 8956. https://doi.org/10.1073/pnas.1606034114
- Anderson HL, Brodsky IE, Mangalmurti NS (2018) The Evolving Erythrocyte: Red
   Blood Cells as Modulators of Innate Immunity. J Immunol 201:1343–
   1351. https://doi.org/10.4049/jimmunol.1800565
- Anderson NK, Gutierrez SO, Bernal XE (2019) From forest to city: urbanization
   modulates relative abundance of anti-predator coloration. J Urban Ecol
   5:juz016. https://doi.org/10.1093/jue/juz016
- Assis VR, Robert J, Titon SCM (2023) Introduction to the special issue Amphibian
   immunity: stress, disease and ecoimmunology. Philos Trans R Soc B Biol
   Sci 378:20220117. https://doi.org/10.1098/rstb.2022.0117
- 543 Bates D, Mächler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects 544 Models Using Ime4. J Stat Softw 67:1–48. 545 https://doi.org/10.18637/jss.v067.i01
Blumstein DT (2010) Flush early and avoid the rush: a general rule of antipredator 546 behavior? 547 Behav Ecol 21:440-442. https://doi.org/10.1093/beheco/arq030 548 Blumstein DT (2014) Attention, habituation, and antipredator behaviour: 549 implications for urban birds. In: Gil D, Brumm H (eds) Avian Urban 550 Ecology, Oxford University Press, p 41-53 551 Bovo RP, Simon MN, Provete DB, et al (2023) Beyond Janzen's Hypothesis: How 552 Amphibians that Climb Tropical Mountains Respond to Climate Variation. 553 Integr Org Biol obad009. https://doi.org/10.1093/iob/obad009 554 555 Brans KI, De Meester L (2018) City life on fast lanes: Urbanization induces an evolutionary shift towards a faster lifestyle in the water flea Daphnia. 556 Funct Ecol 32:2225-2240. https://doi.org/https://doi.org/10.1111/1365-557 2435.13184 558 Brooks ME, Kristensen K, van Benthem KJ, et al (2017) glmmTMB Balances 559 Speed and Flexibility Among Packages for Zero-inflated Generalized 560 Linear Mixed Modeling. R J 9:378-400. https://doi.org/10.32614/RJ-561 2017-066 562 563 Brown D, Christian W, Hanson RM (2024) Tracker: video analysis and modeling tool 564 Bruslé J, Anadon G (1996) The Structure and Function of Fish Liver. In: Fish 565 Morphology. pp 77–93 566 Burraco P, Orizaola G (2022) Ionizing radiation and melanism in Chornobyl tree 567 15:1469-1479. frogs. Evol Appl 568 https://doi.org/https://doi.org/10.1111/eva.13476 569 Canavero A, Arim M (2009) Clues supporting photoperiod as the main 570

determinant of seasonal variation in amphibian activity. J Nat Hist
43:2975–2984. https://doi.org/10.1080/00222930903377539

- Cao L, Liao W, Yuan L, et al (2024) Adaptation of skin structures to environmental
   variations in anurans from southern and southwestern China. Integr Zool
   n/a: https://doi.org/https://doi.org/10.1111/1749-4877.12914
- Carrola J, Fontaínhas-Fernandes A, Matos P, Rocha E (2009) Liver
  Histopathology in Brown Trout (*Salmo trutta* f. fario) from the Tinhela
  River, Subjected to Mine Drainage from the Abandoned Jales Mine
  (Portugal). Bull Environ Contam Toxicol 83:35–41.
  https://doi.org/10.1007/s00128-009-9726-4
- Chaiyasing R, Jinagool P, Wipassa V, et al (2024) Impact of rising temperature
   on physiological and biochemical alterations that affect the viability of
   blood cells in American bullfrog crossbreeds. Heliyon 10:e32416.
   https://doi.org/10.1016/j.heliyon.2024.e32416
- Charmantier A, Demeyrier V, Lambrechts M, et al (2017) Urbanization Is
   Associated with Divergence in Pace-of-Life in Great Tits. Front Ecol Evol
   587 5:
- Chaves MF, Tenório FCMA, Santos ILVL, et al (2017) Correlations of condition
   factor and gonadosomatic, hepatosomatic and lipo-somatic relations of
   Leptodactylus macrosternum (ANURA: Leptodactylidae) in the Brazilian
   Semi-arid. An. Acad. Bras. Cienc. 89
- Churchill TA, Storey KB (1994) Effects of dehydration on organ metabolism in the
   frog *Pseudacris crucifer*. hyperglycemic responses to dehydration mimic
   freezing-induced cryoprotectant production. J Comp Physiol B 164:492–
   498. https://doi.org/10.1007/BF00714587

Collyer ML, Adams DC (2018) RRPP: An r package for fitting linear models to
 high-dimensional data using residual randomization. Methods Ecol Evol
 9:1772–1779. https://doi.org/10.1111/2041-210X.13029

Collyer ML, Adams DC (2013) Phenotypic trajectory analysis: comparison of
 shape change patterns in evolution and ecology. Hystrix, Ital J Mammal
 24:75–83. https://doi.org/10.4404/hystrix-24.1-6298

Cosentino BJ, Gibbs JP (2022) Parallel evolution of urban–rural clines in
 melanism in a widespread mammal. Sci Rep 12:1752.
 https://doi.org/10.1038/s41598-022-05746-2

da Silva TV, Nascimento Silva AL do, Farias KNN, et al (2025) Liver morphometry
 and histopathology effects in *Astyanax lacustris* exposed to lambda cyhalothrin pyrethroid insecticide. Toxicol Reports 14:101919.
 https://doi.org/https://doi.org/10.1016/j.toxrep.2025.101919

Davis AK, Maney DL, Maerz JC (2008) The use of leukocyte profiles to measure
 stress in vertebrates: a review for ecologists. Funct Ecol 22:760–772.

611 https://doi.org/https://doi.org/10.1111/j.1365-2435.2008.01467.x

de Amaral M, Von Dentz MC, Ohlweiler R, et al (2022) Seasonal variations in the
 intermediate metabolism in South American tree-frog *Boana pulchella*. J
 Comp Physiol B 192:297–311. https://doi.org/10.1007/s00360-021 01420-7

De Souza Santos LR, Franco-Belussi L, Zieri R, et al (2014) Effects of Thermal
 Stress on Hepatic Melanomacrophages of *Eupemphix nattereri* (Anura).
 Anat Rec 297:864–875. https://doi.org/https://doi.org/10.1002/ar.22884

Diamond SE, Martin RA (2020) Evolutionary Consequences of the Urban Heat
 Island. Urban Evol. Biol. 92–110

Diamond SE, Martin RA (2021) Physiological adaptation to cities as a proxy to
forecast global-scale responses to climate change. J Exp Biol
224:jeb229336. https://doi.org/10.1242/jeb.229336
Diamond SE, Prileson E, Martin RA (2022) Adaptation to urban environments.

 625
 Curr
 Opin
 Insect
 Sci
 100893.

 626
 https://doi.org/https://doi.org/10.1016/j.cois.2022.100893

Dias KS, Dosso ES, Hall AS, et al (2019) Ecological light pollution affects anuran
 calling season, daily calling period, and sensitivity to light in natural
 Brazilian wetlands. Sci Nat 106:46. https://doi.org/10.1007/s00114-019 1640-y

Diniz FG, Silva M do SF da, Andrade MH da S (2021) Impactos socioambientais
 e a conservação do Córrego Bandeira em Campo Grande - MS. Rev
 Monogr Ambient 1:e5. https://doi.org/10.5902/2236130865737

Duellman WE, Trueb L (1994) Biology of amphibians. The Johns Hopkins
 University Press, Baltimore

# Feder ME, Burggren WW (1992) Environmental physiology of the amphibians. University of Chicago Press, Chicago

Fernández-Juricic E, Jimenez MD, Lucas E (2001) Bird tolerance to human
disturbance in urban parks of Madrid (Spain): Management implications.
In: Marzluff JM, Bowman R, Donnelly R (eds) Avian Ecology and
Conservation in an Urbanizing World. Springer US, Boston, MA, pp 259–
273

Franco-Belussi L, de Oliveira Júnior JG, Goldberg J, et al (2024) Multiple
 morphophysiological responses of a tropical frog to urbanization conform
 to the pace-of-life syndrome. Conserv Physiol 12:coad106.

#### https://doi.org/10.1093/conphys/coad106

Franco-Belussi L, Nilsson Sköld H, de Oliveira C (2016) Internal pigment cells
 respond to external UV radiation in frogs. J Exp Biol 219:1378–1383.
 https://doi.org/10.1242/jeb.134973

Franco-Belussi L, Provete DB, Leão TRF, et al (2022) Hematological parameters
 of a Neotropical wild frog population, with a phylogenetic perspective on
 blood cell composition in Anura. Curr Zool 68:361–369.
 https://doi.org/10.1093/cz/zoab059

Franco-Belussi L, Valverde BSL, Bach NC, et al (2023) Morphological
Responses as a New Tool to Evaluate the Effects of Agrochemicals and
Other Emerging Contaminants in Neotropical Frogs. In: Larramendy ML,
Liwszyc GE (eds) Amphibian Species in Environmental Risk Assessment
Strategies. Royal Society of Chemistry, p 0

Gomes FR, Rezende EL, Grizante MB, Navas CA (2009) The evolution of
jumping performance in anurans: morphological correlates and
ecological implications. J Evol Biol 22:1088–1097.
https://doi.org/https://doi.org/10.1111/j.1420-9101.2009.01718.x

Greven H, Zanger K, Schwinger G (1995) Mechanical properties of the skin of
 *Xenopus laevis* (Anura, Amphibia). J Morphol 224:15–22.
 https://doi.org/https://doi.org/10.1002/jmor.1052240103

Hamer AJ, McDonnell MJ (2008) Amphibian ecology and conservation in the
urbanising world: A review. Biol Conserv 141:2432–2449.
https://doi.org/https://doi.org/10.1016/j.biocon.2008.07.020

Hartig F (2024) DHARMa: Residual Diagnostics for Hierarchical (Multi-Level /
 Mixed) Regression Models. R package version 0.4.7. https://cran.r-

671 project

project.org/web/packages/DHARMa/index.html/DHARMa/

- Haslam IS, Roubos EW, Mangoni ML, et al (2014) From frog integument to
  human skin: dermatological perspectives from frog skin biology. Biol Rev
  89:618–655. https://doi.org/https://doi.org/10.1111/brv.12072
- Hillman SS, Withers PC, Drewes RC, Hillyard SD (2008) Ecological and
   environmental physiology of amphibians. Oxford University Press, New
   York
- Iglesias-Carrasco M, Aich U, Jennions MD, Head ML (2020) Stress in the city:
  meta-analysis indicates no overall evidence for stress in urban
  vertebrates. Proc R Soc B Biol Sci 287:20201754.
  https://doi.org/10.1098/rspb.2020.1754
- Iglesias-Carrasco M, Martín J, Cabido C (2017) Urban habitats can affect body
   size and body condition but not immune response in amphibians. Urban
   Ecosyst 20:1331–1338. https://doi.org/10.1007/s11252-017-0685-y
- Jennette MA, Snodgrass JW, Forester DC (2019) Variation in age, body size, and
   reproductive traits among urban and rural amphibian populations. Urban
   Ecosyst 22:137–147. https://doi.org/10.1007/s11252-018-0801-7
- Kaiser K, Devito J, Jones CG, et al (2015) Effects of anthropogenic noise on
   endocrine and reproductive function in White's treefrog, Litoria caerulea.
   Conserv Physiol 3:cou061. https://doi.org/10.1093/conphys/cou061
- Khan RA (2010) Two Species of Commercial Flatfish, Winter Flounder,
   *Pleuronectes americanus*, and American Plaice, *Hippoglossoides platessoides*, as Sentinels of Environmental Pollution. Bull Environ
   Contam Toxicol 85:205–208. https://doi.org/10.1007/s00128-010-0050-9
   Kosmala GK, Brown GP, Shine R (2020) Thin-skinned invaders: geographic

696	variation in the structure of the skin among populations of cane toads							
697	( <i>Rhinella marina</i> ). Biol J Linn Soc 131:611–621.							
698	https://doi.org/10.1093/biolinnean/blaa128							
699	Landsberg H (1981) The Urban Climate. Urban Clim 275							
700	Laumeier R, Brändle M, Rödel M-O, et al (2023) The global importance and							
701	interplay of colour-based protective and thermoregulatory functions in							
702	frogs. Nat Commun 14:8117. https://doi.org/10.1038/s41467-023-43729-							
703	7							
704	Laurén D, Wails D (1990) Liver Structural Alterations Accompanying Chronic							
705	Toxicity in Fishes: Potential Biomarkers of Exposure. In: Biomarkers of							
706	Environmental Contamination. pp 17–57							
707	Leão T, Siqueira M, Marcondes S, et al (2021) Comparative liver morphology							
708	associated with the hepatosomatic index in five Neotropical anuran							
709	species. Anat Rec 304:860-871.							
710	https://doi.org/https://doi.org/10.1002/ar.24540							
711	Leveau L (2021) United colours of the city: A review about urbanisation impact							
712	on animal colours. Austral Ecol n/a:							
713	https://doi.org/https://doi.org/10.1111/aec.13005							
714	Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a							
715	review and prospectus. Can J Zool 68:619–640.							
716	https://doi.org/10.1139/z90-092							
717	Lindemann B, Voûte C (1976) Structure and Function of the Epidermis. In: Llinás							
718	R, Precht W (eds) Frog Neurobiology: A Handbook. Springer Berlin							
719	Heidelberg, Berlin, Heidelberg, pp 169–210							
720	Lüdecke D, Ben-Shachar MS, Patil I, et al (2021) performance: An R Package for							

721	Assessment, Comparison and Testing of Statistical Models. J Open
722	Source Softw 6:3139. https://doi.org/10.21105/joss.03139
723	Luedtke JA, Chanson J, Neam K, et al (2023) Ongoing declines for the world's
724	amphibians in the face of emerging threats. Nature.
725	https://doi.org/10.1038/s41586-023-06578-4
726	MapBiomas P (2024) Projeto MapBiomas – Coleção [v.8] da Série Anual de
727	Mapas de Cobertura e Uso do Solo do Brasil. https://mapbiomas.org/.
728	Accessed 28 Apr 2023
729	McGowan MM, Patel PD, Stroh JD, Blumstein DT (2014) The Effect of Human
730	Presence and Human Activity on Risk Assessment and Flight Initiation
731	Distance in Skinks. Ethology 120:1081–1089.
732	https://doi.org/https://doi.org/10.1111/eth.12281
733	Méndez-Tepepa M, Morales-Cruz C, García-Nieto E, Anaya-Hernández A (2023)
734	A review of the reproductive system in anuran amphibians. Zool Lett 9:3.
735	https://doi.org/10.1186/s40851-023-00201-0
736	Minias P (2016) Reproduction and survival in the city: which fitness components
737	drive urban colonization in a reed-nesting waterbird? Curr Zool 62:79-
738	87. https://doi.org/10.1093/cz/zow034
739	Minias P (2023) The effects of urban life on animal immunity: Adaptations and
740	constraints. Sci Total Environ 895:165085.
741	https://doi.org/https://doi.org/10.1016/j.scitotenv.2023.165085
742	Mohanty JG, Nagababu E, Rifkind JM (2014) Red blood cell oxidative stress
743	impairs oxygen delivery and induces red blood cell aging. Front Physiol
744	5:84. https://doi.org/10.3389/fphys.2014.00084
745	Møller AP, Tryjanowski P, Díaz M, et al (2015) Urban habitats and feeders both

746	contribute to flight initiation distance reduction in birds. Behav Ecol
747	26:861-865. https://doi.org/10.1093/beheco/arv024
748	Morado CN, Araújo FG, Gomes ID (2017) The use of biomarkers for assessing
749	effects of pollutant stress on fish species from a tropical river in
750	Southeastern Brazil. Acta Sci Biol Sci 39:.
751	https://doi.org/10.4025/actascibiolsci.v39i4.34293
752	Murray MH, Sánchez CA, Becker DJ, et al (2019) City sicker? A meta-analysis of
753	wildlife health and urbanization. Front Ecol Environ 17:575–583.
754	https://doi.org/10.1002/FEE.2126
755	Myers G (2021) Urbanisation in the Global South. In: Shackleton CM, Cilliers SS,
756	Davoren E, du Toit MJ (eds) Urban Ecology in the Global South. Springer
757	International Publishing, Cham, pp 27–49
758	Natt MP, Herrick CA (1952) A New Blood Diluent for Counting the Erythrocytes
759	and Leucocytes of the Chicken. Poult Sci 31:735–738.
760	https://doi.org/https://doi.org/10.3382/ps.0310735
761	Nishiumi N, Mori A (2020) A game of patience between predator and prey: waiting
762	for opponent's action determines successful capture or escape. Can J
763	Zool 98:351–357. https://doi.org/10.1139/cjz-2019-0164
764	Ortonne J (2002) Photoprotective properties of skin melanin. Br J Dermatol
765	146:7–10. https://doi.org/10.1046/j.1365-2133.146.s61.3.x
766	Pérez-Ortega B, Hendry AP (2023) A meta-analysis of human disturbance effects
767	on glucocorticoid hormones in free-ranging wild vertebrates. Biol Rev
768	98:1459–1471. https://doi.org/https://doi.org/10.1111/brv.12962
769	Piano E, Souffreau C, Merckx T, et al (2020) Urbanization drives cross-taxon
770	declines in abundance and diversity at multiple spatial scales. Glob

771	Chang Biol 26:1196–1211. https://doi.org/10.1111/gcb.14934
772	Ponssa ML, Barrionuevo JS, Pucci Alcaide F, Pucci Alcaide A (2017)
773	Morphometric Variations in the Skin Layers of Frogs: An Exploration Into
774	Their Relation With Ecological Parameters in Leptodactylus (Anura,
775	Leptodactylidae), With an Emphasis on the Eberth-Kastschenko Layer.
776	Anat Rec 300:1895–1909.
777	https://doi.org/https://doi.org/10.1002/ar.23640
778	Ranzani-Paiva M, Pádua S, Tavares-Dias M, Egami M (2013) Métodos Para
779	Análise Hematológica em Peixes. Universidade Estadual de Maringá,
780	Maringá, Brazil
781	Réale D, Garant D, Humphries MM, et al (2010) Personality and the emergence
782	of the pace-of-life syndrome concept at the population level. Philos Trans
783	R Soc London Ser B, Biol Sci 365:4051–4063.
784	https://doi.org/10.1098/rstb.2010.0208
785	Ricklefs RE, Wikelski M (2002) The physiology/life-history nexus. Trends Ecol
786	Evol 17:462–468. https://doi.org/10.1016/S0169-5347(02)02578-8
787	Rollins-Smith LA (2017) Amphibian immunity-stress, disease, and climate
788	change. Dev Comp Immunol 66:111–119.
789	https://doi.org/https://doi.org/10.1016/j.dci.2016.07.002
790	Romanova EB, Egorikhina MN (2006) Changes in hematological parameters of
791	Rana frogs in a transformed urban environment. Russ J Ecol 37:188-
792	192. https://doi.org/10.1134/S1067413606030076
793	Rudh A, Qvarnström A (2013) Adaptive colouration in amphibians. Semin Cell
794	Dev Biol 24:553–561.
795	https://doi.org/https://doi.org/10.1016/j.semcdb.2013.05.004

Samia DSM, Nomura F, Blumstein DT (2013) Do animals generally flush early
 and avoid the rush? A meta-analysis. Biol Lett 9:20130016.
 https://doi.org/10.1098/rsbl.2013.0016

Schoenle LA, Downs CJ, Martin LB (2018) An Introduction to Ecoimmunology.
 In: Cooper EL (ed) Advances in Comparative Immunology. Springer
 International Publishing, Cham, pp 901–932

- Stankowich T, Blumstein DT (2005) Fear in animals: a meta-analysis and review
   of risk assessment. Proc R Soc B Biol Sci 272:2627–2634.
   https://doi.org/10.1098/rspb.2005.3251
- Stott I, Salguero-Gómez R, Jones OR, et al (2024) Life histories are not just fast
  or slow. Trends Ecol Evol 39:830–840.
  https://doi.org/10.1016/j.tree.2024.06.001
- Tavares-Dias M, Moraes F (2006) Características hematológicas da *Tilapia rendalli* Boulenger, 1896 (Osteichthyes: Cichlidae) capturada em
   "Pesque-Pague" de Franca, São Paulo, Brasil. Biosci J 19:

Tindal JS (1956) Glycogenolysis in the Liver of the Common Frog, *Rana Temporaria*. J Exp Biol 33:196–210. https://doi.org/10.1242/jeb.33.1.196
Toledo RC, Jared C (1993) The calcified dermal layer in anurans. Comp Biochem
Physiol Part A Physiol 104:443–448.
https://doi.org/https://doi.org/10.1016/0300-9629(93)90444-9

Torroba M, Zapata AG (2003) Aging of the vertebrate immune system. Microsc
Res Tech 62:477–481. https://doi.org/https://doi.org/10.1002/jemt.10409
Varga JFA, Bui-Marinos MP, Katzenback BA (2019) Frog Skin Innate Immune
Defences: Sensing and Surviving Pathogens. Front Immunol 9:

Vences M, Galán P, Vieites D, et al (2002) Field body temperatures and heating

- rates in a montane frog population: The importance of black dorsal
   pattern for thermoregulation. Ann Zool Fennici 39:
- Watters JL, Cummings ST, Flanagan RL, Siler CD (2016) Review of morphometric measurements used in anuran species descriptions and recommendations for a standardized approach. Zootaxa 4072:477–495. https://doi.org/10.11646/zootaxa.4072.4.6
- Wells KD (2007) The ecology and behavior of amphibians. University of Chicago
   Press, Chicago
- Zhelev Z, Popgeorgie G (2021) Use of haematological indicators in anurans for
   assessing their health status when inhabiting conditions of anthropogenic
   stress. *Pelophylax ridibundus* (Amphibia: Ranidae) as an example: a
   review and appraisal. Ecol Balk 13:289–320
- 833

## 834 Author Contributions

- 835
- 836 Marcos R. Severgnini: Writing–Original Draft (Lead), Methodology (Equal), Data
- 837 Curation (Equal), Formal Analysis (Equal).
- 838 **Sabrina F. Gonçalves**: Data Curation (Equal).
- 839 **Carlos E. Fernandes**: Methodology (Equal), Data Curation (Equal).
- 840 **Rafael P. Bovo**: Methodology (Equal)
- Lilian Franco-Belussi: Methodology (Equal), Writing (Equal), Data Curation
- 842 (Equal).
- **Diogo B. Provete**: Methodology (Equal), Writing (Equal), Reviewing and Editing,
- 844 Conceptualization (Lead), Formal Analysis (Equal), Supervision.
- 845

All authors contributed critically to the drafts and gave final approval for publication.

848

## 849 Acknowledgements

- <sup>850</sup> We are grateful to the landowners for allowing access their properties. Bruno S.
- L Valverde helped us with data processing in laboratory. Heloísa M. Rodrigues
- and Leandro B. Cunha-Menezes helped with field work. Yasmin R. Zavelinski
- and Jeane Lima-Passos helped to carry out the experiment.

### 854 **Conflict of interest**

855 The authors declare no conflict of interest.

856

## 857 Data availability

All data and associated R code used to run the analysis will be available at FigShare.

860

### 861 **Funding**

DBP is supported by a CNPq grant (#407318/2021-6) and receives a fellowship from FUNDECT (#83//027.032/2024). This study was funded in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001 to MRS and DBP. During the final stages of the writing, DBP was supported by a fellowship for experienced researchers from the Alexander von Humboldt Foundation.

868

#### 869 Ethics approval

ICMBio provided the collecting permit (#80075-1). All the procedures were
approved by the Institutional Animal Care and Use Committee of our university
(CEUA-UFMS #1.203/2021).

873

## 874 Figures and Table legends

875

Table 1. Results of the Analysis of Covariance. D1stJ: Distance of first jump, FID:
Flight initiation distance. DF: Degrees of freedom, SS: Sum of squares, MS: Mean
squares, F: F-statistics, R<sup>2</sup>: R-squared. Significant *P* values are in bold. Site:
categorical predictor variable with two levels – rural and urban.

	DF	SS	MS	F	R <sup>2</sup>	P value
D1stJ		-				
Body size	1	40.5	40.47	0.1199	0.0049	0.7321
Site	1	181.8	181.76	0.5387	0.0219	0.4701
Body size * Site	1	210.3	210.34	0.6234	0.0253	0.4375
Residuals	24	8098.3	337.43			
Size-free leg length	1	263.3	263.27	0.7775	0.0313	0.3867
Site	1	67.7	67.74	0.2000	0.0082	0.6587
Size-free leg length *	1	72.8	72.79	0.2150	0.0088	0.6471
Site						
Residuals	24	8127.1	338.63			
FID						
Body size	1	7305.8	7305.8	8.8553	0.2695	0.006573
Site	1	1252.3	1252.3	1.5179	0.0594	0.229877

Body size * Site		447.3	447.3	0.5422	0.0220	0.468664
Residuals		19800.5	825.0			
Size-free leg length	1	21.9	21.9	0.0248	0.0010	0.876099
Site	1	7204.0	7204.0	8.1725	0.2540	0.008658
Size-free leg length *	1	424.2	424.2	0.4812	0.0196	0.494535
Site						
Residuals	24	21155.8	881.5			

Table 2. Results of Generalized Linear Mixed-effects models and linear models
used to test the effect of LAGs (Lines of arrested growth) and Site (Rural and
Urban) on several morphological and physiological metrics. Significant *P* values
are in bold. Chisq: chisquare; R<sup>2</sup> m: pseudo-R square marginal; R<sup>2</sup> c: pseudo-R
square conditional.

	DF	Chisq	Р	R <sup>2</sup> m	R <sup>2</sup> c
Percentage of sp			0.063	0.630	
Site	1	1.0679	0.3014		
LAGs	2	0.8251	0.6620		
Site:LAGs	2	0.4672	0.7917		
Locule area				<0.001	<0.001
Site	1	3.1563	0.07564		
LAGs	2	3.3243	0.18973		
Site:LAGs	2	3.3385	0.18839		
Hepatic melanin				0.071	0.653
Site	1	0.0069	0.9338		
LAGs	2	2.7765	0.2495		

Site:LAGs	2	0.6091	0.7375		
EK				0.469	0.776
Site	1	79.8950	0.00001		
LAGs	2	0.8504	0.6536		
Site:LAGs	2	4.1414	0.1261		
EP				0.130	0.554
Site	1	13.2723	0.0002		
LAGs	2	3.9995	0.1353665		
Site:LAGs	2	0.2145	0.8983202		
Total thickness				0.026	0.255
Site	1	1.7825	0.1818		
LAGs	2	2.2334	0.3274		
Site:LAGs	2	3.8172	0.1483		
Darkness				0.471	1.000
Site	1	17.5950	0.00001		
LAGs	2	1.3952	0.4978		
Site:LAGs	2	1.7948	0.4076		
Red cells				0.237	0.237
Site	1	2.4454	0.11787		
LAGs	2	0.2024	0.90376		
Site:LAGs	2	8.3763	0.01517		
White cells	DF	SS	F	R <sup>2</sup>	Р
Site	1	0.7680	2.4749	0.0739	0.12583
LAGs	2	0.0917	0.1478	0.0094	0.86320
Site:LAGs	2	2.0704	3.3361	0.1771	0.04873

**Table 3**. Results of the two-way MANOVA for somatic indices and leukocyte profile. Significant *P* values are in bold. LAGs (Lines of arrested growth) and Site (Rural and Urban).

	DF	SS	MS	R <sup>2</sup>	F	Z	Р
Somatic indice	es						
Site	1	50.724	50.724	0.33017	13.4115	4.4976	0.001
LAGs	2	6.002	3.001	0.03907	0.7934	-0.1504	0.558
Site:LAGs	2	8.101	4.050	0.05273	1.0709	0.3194	0.380
Residuals	25	94.553	3.782	0.61547			
Total	30	50.724					
Leukocyte pro							
Site	1	7.671	7.6709	0.06581	2.0312	1.19674	0.115
LAGs	2	3.875	1.9377	0.03325	0.5131	-0.78335	0.796
Site:LAGs	2	10.605	5.3024	0.09098	1.4040	0.77209	0.219
Residuals	25	94.412	3.7765	0.80997			
Total	30	116.564					

890

Table 4. Results of the Phenotypic Trajectory Analysis for somatic indices and
leukocyte count, showing the respective statistics. Bold numbers indicate
significant values.

Correlation	Angle	Difference	in	Differences	in
between	( <i>θ</i> )	Length (⊿d)		Shape ( <i>Dp</i> )	
trajectories (r)					



count

894



895

Figure 1. Map of the sampling sites (black dots) in the urban perimeter of Campo
Grande. Map features extracted from Instituto Brasileiro de Geografia e

898 Estatística (IBGE) and MapBiomas 2024; and prepared on QGIS v. 3.22.1.



Figure 2. Scheme in lateral view showing the measurements taken during the behavioral experiment. ID: starting walking point towards the frog; FID: flight initiation distance before human approximation; D1stJ: Distance of the first jump after human approximation. Grey silhouettes represent movement, while black ones represent initial position. Frog and human silhouettes are CC-BY from PhyloPic. Digital camera created by Khairuman.



Figure 3. Linear relationship between flight initiation distance and distance of first
jump in relation to area (rural or urban) after accounting for body size and sizefree leg length.



Figure 4. Boxplot of the relationship between LAGs (Lines of arrested growth)
and Site (Rural and Urban) on several morphological and physiological metrics.
Colors: green – rural site; red – urban.



Figure 5. Ordination diagram showing the phenotypic trajectories for somatic indices (A) and leukocyte count (B) of two populations
 of *Leptodactylus podicipinus*, showing the centroids of each age class and site. Numbers represent the age (LAGs – Lines of arrested
 growth) of the frogs. Variables shown beside axes have correlation > 0.4. ESI = Splenosomatic; HSI = Hepatosomatic Index; RVM =

Relative Ventricle Mass. For somatic index, rural and urban population occupy distinct positions in the ordination and change
 throughout adult life span in opposite ways. For leukocyte count, the two population have distinct trajectory along adult life span.

- 924 Supporting Information

- 926 Supplementary methods
- 927 Experimental design

We did not use a reciprocal transplant because the air temperature, 928 humidity, and land surface temperature in both rural and urban areas were very 929 similar. We used a thermohygrometer (Minipa MTH-1300) to take daytime and 930 nighttime temperature and humidity in both areas, which were: 36.9° C and 48.4% 931 (day); 25.1°C and 86.1% (night) for the rural area. For the urban area, it was: 932 35.1°C and 47.2% (day); 27.2°C and 74% (night). To check if these point 933 934 measures corresponded to historical variation in mean temperature, we used 935 QGIS v. 3.22.1 (QGIS.org 2020) to extract annual mean temperature of each site from Worldclim (Fick and Hijmans 2017). Mean temperature varied little between 936 sites from 1970 to 2000: 23.7 °C (20.2-24.7) for urban area, and 23.6 °C (range 937 21.10-25.79) for the rural area. 938



**Figure S1.** Residuals of generalized Linear Mixed-effects models and linear models used to test the effect of LAGs (Age) and Site on several morphological and physiological metrics. A: Locule area; B: Percentage of sperm; C: Hepatic melanin; D: Red cells; E: White cells; F: EK-Layer; G: Total thickness; H: Epidermis; I: Darkness.

# CONCLUSÃO GERAL

- Os padrões encontrados para o efeito da urbanização no fenótipo dos anfíbios são opostos àqueles para regiões temperadas do globo.
- Cidades tropicais com processos de urbanização recentes, porém intensos, parecem ter menor impacto nas mudanças morfológicas médias. Por outro lado, a variabilidade de atributos como tamanho de corpo, comprimento da perna e forma da cabeça nas áreas urbanas foi menor em áreas mais urbanizadas. Portanto, além da homogeneização biótica, as cidades podem também gerar homogeneização funcional. Entretanto, não encontramos um padrão espacial claro da urbanização sobre o fenótipo das populações avaliadas.
- A frequência dominante não diferiu entre áreas rurais e urbanas como esperado. Porém, sua variabilidade foi menor em áreas urbanas. Ainda, apesar de haver seleção direcional ao longo de todo gradiente, sua fraca intensidade pode ter permitido uma maior variação dos atributos acústicos. No entanto, como indivíduos que vocalizavam em frequências maiores tiveram menor fitness, talvez a seleção sexual esteja atuando de maneira mais intensa do que a seleção natural nas populações em áreas rurais e urbanas.
- A distância de fuga em áreas urbanas foi menor do que em áreas rurais, sugerindo um possível processo de habituação à presença humana, que pode impactar positivamente no comportamento de forrageio.
- As alterações morfofisiológicas encontradas nesse estudo, como altos níveis de glicose, índice hepatosomático elevado, pele dorsal com coloração mais escura e espessa, menor número de células vermelhas e brancas em indivíduos mais velhos apontam para possíveis consequências de um ritmo de vida mais acelerado nas cidades. Algumas dessas adaptações podem ter impactos positivos em termorregulação, osmorregulação e proteção contra

patógenos em áreas urbanas. Por outro lado, outros sugerem um possível estresse crônico em ambientes urbanos que impactam severamente atividades regulatórias do sistema imune e taxa metabólica.