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Impacto das mudanças climáticas na fisiologia térmica de anuros: o que podemos esperar para o futuro?

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Tese apresentada como requisito parcial para obtenção do título de Doutora, ao Programa de Pós-Graduação em Ecologia e Evolução, da Universidade do Estado do Rio de Janeiro.

Orientador: Prof. Dr. Carlos Frederico Duarte da Rocha

Coorientadora: Prof.ª Dra. Luisa Maria Diele-Viegas

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(Michelle Obama)

RESUMO

ALVES, Ivy Miranda do Amaral. **Impacto das mudanças climáticas na fisiologia térmica de anuros**: o que podemos esperar para o futuro? 2023. 95f. Tese (Doutorado em Ecologia e Evolução) – Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, 2023.

As mudanças climáticas são alterações no clima ou sem suas propriedades em decorrência de processos naturais ou causados por ação do homem. A principal causa para estas mudanças é o aumento das emissões de gases de efeito estufa desde a Revolução Industrial, o que pode levar a um potencial aumento de mais de 1,5°C no século XXI. Como consequência, os sistemas ecológicos e fisiológico de diversas espécies podem sofrer perturbações, causando grande perda na biodiversidade. Os anfíbios, como seres ectotérmicos, são o grupo de vertebrados mais ameaçado pelas mudanças climáticas devido à sua estreita relação com as condições ambientais. As mudanças climáticas podem afetar diferentes aspectos, como seu comportamento, sua distribuição e suas respostas fisiológicas e imunológicas. No presente estudo, buscou-se investigar como as mudanças climáticas afetam os anfíbios quanto à sua distribuição e fisiologia térmica. Sendo assim, o primeiro capítulo apresenta uma revisão narrativa que explora o impacto das mudanças climáticas na fisiologia térmica de anuros ao redor do mundo. No segundo capítulo investigamos o impacto das mudanças climáticas em uma assembleia de anuros que habitam um remanescente de Mata Atlântica no estado de Alagoas, investigando áreas termicamente adequadas para sua ocorrência através de uma Modelagem de Nicho Ecológico (MNE) correlativa em diferentes cenários. Por fim, no terceiro capítulo utilizamos uma abordagem de MNE mista, considerando aspectos macro e microclimáticos, bem como características fisiológicas para determinar áreas termicamente adequadas. Este estudo reforça a vulnerabilidade de anuros aos possíveis impactos das mudanças climáticas em cenários futuros, uma vez que estes indivíduos podem não ser capazes de suportar os efeitos do clima em cenários do futuro.

Palavras-chave: Mudanças climáticas. Ectotermia. Vulnerabilidade. Anura. Fisiologia térmica.

ABSTRACT

ALVES, Ivy Miranda do Amaral. **Climate change impact on anuran thermal physiology**: what can we expect for the future? 2023. 95f. Tese (Doutorado em Ecologia e Evolução) – Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, 2023.

Climate change is described as any change in the climate or its properties due to natural processes or caused by human action. The main cause for these changes is the increase in greenhouse gas emissions since the Industrial Revolution, which could lead to a potential increase of more than 1.5°C in the 21st century. Therefore, ecological and physiological systems of several species may suffer disturbances, causing a great biodiversity loss. Amphibians, as ectothermic organisms, represent the most threatened group of vertebrates to climate change due to their close relationship with environmental conditions. Climate change can affect different aspects, such as their behavior, distribution and physiological and immunological responses. In the present study, we sought to investigate how climate change affects anurans regarding their distribution and thermal physiology. Therefore, the first chapter presents a narrative review that explores the impact of climate change on the thermal physiology of anurans around the world. In the second chapter, we investigate the impact of climate change on an assemblage of anurans that inhabit a remnant of the Atlantic Forest in the state of Alagoas, investigating areas thermally suitable for its occurrence through correlative Ecological Niche Modeling (ENM) in different scenarios. Finally, in the third chapter we use a mixed ENM approach, considering macro and microclimatic aspects, as well as physiological traits to determine thermally suitable areas. This study reinforces the vulnerability of anurans to the possible impacts of climate change in future scenarios, as these individuals may not be able to withstand the effects of climate in future scenarios.

Keywords: Climate change. Ectothermy. Vulnerability. Anura. Thermal physiology.

SUMÁRIO

INTRODUÇÃO GERAL

As mudanças climáticas são caracterizadas por qualquer alteração no clima ou na variabilidade de suas propriedades e efeitos por um determinado período, em decorrência de processos naturais ou antrópicos (CUBASCH et al., 2013). O aumento das emissões de gases do efeito estufa e acúmulo na atmosfera desde a revolução industrial do século XVIII (ca. 1760-1840) constitui um dos principais fatores causadores das mudanças climáticas globais recentes (CROWLEY, 2000; SALA et al., 2000). Estima-se um potencial aquecimento superior a 1,5 °C da superfície terrestre durante o século XXI, com crescente aumento de temperatura após 2100 (IPCC, 2023). Dentre as consequências previstas estão uma forte alteração em diferentes processos dos sistemas ecológicos e fisiológicos das espécies, que poderão causar grande perda da biodiversidade e a ocorrência de eventos climáticos extremos que alteram de forma significativa o equilíbrio das regiões e ecossistemas do planeta (CROWLEY, 2000; SALA et al., 2000; LI; COHEN; ROHR, 2013).

Diante do iminente risco, desde 1990 o Painel Intergovernamental em Mudanças Climáticas (IPCC) elabora relatórios com o propósito de analisar e esclarecer os possíveis cenários sobre a situação climática, sendo o mais recente o Sexto Relatório de Avaliação (AR6, IPCC, 2023). Cinco possíveis cenários futuros foram disponibilizados no AR6, chamados *Shared Socio-economic Pathways* (SSP). Tais cenários são versões atualizadas dos *Representative Concentration Pathways* (RCPs), os quais representavam o valor total aproximado da forçante radiativa para o futuro tendo como comparação o ano de 1750, referência para o período pré-revolução industrial (ALEXANDER et al., 2013). O AR6, por sua vez, leva em consideração cenários de emissões de gases de efeito estufa (GEE) influenciados por fatores socioeconômicos (IPCC, 2023). Estes cenários incluem: (1) SSP1-1.9, cenário otimista, no qual haveria mais investimentos em áreas de educação e saúde e a sociedade aderiria a um modo de vida mais sustentável, apresentando emissões de CO2 reduzidas a zero até 2050; (2) SSP1-2.6, cenário comparativamente menos otimista que o anterior, mas que ainda apresentaria emissões globais consideravelmente mais baixas de CO2, atingindo emissões líquidas iguais a zero após período de 2050 e que ainda considera condições mais sustentáveis; (3) SSP2-4.5, cenário intermediário, no qual as emissões variam entre altas e baixas nas emissões, sem contudo zerar as emissões, até o fim do século e no qual o avanço sustentável ocorre de maneira lenta; (4) SSP3-7.0, no qual a competição aumenta de maneira global, com cada país centralizando suas preocupações internamente, havendo aumento considerável nas temperaturas e emissões de CO2 até 2100; (5) SSP5-8.5, que prevê um futuro onde as emissões atingem aproximadamente o dobro das emissões atuais até a metade do século, em decorrência de um crescimento econômico acelerado no qual os níveis de energia utilizadas pela sociedade aumentariam sem mitigações, bem como a contínua exploração de recursos não renováveis (IPCC, 2023). O cenário SSP 1-1.9 trata-se do único que se encaixaria nas exigências propostas pelo Acordo de Paris, no qual o aquecimento atingiria 1,5°C, porém cairia para 1,4°C ao final do século XXI, enquanto os demais cenários estabilizariam em aproximadamente 1,8°C (SSP1-2.6) e aumentariam 2,7°C, 3,6°C e 4,4°C (SSP2-4.5, SSP3-7.0 e SSP5-8.5, respectivamente; IPCC, 2023).

A vulnerabilidade de um sistema, que trata do grau em que este é suscetível a danos e incapaz de lidar com os efeitos nocivos de uma exposição a um risco, teve seu conceito amplamente discutido ao longo das últimas décadas (CUTTER, 1996; ADEJUWON et al., 2001; MCCARTHY et al., 2001; WILLIAMS et al., 2008). Para uma análise de vulnerabilidade, considera-se o grau de exposição de um organismo, ou um sistema, a uma determinada perturbação ou estresse; a sensibilidade à essa perturbação; e sua capacidade de responder aos efeitos da mesma (ADEJUWON et al., 2001; TURNER et al., 2003). Assim, no contexto de mudanças climáticas, será vulnerável um sistema ou indivíduo exposto às alterações climáticas preditas pelos modelos e que responda com uma baixa capacidade de se adaptar (ADEJUWON et al., 2001; WILLIAMS et al., 2008).

Apesar dos efeitos das mudanças climáticas não serem tão explicitamente observados, eles representam uma séria ameaça à biodiversidade, podendo se tornar irreversíveis no futuro (KAPPELLE; VAN VUUREN; BAAS, 1999). A velocidade de aumento da temperatura global recente supera as taxas de aquecimento natural dos últimos 18 milhões de anos (KAPPELLE; VAN VUUREN; BAAS, 1999). Devido a este aumento, as condições climáticas tornaram-se inadequadas para muitas espécies, assumindo um importante papel no declínio da biodiversidade (NUNEZ et al., 2019). Características do clima (*e.g.*, temperatura, umidade, incidência solar) influenciam na fenologia, fisiologia e distribuição das espécies (PEARSON; DAWSON, 2003; BELLARD et al., 2012). Sendo assim, uma possível incapacidade das espécies em se adaptarem a estas condições com a mesma rapidez pode levar à extirpação local das espécies (WEBB, 1992; BELLARD et al., 2012).

Com os anfíbios não é diferente e, há cerca de cinco décadas atrás já se percebia a inter-relações (CAREY, 1978) e, no início do presente século, já se avançava na compreensão da forte ligação entre as mudanças climáticas e os processos afetando negativamente este grupo de organismos (*e.g.*, WEYGOLDT, 1989; POUNDS; CRUMP, 1994; CAREY; ALEXANDER, 2003). Um considerável número de espécies de anfíbios encontra-se atualmente em declínio (READING, 2007) e sua distribuição geográfica também pode sofrer alterações em resposta aos efeitos climáticos (PARMESAN, 2006). A construção do conhecimento sobre o tem mostrado avanços mas também tem incluído controvérsias e muitas partes ou processos ainda pouco compreendidos (LI; COHEN; ROHR, 2013).

Anfíbios representam um grupo altamente vulnerável às alterações climáticas, uma vez que sua habilidade de termorregular através do comportamento é reduzida devido ao trade-off entre temperatura e hidratação (CAREY, 1978; CAREY; ALEXANDER, 2003). Anuros são importantes componentes ecossistêmicos, uma vez que, em sua maioria, apresentam ciclo bifásico e conectam vários níveis na cadeia trófica (DUELLMAN; TRUEB, 1994). As mudanças climáticas podem influenciar diferentes processos e características comportamentais, fisiológicas e imunológicos (BEECHER; DEMAS, 2004) e aumento de eventos climáticos de curto prazo pode ter efeito nas distribuições deste grupo (HEYER et al., 1988). Com o aumento de eventos extremos, as condições climáticas necessárias para a sobrevivência das espécies podem se mover para áreas que não apresentem os recursos necessários para seu estabelecimento (SCHIVO et al., 2019). Por sua baixa vagilidade, é possível que as espécies não consigam se locomover para estas regiões onde as condições climáticas favoreceriam sua sobrevivência (SINSCH, 1990; ENRIQUEZ -URZELA 2019). Além disso, a interação das condições climáticas com outros fatores, tais quais a perda de habitat, decréscimo de recursos alimentares localmente e a presença de patógenos, podem potencializar os efeitos negativos em anuros (NAVAS; OTANI, 2007; BLAUSTEIN et al., 2010; HOF et al., 2011) .

Por serem animais ectotérmicos, a temperatura corpórea (T_c) dos anuros é diretamente influenciada por fatores ambientais como temperatura e precipitação (CAREY; ALEXANDER, 2003). Além disso, seu desempenho locomotor é regulado através da interação de sua T_c com as relações hídricas (TITON et al., 2010). Devido à elevada permeabilidade de sua pele, estes indivíduos são suscetíveis à dessecação, colocando-os em risco frente à alteração das condições climáticas (WINTER et al., 2016) . Com o aumento na temperatura e na frequência de eventos extremos projetados para o futuro, a capacidade de aclimatação deste grupo às condições adversas (conhecidamente baixa, GUNDERSON; STILLMAN, 2015) pode não evoluir suficientemente rápido para suportar estas mudanças (DIFFENBAUGH; FIELD, 2013).

Os declínios de diversas populações, atribuídos às mudanças climáticas, gerou a necessidade do avanço de metodologias para avaliar e aprender sobre estes efeitos, uma vez que os dados históricos de distribuição de grande parte das espécies encontra-se impreciso, ou ausente (LI; COHEN; ROHR, 2013; VASCONCELOS; DO NASCIMENTO; PRADO, 2018). Assim, a metodologia de Modelagem de Nicho Ecológico (Araújo e Prado 2012) vem sendo cada vez mais utilizada mundialmente para entender efeitos das mudanças climáticas sobre a distribuição das espécies no presente e em um cenário futuro de aquecimento global (*e.g.* ARAÚJO et al., 2011; LEMES; LOYOLA, 2013; FOIS et al., 2016). Esta metodologia permite estimar áreas onde as espécies potencialmente estariam distribuídas, bem como determinar áreas prioritárias para preservação (THUILLER et al., 2005; HEINRICHS et al., 2010; JIMÉNEZ-VALVERDE et al., 2011).

Neste estudo, visamos avaliar o impacto de mudanças climáticas nos anuros, considerando sua fisiologia térmica e distribuição geográfica. Para isso, o trabalho está dividido em três principais seções: no primeiro capítulo apresento uma revisão narrativa acerca do impacto de mudanças climáticas na fisiologia térmica de anuros ao redor do mundo; no segundo capítulo avalio este impacto em espécies de anuros de um remanescente da Mata Atlântica; e, no terceiro capítulo, apresento uma modelagem de nicho ecológico considerando estas mudanças climáticas em diferentes cenários.

Objetivo geral

Avaliar o impacto das mudanças climáticas em características da fisiologia térmica e na distribuição potencial de uma assembleia de anuros no bioma Mata Atlântica.

Objetivos específicos

1) Analisar a literatura sobre o efeito das mudanças climáticas na fisiologia térmica de anuros;

2) Identificar áreas climaticamente adequadas para a ocorrência de uma assembleia de anuros ao longo do tempo, através de uma modelagem correlativa;

3) Identificar áreas de adequabilidade considerando características de fisiologia térmica através de uma modelagem mecanicista comparando passado, presente e futuro.

4) Descrever as áreas de acúmulo de espécies identificadas em ambas as abordagens correlativa e mecanicista.

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1 EFFECTS OF CLIMATE CHANGE ON ANURANS THERMAL PHYSIOLOGY

(Manuscrito sob revisão de co-autores: AMARAL-ALVES, I. M.; ROCHA C. F. D.; DIELE-VIEGAS, L. M. **Effects of climate change on anurans thermal physiology**).

Abstract

The world is facing a 1.25°C increase in the global average temperature compared to industrial age, and it is likely to reach a climatic tipping point where it will no longer be able to recover by the end of the 21st century. The ongoing thermal increase is affecting environmental conditions and harming biodiversity. Amphibians are among the most vulnerable groups to climate change due to their thermal and hydric dependence on external factors, which influences different physiological processes. By focusing on their dependence on environmental temperatures, here we assess the possible effects of climate change on the thermal physiology of adult anuran amphibians through a theoretical literature review. We thus synthesized techniques, physiological and environmental variables explored by the studies, as well as the most studied families, and methods used to test studies' hypotheses. We identified 16 articles dealing specifically with the topic of interest, among which two highlighted techniques used in this field of research, and three reported potential behavioral or adaptation strategies to cope with the future effects of climate change. Eleven studies indicated some degree of negative influence of climate change on the thermal physiology and survival of at least one of the analyzed species. We reinforce the need for studies assessing the vulnerability of highly restricted species with low vagility and differences between populations of widely distributed species. Finally, we indicate that including microclimatic data, thermal performance curves, and temperature-hydration interaction into vulnerability assessments is necessary to obtain consistent, reliable, and realistic information that is vital to amphibian conservation.

Keywords: Anura, Ecophysiology; Thermal Ecology; Review.

1.1 Introduction

According to the Sixth Assessment Report (AR6; IPCC, 2021), which considers greenhouse gas (GHG) emission scenarios influenced by socioeconomic factors, current warming rates have already surpassed 1.1°C compared to pre-industrial revolution (IPCC, 2023). Such change is related to the increase in frequency and intensity of extreme weather events that are already experienced worldwide (IPCC, 2023). Temperatures are predicted to increase throughout time, possibly reaching the 1.5°C temperature limit established by the Paris Agreement for the next two decades and up to 5.7°C of average global warming by the end of the 21st century (IPCC, 2023). Climate change affects the general biology of different groups of organisms (CORN, 2005; HELLER; ZAVALETA, 2009), including changes in animal reproductive cycles, geographic distributions, and physiological performance (CAREY; ALEXANDER, 2003; CORN, 2005; WILBY; PERRY, 2006). Amphibians are among the most vulnerable groups to climate change (NAVAS; OTANI, 2007; DEUTSCH et al., 2008). Environmental temperature influences different physiological processes and functions, such as energy acquisition, enzyme functioning and performance, among others (FEDER; BURGGREN, 1992; CAREY; ALEXANDER, 2003). A thermal tolerance range describes an animal's physiological performance, ranging from minimum (CT_{min}) to maximum (CT_{max}) temperature limits that the animal is capable of enduring (HUEY; STEVENSON, 1979). In order for their metabolism to work adequately, without compromising them negatively, there is a temperature breadth comprising an optimal temperature (T_{opt}) at which their performance reaches its maximum (HUEY; STEVENSON, 1979). Amphibians' body temperature (T_b) is intensely associated with the air and substrate temperature, and species are usually considered thermoconformers since they hardly display any active thermoregulatory ability (LAMBRINOS; KLEIER, 2003). Additionally, relative humidity (rH) and precipitation are significant factors affecting the survival of this group because their permeable skin renders them highly vulnerable to dehydration (WINTER et al., 2016). In this sense, large variations in environmental temperature and relative humidity can make amphibians more sensitive to diseases, such as those caused by the fungus *Batrachochytrium dendrobatidis* (BERGER et al., 2004; WARDZIAK et al., 2013).

The First World Congress of Herpetology in 1989 opened doors provoked debates regarding on the reasons for amphibian declines worldwide (WAKE, 1991). Although discussion on the matter is relatively recent, some amphibian declines are dated to years before that period (PECHMANN; WILBUR, 1994). In addition, studies acknowledging the relationship between amphibians' ecophysiology and climate started being developed between the 60s and 80s (BRATTSTROM, 1968; BEEBEE, 1977). Even though pioneer studies on the matter had already displayed a fair amount of information on the potential effects of climate change and its scenarios, we still lack an overview of how these effects can synergistically be expressed, especially on adult amphibians' thermal physiology and their vulnerability to climate change.

In the present study, due to a relatively wide misalignment of methodologies used to study the effect of climate change on amphibians, we reviewed methodologies and variables explored by studies on the effect of climate change on anuran ecophysiology. Additionally, we seek to understand the reason concerning choices of the studied families (*e.g.*, possible threats to the species, great availability of individuals for research, phylogenetic relationship in relation to vulnerability between close groups), the tested hypotheses and the predicted impact of climate change on frogs.

1.2 Methodology

We focused our analysis on studies concerning the influence of climate change on the thermal physiology of adult anurans and performed a broad bibliographic search. We restricted our search to articles published in scientific journals, excluding unpublished literature (*e.g.*, grey literature as Theses, Dissertations, and Conference proceedings). The search was performed through PubMed, Scholar Google, SciELO, Web of Science and Wiley Online Library databases with the following keywords: [Climate Change OR Climate Warming OR Global warming] AND [Frog* OR Toad* OR Amphibia OR Amphibian OR Anura OR Lissamphibia] AND [Thermal Physiology] NOT [Bd* OR Batrachochrytrium] NOT [Tadpole*] (Table 1).

Table 1- Databases and amount of articles recovered

Databases	Number of studies
SciELO	1
Pub Med	86
Wiley	143
Google Scholar	1330
Web of Science	7344
Total	**8904**

We retrieved studies evaluating the effects of climate change on thermal physiology of adult anurans. Therefore, we did not include studies focusing on anuran's larval stages. Furthermore, we did not consider articles focusing on evaluating these effects while investigating the fungus *Batrachochytrium dendrobatidis*.

Subsequently, to evaluate the effects of climate change on anuran physiology, we extracted the following information from the selected articles: families of interest, hypotheses explored by the authors, thermal physiology data (body temperature, preferred temperature, optimal performance temperature, critical thermal minimum and maximum), environmental data (*e.g.*, air temperature, substrate temperature, and bioclimatic variables) and environment type where species lived in.

We based our evaluation on TAYLOR et al. (2020) to assess the discussion on the methods used by studies to measure thermal variables. TAYLOR et al. (2020) discuss the most appropriate variables and techniques to perform tests on the effect of climate on amphibians' thermal physiology. We also evaluated the choice criteria on studied families in the articles, displaying (when present) the possible reasons for such choices and discussing the hypotheses explored and whether these were proven or refuted according to the obtained results.

1.3 Results and Discussion

Our search initially retrieved 8904 articles, but only 39 studies met the objectives' criteria and were kept for the analyses. These articles were further submitted to an additional filter where we verified which studies addressed both climate change and thermal physiology simultaneously, restricting to 16 the final number of articles considered in this review. Hereafter, we discuss how studies have assessed the risk and vulnerability evaluation for amphibians in the face of climate change, considering their thermal physiology, evaluating the methodologies used (variables, tests) and discussing their hypotheses and group-choice criteria.

1.3.1 *Metrics*

Body, environmental/air and substrate temperatures)

The body temperature (T_b) is a highly important variable for studies that investigate the relationship between climate change and ecophysiology since it represents the temperature at which the animal displays in its natural environment. Thus, T_b must be measured through an instrument that causes minimal stress or interference to the individual (TAYLOR et al., 2020). Preferably, this measurement should occur in the field through an instrument capable of measuring the animal's internal temperature. However, surface temperature may be an alternative for individuals that cannot be manipulated as easily (TAYLOR et al., 2020).

Despite its importance, only one study measured their T_b in the field (SANABRIA; QUIROGA, 2019). The other studies indicate the use of estimates through biophysical models (NOWAKOWSKI et al., 2017) or procedures conducted in the laboratory (*e.g.*, SAUER; SPERRY; ROHR, 2016; BARRIA; BACIGALUPE, 2017; ENRIQUEZ-URZELAI et al., 2018). Two of the studies did not record T_b under either field or laboratory conditions. One of these used agar models and recorded the operative temperature (LERTZMAN-LEPOFSKY et al., 2020), the other used measures of performance acclimated to a specific temperature and defined the anuran's optimal performance through maximum exertion capacity and jumping force (detailed in the thermal performance curve section; (PADILLA et al., 2019).

The recording of environmental temperatures is necessary for assessments on the impact of climate change on amphibians due to their ectothermy, since this temperature is known to directly influence their physiological functions (HUTCHINS et al., 2003). The studies assessed referred to environmental temperature in multiple forms: T_{env}, T_a (air temperature) and T_s (substrate temperature), and some studies considered more than one of them. Although much is questioned about the reliability of air temperature as a *proxy* (a variable that represents the interest variable) for T_b of these individuals (*e.g.*, TRACY, 1976), a study argued that air temperature was the one that best represented T_b in amphibians during activity (NAVAS, 1996). Thirteen articles included at least one environmental variable. The variables analyzed included air temperature within laboratory (*e.g.*, RUIZ-ARAVENA et al., 2014), habitat or microhabitat temperature (*e.g.*, NOWAKOWSKI et al., 2017; VIDAL et al., 2017; VON MAY et al., 2019), air temperature from weather stations (*e.g.*, VIDAL et al., 2017) or bioclimatic variables (*e.g.*, FRISHKOFF; HADLY; DAILY, 2015; SANABRIA; QUIROGA, 2019; GONZÁLEZ-DEL-PLIEGO et al., 2020, Table 2). Three of the analyzed studies did not measure any environmental temperature metric (HERREL; BONNEAUD, 2012; SAUER; SPERRY; ROHR, 2016; ENRIQUEZ-URZELAI et al., 2018; PADILLA et al., 2019).

Thus, environmental temperatures may be important factors for amphibians' thermal physiology studies due to heat exchange between the individuals' skin and the sites where they may be found (CATENAZZI; LEHR; VREDENBURG, 2014). It was previously shown that T_b was highly associated with substrate temperature (hereafter, T_s) during their inactive period (NAVAS et al. 1996). Furthermore, measuring T_s along with T_b can provide important information about the time required by an ectotherm at a particular location for their T_b to adapt to environmental conditions (SAUER; SPERRY; ROHR, 2016). Only three of the studies explored this variable. Those studies varied in terms of measurement methods: iButtons, using T_s as a proxy for operative temperature (CATENAZZI; LEHR; VREDENBURG, 2014), the use of a probe (TES TP-K03) on the substrate where individuals were found (SANABRIA; QUIROGA, 2019) and an infrared thermometer (Extech®, accuracy: ±2% of rdg <932°F) at the closest place to where the animal was positioned under laboratory conditions (SAUER; SPERRY; ROHR, 2016). The other studies did not register this variable.

Preferred temperature

Preferred temperature usually refers to the mean or median T_b or a series of T_bs selected by the study animal over a given time period in a lab experiment (TAYLOR et al., 2020). Only four studies performed tests to record the preferred temperature of anuran species. Although the researched articles treat this variable as T_{pref}, based on TAYLOR et al. (2020), we hereafter refer it as set point T_b (set-point body temperature, T_{set}).

In order to determine T_{set}, researchers must make sure that the animal has access to a temperature gradient, allowing it to voluntarily move along the gradient and select sites where temperature is most suitable within a period (TAYLOR et al., 2020). All studies that measured T_{set} maintained the gradient within a variation of 20-23°C from the cooled end to the heated ends (RUIZ-ARAVENA et al. 2014, SAUER; SPERRY; ROHR, 2016, BARRIA; BACIGALUPE 2017, ENRIQUEZ-URZELAI et al. 2018).

Following CAMACHO; RUSCH (2017), TAYLOR et al. (2020) indicated the use of some type of thermal device, which must be inserted in the cloacal opening of individuals, or the use of thermal cameras above the gradient to record the temperature. None of the evaluated studies used such devices to measure their T_b, preferring infrared thermometers pointed at the animal’s dorsal portion. Some of these studies have argued that dorsal temperature is highly related to cloacal temperature (RUIZ-ARAVENA et al. 2014, SAUER; SPERRY; ROHR 2016, BARRIA; BACIGALUPE 2017).

Two of the studies varied on the choice of the acclimation temperatures prior to performing T_{set} tests. Acclimation refers to the physiological adjustments organisms undergo as a response to changes in its environment, in order to reduce stress and enhance performance HUEY et al. 1999, IUPS 2003). Authors presented the following reasons for the acclimation temperature choice: (1) temperature regarding the annual thermal average and annual maximum, and (2) annual thermal average during the breeding period, locality’s average, and maximum temperature under a scenario of high greenhouse gas emissions (1,250 ppm: SOLOMON et al., 2007; RUIZ-ARAVENA et al., 2014). One of the studies reported the reason being animal acclimation to a common temperature (ENRIQUEZ-URZELAI et al. 2018). Only one study did not submit these individuals to temperatures prior to their placement on the gradient (SAUER; SPERRY; ROHR 2016).

The time taken by the experiments also varied among the studies but failed to present justification as to why those periods were stipulated. It has been considered that the optimal test duration would be 24 hours in order to have a wide variety of temperatures, although shorter periods (*e.g.*, measuring only during the active period of the animal) may be used depending on the type of study performed and the test's purpose (TAYLOR et al., 2020). Test details of each study are displayed in Table 3.

Critical thermal minimum and maximum

The temperatures that define an individual's thermal tolerance limits are known as critical thermal minimum and maximum (CT_{min} and CT_{max}, respectively; TAYLOR et al. 2020), below or above which animals lose the ability to perform their activities. To obtain such variables, most studies considered lower and upper body temperatures as tolerance limits after which the animal lost the ability to present a locomotor response after being flipped onto its back, being unable to return to its original position (TAYLOR et al., 2020). The differences between the methodological aspects for CT_{min} e CT_{max} measurements are detailed in Table 4.

Among the analyzed studies, 11 assessed either CT_{min} or CT_{max} (or both). These studies varied as to the criteria to reach this measurement, including (1) lack of righting response (*e.g.*, HERREL; BONNEAUD, 2012; RUIZ-ARAVENA et al., 2014; VON MAY et al., 2019); (2) lack of muscle stimulus (ENRIQUEZ-URZELAI et al., 2018), and (3) some type of behavioral change (unspecified; VIDAL et al., 2017). Moreover, studies also varied in aspects such as cooling/heating rates and temperature recording devices. A certain methodological disagreement was observed between the articles; eight among the 11 studies determine the animal's lack of righting response after a given time or physical stimulus. Five of these studies point out a waiting time of 5 seconds (CATENAZZI; LEHR; VREDENBURG, 2014; NOWAKOWSKI et al., 2017; VON MAY et al., 2017; GONZÁLEZ-DEL-PLIEGO et al., 2020). Three of the studies report waiting for one minute (RUIZ-ARAVENA et al., 2014; BARRIA; BACIGALUPE, 2017), and finally, one of them determines the lack of stimulus after touching the animal gently 10 times while turned on its back (ENRIQUEZ-URZELAI et al. 2018). The remaining studies did not mention the reason for these choices (HERREL; BONNEAUD, 2012; FRISHKOFF; HADLY; DAILY, 2015; VIDAL et al., 2017). Thus, we observed a clear divergence among some of the studies and curiously, no reason regarding these "choices" was given by the studies, which raises questions regarding these criteria's true relevance to determine the lack of righting response.

Literature also evidences the need to measure heating/cooling rates during critical thermal tests, keeping them constant and controlled (TAYLOR et al., 2020). Only one study (VIDAL et al. 2017) did not present data regarding the heating/cooling rate. The apparatus used for body temperature measurements also differed consistently among studies. Although TAYLOR et al. (2020) indicate those different types of devices may be employed, the use of resistance temperature detectors (RTDs) or thermistors is suggested due to their high accuracy. A wide variety of measuring instruments were used for this test among the studies but none operated with either of those recommended by TAYLOR et al. (2020).

Thermal Performance Curve

The thermal performance curve (TPC) encompasses the continuous response of any performance characteristic of an individual regarding a spectrum of different temperatures (HUEY; STEVENSON, 1979). Such characteristics may relate to different variables, such as locomotion and oxygen consumption (TAYLOR et al., 2020). TPCs are described by the interval between CT_{min} and CT_{max}. Another important parameter presented by TPC is the optimal temperature (T_{opt}), where performance reaches its maximum (TAYLOR et al., 2020). Additionally, aspects considered more informative can also be retrieved by the TPC, such as the range of thermal performance at 80% (80%T_{br}), *i.e.* the temperatures comprising 80% of the maximum expected performance. This measurement can be more informative than the critical limits since different species with identical or similar critical limits can present 80% larger or narrower T_{br}, characterizing them as thermal generalists or specialists, respectively (TAYLOR et al., 2020).

Table 2 – Studies that registered environmental variables and T_b.

Studies	Authors	Field / lab T_b	T_a	T_s	T_{env} (Environmental temperature)
Intraspecific geographic variation in thermal limits and acclimatory capacity	BARRIA; BACIGALUPE 2017	Measured only during T_{set} test	-	-	Monthly mean, maximum and minimum temperature
Thermal Physiology, Disease, and Amphibian Declines on the Eastern Slopes of the Andes	CATENAZZI; LEHR; VREDENBURG, 2014	Measured only during critical limits test	Inference through daily mean regression from 2001 and 2006 in 4 metereological stations from 520 to 3485 m.a.s.l.	T_e as proxy for T_s, through data loggers iButons	-
Hindered and constrained: limited potential for thermal adaptation in post-metamorphic and adult *Rana temporaria* along elevational gradients	ENRIQUEZ-URZELAI et al. 2018	Measured only during critical limits test and performance	-	-	-
Temperature dependence of locomotor performance in the tropical clawed frog, *Xenopus tropicalis*	HERREL; BONNEAUD 2012	Measured only during T_{set} and critical limits test and performance	-	-	-
Thermal physiological traits in tropical lowland amphibians: Vulnerability to climate warming and cooling	VON MAY et al. 2019	Measured only during critical limits test	Maximum air temperature from local climate station	-	iButtons – leaf-litter and understory forest microhabitats. HOBO data loggers – leaf-litter, floodplain and terra firme forest.
Modeling warming predicts a physiological threshold for the extinction of the living fossil frog *Calyptocephalella gayi*	VIDAL et al. 2017	Measured only during critical limits test	Average daily maximum air temperature (T_{a1}) 10 cm below water surface; mean annual air temperature (external, T_{a2}): data from environmental monitoring station of the Ministry of Agriculture of Chile. Both used for T_{set} tests.	-	Average daily maximum temperature as environment temperature (Ta). Used for T_{set} tests.
Divergence of thermal physiological traits in terrestrial breeding frogs	VON MAY et al., 2017	Measured only during critical limits test	Maximum air temperature estimated for altitudinal gradient through regression	-	-

along a tropical elevational gradient			of average daily temperature vs a elevation of 4 metereological stations in Peru from 520 to 3845 m.a.s.l.		
Water loss and temperature interact to compound amphibian vulnerability to climate change	LERTZMAN-LEPOFSKY et al. 2020	-	Air temperature obtained every hour from the nearest SNOTEL climate station to expedition sites.	-	-
Thermal tolerance and the importance of microhabitats for Andean frogs in the context of land-use and climate change	GONZÁLEZ-DEL-PLIEGO et al. 2020	Measured only during critical limits test	-	-	Bioclimatic variables: monthly average maximum temperature and annual precipitation from WordlClim for 2070 (Fick and Hijmans 2017).
Inter-population variation in thermal sensitivity of the tropical toad *Duttaphrynus melanostictus*, across a small spatial scale in Sri Lanka	ALGIRIYAGE, JAYAWEERA AND WIJESINGHE 2020	Measured only during critical limits test and performance.	-	-	Maximum and minimum monthly temperatures, mean maximum and minimum monthly temperatures through metereological department in Sri Lanka
The body temperature of active desert anurans from hyper-arid environment of South America: The reliability of WorldClim for predicted body temperatures in anurans	SANABRIA; QUIROGA 2019	T_b in activity – data from literature and through a catheter TES TP-K01 coupled with a digital thermometer (TES 1312)	-	Measured through a TES TP-K03 substrate probe.	Seven Worldclim variables: mean temperature of the last 50 years, maximum temperature of the warmest month, minimum temperature of the coldest month, mean temperature of the wettest quarter, mean temperature of the driest quarter, mean temperature of the warmest quarter and mean temperature of the coldest month.
An efficient and inexpensive method for measuring long-term thermoregulatory behavior	SAUER; SPERRY; ROHR 2016	Measured under lab condition during T_{set} test: infrared thermometer Extech® (acc: ± 2% do rdg <932 ° F)	-	Infrared thermometer Extech® (acc: ± 2% do rdg <932 ° F) used at the nearest distance from the animal under laboratory conditions.	-

Acclimation temperature effects on locomotor traits in adult aquatic anurans (*X. tropicalis* and *X. laevis*) fromdifferent latitudes: possible implications for climate change	PADILLA et al. 2019	-	-	-	-
Thermal niche predicts tolerance to habitat conversion in tropical amphibians and reptiles	FRISHKOFF; HADLY; DAILY, 2015	Estimated from the water temperature measured prior to performance test.	-	-	WorldClim variables: annual mean temperature, annual precipitation, temperature annual range and precipitation seasonality.
Impact of global warming at the range margins: phenotypic plasticity and behavioral thermoregulation will buffer an endemic amphibian	RUIZ-ARAVENA et al. 2014	Measured only during performance test.	-	-	Measured only inside the critical limits test chamber through a HOBO data logger.
Tropical amphibians in shifting thermal landscapes under land-use and climate change	NOWAKOWSKI et al. 2017	NicheMapR to estimate maximum T_bs.	Microclimate data through data loggers coupled to agar models: maximum and minimum air temperature.	-	Projected diurnal temperature in six vegetation cover types through MarkSim software.

Table 3 – Studies that performed preferred temperature tests (T_{set}).

Studies	Authors	Measurement device	Gradient	Acclimation temperature	Test period
Intraspecific geographic variation in thermal limits and acclimatory capacity	BARRIA; BACIGALUPE 2017	Infrared thermometer (UEi INF155 Scout I)	From 10°C to 30°C	Either 10°C or 20°C for two weeks for each temperature.	After first acclimation, T_bs were registered 45min after being in the gradient. Later, individuals were exposed to a second acclimation temperature and the procedure was repeated.
Hindered and constrained: limited potential for thermal adaptation in post-metamorphic and adult *Rana temporaria* along elevational gradients	ENRIQUEZ-URZELAI et al. 2018	Infrared thermometer (± 1.8% acc. IR-750 Amprobe)	From 7°C to 30°C	14°C for at least a week.	150min: 30min for acclimation and 120min for measurements, performed every 15 minutes.
An efficient and inexpensive method for measuring long-term thermoregulatory behavior	SAUER; SPERRY; ROHR 2016	Infrared thermometer (±2% acc. Extech® High Temperature)	From 12°C to 33°C)	Not performed	T_bs registered every fur hours, five days a week: three weeks total.
Impact of global warming at the range	RUIZ-ARAVENA	Infrared thermometer	From 10°C	Exposure at 10°C,	After first acclimation, T_bs were registered

margins: phenotypic plasticity and behavioral thermoregulation will buffer an endemic amphibian	et al. 2014	(UEi INF155 Scout I)	to 30°C	20°C, e 25°C for two weeks each for every temperature.	45min after being in the gradient. After termal traits measurements, procedure was repeated for the second and thrid temperatures.

Table 4 – Studies that performed Critical Thermal Limits test. * (Data from ENRIQUEZ-URZELAI, 2018).

Studies	**Authors**	**CT$_{max}$?**	**CT$_{min}$?**	**Moment individual reaches critical limits**	**Tempo da perda de estímulo de resposta**	**Measurement device**	**Warming/Cooling rate**
Intraspecific geographic variation in thermal limits and acclimatory capacity	BARRIA; BACIGALUPE 2017	Yes	Yes	Loss of righting response	After 1 minute	HOBO data logger (termorregulated bath temperature)	0,8°C/min
Thermal Physiology, Disease, and Amphibian Declines on the Eastern Slopes of the Andes	CATENAZZI; LEHR; VREDENBURG, 2014	Yes	No	Loss of righting response	After 5 seconds	Quick-recording thermometer	0,6-0,8° C/min
Hindered and constrained: limited potential for thermal adaptation in post-metamorphic and adult *Rana temporaria* along elevational gradients	ENRIQUEZ-URZELAI et al. 2018	Yes *	Yes*	Loss of righting response	After 10 touches at individuals' urostyle	Quick-recording thermometer	Not mentioned
Temperature dependence of locomotor performance in the tropical clawed frog, *Xenopus tropicalis*	HERREL; BONNEAUD 2012	Yes	Yes	Loss of righting response	Not mentioned	not mentioned	1°/10min (0.1/min)
Thermal physiological traits in tropical lowland amphibians: Vulnerability to climate warming and cooling	VON MAY et al. 2019	Yes	Yes	Loss of righting response	After 5 seconds	Quick-recording thermometer	1°C/min
Modeling warming predicts a physiological threshold for the extinction of the living fossil frog *Calyptocephalella gayi*	VIDAL et al. 2017	Yes	No	Behavioral change (not specified)	Not mentioned	Laser thermometer	not mentioned
Divergence of thermal physiological traits in terrestrial breeding frogs along a tropical elevational gradient	VON MAY et al. 2017	Yes	Yes	Loss of righting response	After 5 seconds	Quick-recording thermometer	1°C/min
Thermal tolerance and the importance of microhabitats for Andean frogs in the context of land-use and climate change	GONZÁLEZ-DEL-PLIEGO et al. 2020	Yes	No	Loss of righting response	After 5 seconds	Two-channel digital thermometer (K-type; resolution: 0.1°C, acc. ±0.1°C)	0,45°C/min
Thermal niche predicts tolerance to habitat conversion in tropical amphibians	FRISHKOFF; HADLY; DAILY,	Yes	Yes	Loss of righting response	Not mentioned	Not mentioned	0,2°C/min

and reptiles	2015						
Impact of global warming at the range margins: phenotypic plasticity and behavioral thermoregulation will buffer an endemic amphibian	RUIZ-ARAVENA et al. 2014	Yes	Yes	Loss of righting response	After 1 minute	HOBO data logger (termorregulated bath temperature)	0,8°C/min
Tropical amphibians in shifting thermal landscapes under land-use and climate change	NOWAKOWSKI et al. 2017	Yes	No	Loss of righting response	after 5 seconds	Thermocouple	0,5°C/min

Subtitles: *Data from ENRIQUEZ-URZELAI et al. 2018

Six studies performed locomotor performance tests for the designing of TPCs (HERREL; BONNEAUD, 2012; RUIZ-ARAVENA et al., 2014; FRISHKOFF; HADLY; DAILY, 2015; ENRIQUEZ-URZELAI et al., 2018; PADILLA et al., 2019; ALGIRIYAGE; JAYAWEERA; WIJESINGHE, 2020). Although there are different ways to perform this task, some criteria must be followed in order to provide a TPC design properly. It is strongly recommended that each individual should be tested under at least five different temperatures (ANGILLETTA, 2009), which ideally should be more similar to those experienced by the species in their habitat (TAYLOR et al., 2020). Considering this criterion, five studies met the expectations with at least five categorized temperatures (ALGIRIYAGE; JAYAWEERA; WIJESINGHE, 2020; ENRIQUEZ-URZELAI et al., 2018; FRISHKOFF; HADLY; DAILY, 2015; HERREL; BONNEAUDHERREL; BONNEAUD, 2012; Ruiz-Aravena et al., 2014), while only one tested two species under two temperatures (PADILLA et al., 2019). Temperature adjustments are usually performed in heating or cooling chambers.

Among assessed studies, we observed different methods for quantifying performance and establishing TPCs, including maximum exertion capacity, jump distance, jump force, maximum performance capacity, maximum jump speed, and contact time. For maximum exertion capacity, researchers recorded time and distance covered by the animal along a circular track until exhaustion or lack of righting response. In jump distance, they recorded either the distance an individual covered over a given period or the number of times the animal jumped. Jump force was recorded with a custom force plate that registered the difference between the output before and after the animal left the plate or the strongest among a group of performed jumps. Maximum performance capacity is measured through individuals' maximum swimming speed and acceleration (HERREL; BONNEAUD, 2012). Maximum jump speed is measured by the number of jumps during a five-minute period, also quantifying average jump distance (RUIZ-ARAVENA et al., 2014). Finally, contact time is characterized by the time between a touching stimulus at the urostyle and the exact moment the individual's feet left the platform (ALGIRIYAGE; JAYAWEERA; WIJESINGHE, 2020). Some of the articles addressed multiple tests in order to quantify performance. Further details on the different approaches are displayed in Table 5.

Hydration level is a crucial variable when considering anurans' thermal performance once these animals are highly sensitive to desiccation (PREEST; POUGH, 1989; WINTER et al., 2016). Among the analyzed studies, though every single one kept anurans in water-filled recipients prior to testing, none of them quantified hydration level under any temperature.

Table 5 – Studies that performed locomotor performance tests to build TPCs and performance measurement.

Studies	Authors	Performance measurement method
Hindered and constrained: limited potential for thermal adaptation in post-metamorphic and adult *Rana temporaria* along elevational gradients	ENRIQUEZ-URZELAI et al. 2018	Jumping distance for 5 minutes at a circular track (1m diameter) covered with moist soil.
Temperature dependence of locomotor performance in the tropical clawed frog, *Xenopus tropicalis*	HERREL; BONNEAUD 2012	**Maximum exertion capacity:** Time and distance spent by the individual at a circular track (3m diameter) until its exhaustion. Exhaustion: loss of righting response. **Maximum performance capacity:** Maximum swimming speed and acceleration.
Inter-population variation in thermal sensitivity of the tropical toad *Duttaphrynus melanostictus*, across a small spatial scale in Sri Lanka	ALGIRIYAGE; JAYAWEERA; WIJESINGHE 2020	**Contact time:** time between an urostyle touch and the feet leaving the platform. **Jumping distance:** distance after two jumps. **Jumping force:** Difference between tension before and after a jump through a force plate platform.
Acclimation temperature effects on locomotor traits in adult aquatic anurans (*X. tropicalis and X. laevis*) fromdifferent latitudes: possible implications for climate change	PADILLA et al., 2019	**Maximum exertion capacity:** (only for *X tropicalis*): Time spent by the individual at a circular track (3m diameter) until its exhaustion. Exhaustion: loss of righting response. **Maximum jump force:** Strongest jump in two session of three or five jumps registered through a force plate platform.
Thermal niche predicts tolerance to habitat conversion in tropical amphibians and reptiles	FRISHKOFF; HADLY; DAILY, 2015	**Longest jump distance:** Most distant jump in four jumps performed by an individual at a determined temperature. First jump distance also considered.
Impact of global warming at the range margins: phenotypic plasticity and behavioral thermoregulation will buffer an endemic amphibian	RUIZ-ARAVENA et al. 2014	**Maximum jumping speed following NAVAS et al 2007:** Number of jumps performed during five minutes, Mean speed and distance registered.

Other significant variables

Several other variables may be especially useful and informative when it comes to amphibians’ thermal physiology and their vulnerability to climate change. Those variables may include operative temperature (T_e, temperature an animal that does not thermoregulate may reach in an environment), minimum and maximum voluntary temperature (VT_{min} and VT_{max}, coldest and hottest temperatures an animal is capable of tolerating and retreats to avoid temperatures beyond its tolerance, panting and gaping threshold (T_{pant} and T_{gape}, *i.e.* elevated T_bs where individuals rapidly breathe or gapes to increase evaporative cooling, warming tolerance (WT, difference between CT_{max} and active field T_b), thermal safety margin (TSM, the difference between $CT_{max}/VT_{max}/T_{pant}$ and environment air temperature; TAYLOR et al., 2020).

None of the assessed articles recorded VT_{max}, VT_{min}, T_{pant} or T_{gape}, although two studies may have included the rate evaporative water loss (EWL), a variable which approximates to T_{pant} and T_{gape} since it is related to organisms’ hydration level (RUIZ-ARAVENA et al., 2014; LERTZMAN-LEPOFSKY et al., 2020). Nevertheless, differently from T_{pant} and $T_{gape,}$ EWL aims to quantify organisms’ water loss using agar model simulations by inserting those models in the microhabitat these animals operate (RUIZ-ARAVENA et al., 2014; LERTZMAN-LEPOFSKY et al., 2020). The higher the T_b, the higher the EWL, which, in turn, increases the rate of evaporative cooling (ROME; STEVENS; JOHN-ALDER, 1992). These variables have been suggested to provide ecologically more expressive information on limitations when compared to CT_{min} and CT_{max}.

Regarding thermal physiology tests, we propose a sequence of approaches. Upon individuals’ observation, Tbs should be assessed in the field, alongside Ta and Ts - important environmental variables - in order to obtain more reliable measures (Taylor et al., 2020). We also recommend using devices to capture Te in microhabitats used by interest species, when possible. Laboratory tests should encompass Tset tests and the calculation of variables such as VTmax and VTmin. Researchers should ponder whether to include critical limits (CTmin and CTmax) and performance tests when conducting Tset tests. Due to the potential thermal stress that these approaches may cause to the organisms, it is advisable to opt for the most appropriate approach based on the study's goal. When performing critical limits, it is pertinent to calculate variables such as WT and TSM, although the latter may be calculated through VTmax instead of CTmax. Furthermore, a somewhat neglected yet crucial variable for anuran studies is water loss or hydration level, given the high susceptibility of these organisms to dehydration (WINTER et al., 2016).Considering the factors discussed in this section, we believe an integrative methodology such as mechanistic Ecological Niche Modelling, in which researchers may use climatic, microclimatic and physiological variables tested either in the field or under laboratory conditions, would be the most appropriate approach to obtain substantially informative data for predictions on climate change impacts on anurans.

1.3.2 *Criteria for studied families' selection*

Amphibians make great models for thermal physiology studies due to their high diversity of species and physiological needs, their life history, the different habitats that they may occupy, and their ectothermy (TAYLOR et al., 2020). The choice of a particular study group may occur due to its abundance, distribution in a particular location, easy handling, among others (*e.g.*, FRISHKOFF; HADLY; DAILY, 2015; RUIZ-ARAVENA et al., 2014). In this section, we direct attention to the families assessed with the most frequency among studies as well as the choice criteria we identified.

Studies assessed 20 families in total, from which the most cited were Bufonidae (n=6), Leptodactylidae and Strabomantidae (n=5), and Hylidae (n=4). The Ranidae family was also cited as a study group by four studies. However, one of the studies did not test on living animals but rather at agar models based on the species *Rana cascadae* (LERTZMAN-LEPOFSKY et al., 2020). The remaining families were assessed in one or two studies (Table 6).

Species distribution was considered a valid reason for species chosen by ten studies, either due to their wide or narrow distribution range. VIDAL et al. (2017) reported the choice of a species from the Calyptocephalidae family that presents a relictual distribution, *i.e.* a more reduced distribution than in the past. In other study, (CATENAZZI; LEHR; VREDENBURG, 2014) also reported the choice of species due to their narrow distribution. In contrast, three studies selected widely distributed species as study groups (RUIZ-ARAVENA et al., 2014; BARRIA; BACIGALUPE, 2017; ALGIRIYAGE; JAYAWEERA; WIJESINGHE, 2020). Further, six studies include species that are distributed along an altitudinal gradient, which might have also been considered as a criterion (FRISHKOFF; HADLY; DAILY, 2015; VON MAY et al., 2017; ENRIQUEZ-URZELAI et al., 2018; SANABRIA; QUIROGA, 2019; ALGIRIYAGE; JAYAWEERA; WIJESINGHE, 2020; GONZÁLEZ-DEL-PLIEGO et al., 2020). These likely criteria are valid for a vast set of reasons. Widely distributed species are satisfactory models to evaluate climate change impact because they allow comparison among climatic and thermal physiology variables in areas with different climatic regimes (BROWN, 1995). A similar variety of conditions may be obtained when species are distributed along an altitudinal gradient since habitats at different mountain ranges are considerably heterogeneous (CORDIER et al., 2020). In turn, species restricted in space may work as more appropriated and adherent models since they are exposed to a narrower variety of thermal conditions, *i.e.* these species are more specialized and therefore comparatively more vulnerable to changes (JOHNSON, 1998; ISAAC et al., 2009). We could also establish a parallel when we assessed the species' diversity of habitats, which preferences could be more generalist, occupying habitat with different structures, or specialist, occupying a specific type of habitat (*e.g.*, exclusively terrestrial or aquatic, RUIZ-ARAVENA et al., 2014; BARRIA; BACIGALUPE, 2017; NOWAKOWSKI et al., 2017; VIDAL et al., 2017). Species with a wider distribution may occur in areas where habitat structures are more heterogeneous and thus, be adapted to a greater range of climatic conditions (JOHNSON, 1998; ISAAC et al., 2009). In contrast, specialists of a particular habitat – especially those tropical ones restricted to top of mountains – can also make good models because they are more vulnerable due to the climatic stability in the tropics (JANZEN, 1967).

We identified the phylogenetic relationship as a criterion for the choice of families in five studies. Four of these studies use either one species (CATENAZZI; LEHR; VREDENBURG, 2014) or highly related species, likely in order to decrease phylogenetic effects when evaluating thermal differences between them (FRISHKOFF; HADLY; DAILY, 2015; VON MAY et al., 2017; PADILLA et al., 2019). The remaining study decided on the opposite approach by assessing species from eight different families through phylogenetic comparative methods to quantify differences in strategies to deal with predicted change in climate (VON MAY et al., 2019).

Table 6 – Families selected by the studies.

Studies	Authors	Família(s) de interesse
Intraspecific geographic variation in thermal limits and acclimatory capacity	BARRIA; BACIGALUPE 2017	Leptodactylidae
Thermal Physiology, Disease, and Amphibian Declines on the Eastern Slopes of the Andes	CATENAZZI; LEHR; VREDENBURG, 2014	Strabomantidae
Hindered and constrained: limited potential for thermal adaptation in post-metamorphic and adult *Rana temporaria* along elevational gradients	ENRIQUEZ-URZELAI et al. 2018	Ranidae
Temperature dependence of locomotor performance in the tropical clawed frog, *Xenopus tropicalis*	HERREL; BONNEAUD 2012	Pipidae

Thermal physiological traits in tropical lowland amphibians: Vulnerability to climate warming and cooling	VON MAY et al. 2019	Aromobatidae Bufonidae Dendrobratidae Hylidae Leptodactylidae Microhylidae Phyllomedusidae Strabomantidae
Modeling warming predicts a physiological threshold for the extinction of the living fossil frog *Calyptocephalella gayi*	VIDAL et al. 2017	Calyptocephalellidae
Divergence of thermal physiological traits in terrestrial breeding frogs along a tropical elevational gradient	VON MAY et al., 2017	Craugastoridae
Water loss and temperature interact to compound amphibian vulnerability to climate change	LERTZMAN-LEPOFSKY et al. 2020	Ranidae
Thermal tolerance and the importance of microhabitats for Andean frogs in the context of land-use and climate change	GONZÁLEZ-DEL-PLIEGO et al. 2020	Strabomantidae
Inter-population variation in thermal sensitivity of the tropical toad *Duttaphrynus melanostictus*, across a small spatial scale in Sri Lanka	ALGIRIYAGE; JAYAWEERA; WIJESINGHE 2020	Bufonidae
The body temperature of active desert anurans from hyper-arid environment of South America: The reliability of WorldClim for predicted body temperatures in anurans	SANABRIA; QUIROGA 2019	Bufonidae Ceratophyridae Leptodactylidae Odontophrynidae
An efficient and inexpensive method for measuring long-term thermoregulatory behavior	SAUER; SPERRY; ROHR 2016	Bufonidae Hylidae
Acclimation temperature effects on locomotor traits in adult aquatic anurans (*X. tropicalis* and *X. laevis*) from different latitudes: possible implications for climate change	PADILLA et al. 2019	Pipidae
Thermal niche predicts tolerance to habitat conversion in tropical amphibians and reptiles	FRISHKOFF; HADLY; DAILY, 2015	Craugastoridae
Impact of global warming at the range margins: phenotypic plasticity and behavioral thermoregulation will buffer an endemic amphibian	RUIZ-ARAVENA et al. 2014	Leptodactylidae
Tropical amphibians in shifting thermal landscapes under land-use and climate change	NOWAKOWSKI et al. 2017	Bufonidae Craugastoridae Centrolenidae Dendrobatidae Eleutherodactylidae Hylidae Phyllomedusidae Ranidae Strabomantidae

The abundance of the chosen species is also likely to have influenced their choice for the studies. Two studies mention species that are abundant in the study areas (VON MAY et al., 2017; GONZÁLEZ-DEL-PLIEGO et al., 2020). Testing on these species may be a good choice in order not to harm their survival and perpetuation since they are highly available.

Dispersal ability, or vagility, was also mentioned in three studies (CATENAZZI; LEHR; VREDENBURG, 2014; RUIZ-ARAVENA et al., 2014; VON MAY et al., 2017). In general, amphibians display low vagility, which renders them unable to move over long distances (BLAUSTEIN; WAKE; SOUSA, 1994). In one study, the authors mention that the studied population inhabits an oasis in the desert, making dispersion practically impossible (RUIZ-ARAVENA et al., 2014).

The lack of data on the body temperature of desert anurans seems to have been the main motivation of SANABRIA; QUIROGA (2019), who sought to use climate variables to predict these body temperatures. The use of agar models to simulate physiological characteristics is an effective option since such models are able to represent the thermal characteristics and permeability of amphibian skin (NAVAS; ARAUJO, 2000). By exploring this option, researchers may avoid performing certain tests on living animals, which may have been a potential motivation in the study carried out by LERTZMAN-LEPOFSKY et al. (2020), which used these models in four different conditions of exposure to moisture and light.

1.3.3 *Hypotheses tested and resolutions*

Hypotheses are fundamental in a scientific study, once they present proposition to explain causes of an event (in the present case, a biological event). Thus, these hypotheses are investigated regarding their effectiveness in representing cause-effect relationships between a proposed idea and an observed phenomenon in nature (GOTELLI; ELISSON, 2011). In this section, we identify categories of hypothesis explored by the studies about the effects of climate change on anurans' thermal physiology and comment on their basis and results.

Distribution-based

Environmental stability and narrow TPCs (HERREL; BONNEAUD 2012) – This hypothesis assumes that species inhabiting stable environments tend to display narrow TPCs, and, therefore, are vulnerable to climate change (WILSON; JAMES; JOHNSTON, 2000; NAVAS; GOMES; CARVALHO, 2008). This study investigates the thermal sensitiveness of *Xenopus tropicalis* performance through maximum exertion capacity (MEC) and maximum performance (MP). Results indicated locomotor performance sensitiveness to temperature in regards to jump time until exhaustion (MEC variable) and both maximum speed and acceleration (MP variables). Both TPC and raw data suggest optimal temperature and performance breadth were narrower for MEC variables when compared to MP variables. Thus, the study species presented a narrow TPC, which corroborates the tested hypothesis that tropical species – inhabiting stable climatic areas – would be more thermally sensitive than temperate species (NAVAS; GOMES; CARVALHO, 2008).

Reduced plasticity in low-latitude species (RUIZ-ARAVENA et al., 2014) – This hypothesis considered that low-latitude species might have higher maximum critical limits and lower plasticity for this trait, therefore being rendered more vulnerable to climate change.

Authors assessed individuals from a population of *Pleurodema thaul* located in an oasis in Carrera Pinto, Chile, in order to investigate whether they had plasticity for any thermal trait as well as any level of thermoregulation. Results revealed that individual acclimated at 20°C and 25°C displayed larger thermal performance breadth, which dislocated their CT_{max} to a higher degree. Results indicate that *P. thaul* had plasticity in TPC and some thermoregulatory behavior, suggesting the species ability of resisting unfavorable climatic conditions.

Climatic variability (BARRIA; BACIGALUPE 2017 and PADILLA et al. 2019) **–** Two studies explored the hypothesis that suggests that species living in higher latitudes would display larger thermal tolerance and better acclimation ability due to a greater climatic variability in these areas.

BARRIA; BACIGALUPE (2017) assessed thermal tolerance and metabolic rate in populations from two different areas in Chile, El Caulle (40°39'S, 72°10’W) and Carrera Pinto (27°06'S, 69°53’W). Results revealed significant geographical variation with the higher latitude population displaying lower CT_{min} and lower latitude population presenting higher CT_{min}, thermal tolerance, and metabolic rate. The population located in Carrera Pinto had broader thermal tolerance than El Caulle population, which opposed to the climatic variability hypothesis (GASTON; CHOWN, 1999). Authors argue that the broad thermal variation of Carrera Pinto may justify the fact that the hypothesis could not be confirmed. Only CT_{max} acclimation responded accordingly, with higher values for El Caulle (CHOWN; GASTON; ROBINSON, 2004). The acclimation response rate was low for both populations, which suggests that the species’ ability to adjust thermally may be insufficient to endure future climatic scenarios.

PADILLA et al. (2019) investigated locomotor performance under different acclimation and test temperatures of two phylogenetically related species, *Xenopus tropicalis,* and *X. laevis*, from different areas (Cameroon and France, respectively). This study investigated an additional hypothesis that indicates the complete opposite of the initial one: tropical species that inhabit stable areas would present better acclimation ability (SEEBACHER; WHITE; FRANKLIN, 2015).

Both acclimation and test temperatures influenced *Xenopus tropicalis* maximum exertion capacity (MEC), and this species had greater resistance to lower temperatures. Burst performance, measured through jump force for this species increased under lower acclimation and test temperatures. For *X. laevis*, only burst performance was tested and jump force was greater under lower test temperatures, regardless of acclimation temperatures.

Results obtained in this study indicate that *X. tropicalis* exhibited better acclimation ability when compared to the temperate species, *X. laevis*. This opposes to Janzen's hypothesis (JANZEN, 1967) that postulates that tropical areas' stability renders species more vulnerable but confirms the additional hypothesis tested. However, authors do not eliminate the possibility that *X. tropicalis* may have its performance restrained under high temperatures, once individuals' physical resistance was lower under high acclimation temperatures. *Xenopus laevis* did not exhibit any influence from different acclimation temperatures on its performance, suggesting a higher vulnerability to climate change. Finally, the study argues that differences observed in the acclimation of the two species may have been influenced by the fact that *X. tropicalis* was sampled at different ponds while *X. laevis* was sampled at a sing pond, presenting lower genetic variability and, therefore, responding differently to acclimation temperatures.

Host thermal sensitivity and lower plasticity of upper critical limits in higher elevations (CATENAZZI; LEHR; VREDENBURG 2014 and VON MAY et al. 2017). – Although not directly related to pathogen infection, the first hypothesis discussed by CATENAZZI; LEHR; VREDENBURG takes this name, postulating that increases in temperature along an altitudinal gradient would drive population declines, *i.e.* species in high elevation would be more sensitive to heating. In order to test that, the study quantified heat sensitivity (Maximum daily average – CT_{max}) and warming tolerance (CT_{max} – annual air temperature) of 15 species in different altitude ranges (from 1220 to 3800 m.a.s.l.) in Parque Nacional Manu, in Peru.

Results found that species from lowlands were more sensitive to warming than higher elevation species, and therefore is in discordance with this hypothesis and previous findings that indicate most threatened species inhabit high elevations both in the tropics (IUCN, 2013) and Peru (VON MAY et al., 2008). Despite the great thermal variability of the Andes, previous work has reported that average warming tended to decrease in altitudes over 3500 m (VUILLE et al., 2003; OHMURA, 2012). This region encompasses varied thermal environments that would provide anurans with a range of microhabitat to be explored as thermal refuge (HUEY et al., 2009).

A similar hypothesis was explored by VON MAY et al. (2017) where it suggests that anurans' upper critical limits (CT_{max}) display lower plasticity in more elevated areas. The study assessed CT_{max} and CT_{min} of 22 Andean terrestrial species in Peru, as well as life history traits, elevation and operative warming tolerance (OWT). Results revealed that body size and mass and elevation range did not explain variation in neither CT_{min} nor CT_{max}. However, critical thermal limits were influenced by elevation (maximum, minimum and average) and OWT when coupled together. Both critical limits tended to decrease with crescent elevation, whereas OWT tended to increase. This suggests that individuals of species from lowlands would be more threatened by temperature as these individuals live in environments where thermal conditions approach their upper limits (HUEY et al., 2009; SUNDAY et al., 2014). Phylogenetic analysis indicated that related species did not present significant similarities for either CT_{max} or CT_{min}, suggesting that changes in these traits may have happened in a short evolutionary period.

"Hotter is better" and "specialist-generalist" (ENRIQUEZ-URZELAI et al. 2018) – The study examined the two trade-off hypotheses by using the eight populations of the species *Rana temporaria* from different altitude ranges in Spain. The first hypothesis defends the assumption that species from warmer areas perform better due to temperature's effects on molecules and chemical reactions (ANGILLETTA; HUEY; FRAZIER, 2010). The second postulates that thermal generalists display low maximum performance but perform satisfactorily in a vast range of temperatures, while specialist would had the opposite behavior (HUEY; KINGSOLVER, 1989; ANGILLETTA, 2009). The study also investigated seasonal variation as it varies differently according to latitude and altitude (DAHL et al., 2012; MIKOLAJEWSKI; DE BLOCK; STOKS, 2015). Thus, it may therefore influence the thermal adaptation process (CONOVER; SCHULTZ, 1995; DAHL et al., 2012).

The study aimed to investigate how locomotor performance's thermal sensitivity would vary geographically in the eight populations from different altitude ranges. Authors performed an analysis through a Template Mode of Variation (TMV) to detail TPC in three categories: one focused on time (seasonal activity windows, SAWs; slow-fast), one focused on temperature (hot-cold), and the other focused on the generalist-specialist *trade-off* (IZEM; KINGSOLVER, 2005). Specialist-generalist *trade-off* explained most of the variation in performance's thermal sensitivity with populations displaying broad TPC. The 'hotter is better' hypothesis was also corroborated, to a lesser degree, with better performance at higher optimal temperatures. Post-metamorphic stages displayed higher optimal temperatures when compared to adults, reflecting differences in thermal biology at different life stages (KINGSOLVER et al., 2011; KLOCKMANN; GÜNTER; FISCHER, 2017; LOCKWOOD; GUPTA; SCAVOTTO, 2018). In addition, environmental traits as well as altitude did not significantly influence thermal traits.

Finally, the population with narrower SAWs had narrower TPCs, higher optimal temperatures and maximum performance. According to the author, this suggests specialization in higher temperatures due to the efficient use of food resources, and, in a future warming scenario, these species could benefit by becoming active for shorter periods.

Colder temperatures favor better performance in lower temperatures (ALGIRIYAGE; JAYAWEERA; WIJESINGHE, 2020) – This hypothesis postulates that climatic differences between two areas implicates in changes in different anuran populations of the same species, even if these areas are geographically close. The study assessed the thermal locomotor performance and thermal safety margin (TSM: optimal temperature – environmental temperature) of the species *Duttaphrynus melanosticus* from two different areas in Sri Lanka (Nuwara-Eliya and Polonnaruwa) under different temperatures.

Results confirmed the hypothesis and revealed that the two populations responded differently to temperatures despite their geographical proximity; the warmer area (Polonnaruwa) population performed better at higher temperatures, while the colder area (Nuwara-Eliya) population displayed better performance under cold temperatures. This suggests that altitudinal isolation may have an important role in justifying this divergence. This is an interesting finding since the short distance that separates both populations would not be enough to cause such differences since thermal sensitivity would be conserved evolutionarily (*e.g.,* HERTZ; HUEY; NEVO, 1983; CROWLEY, 1985; JOHN-ALDER; MORIN; LAWLER, 1988). The Polonnaruwa population displayed lower optimal temperature when compared to environmental temperatures and narrow TSM, rendering it vulnerable to a temperature increase (SOMERO, 2010).

Increased amphibian declines during hotter years (VIDAL et al. 2017) – This hypothesis argues that in hotter years, anuran populations would decline due to temperature increases that would surpass their critical limits in case of an extreme climatic event (ZIPPEL; ARK; VALLEY, 2010). The study analyzed thermoregulatory strategies of the anuran *Calyptocephalella gayi*, a vulnerable species from Chile (IUCN) in response to future climatic scenarios.

Results indicated adults and sub-adults have higher tolerance than larvae. However, despite having higher tolerances, the species displayed a limited ability to adapt to climate change short term (GIBBS; BREISCH, 2001; BUCKLEY; EHRENBERGER; ANGILLETTA, 2015), which may not be enough to cope with higher temperatures (KUSANO; INOUE, 2008; PAU et al., 2011). The authors concluded that a significant percentage of adults (47%) and sub adults (40%) would not be able to survive the warming predicted for the next 85 years.

<u>Microhabitat-based</u>

Deforestation favors species submitted to warmer temperatures (FRISHKOFF; HADLY; DAILY, 2015) – This hypothesis defends that species more tolerant to heat are found in warmer areas, and display higher critical limits and perform better at a higher temperature. Moreover, in an altitudinal gradient, the temperature would be a determinant of species association to deforested areas. The study assessed the deforestation tolerance of two species from different areas of Costa Rica, *Craugastor crassidigitus* (found in forested areas) and *C. fiztingeri* (found in deforested areas). The authors performed an Ecological Niche Modelling to understand whether temperature, precipitation, and their variation along the altitudinal gradient would differ between the species. The study also conducted critical thermal limits and locomotor performance to understand their difference in thermal niches.

Results showed that species adapted to heat would be favored in deforested areas and that temperature was the main determinant of this tolerance for both species. Species did not differ significantly in thermal tolerance: there was a tendency for both species to associate with deforested and warm areas as environmental temperature decreased and altitude rose. Nevertheless, *C. fiztingeri* displayed CT_{max} 2°C higher than *C. crassidigitus*, which is concordant with habitat associations: according to findings, pasture leaf-litter displays on average a higher temperature than forest leaf-litter (~3°C).

Vegetation cover buffers the adverse effects of temperature increase (NOWAKOWSKI et al. 2017 and GONZÁLEZ-DEL-PLIEGO et al. 2020) – Two studies tested a similar hypothesis, where species would be threatened by a future thermal stress in case microclimate was not capable of offering enough vegetation cover so that species could maintain their T_b under their CT_{max} (NOWAKOWSKI et al., 2015). Authors believed that the coupled effect of climate change and vegetation cover would play a greater role in lowering the suitable areas than the two variables isolated.

The first study (NOWAKOWSKI et al., 2017) analyzed the relationship between thermal tolerances and both climate change and vegetation cover to understand how sensitive the frog assemblage from Costa Rica would be to these conditions. Results show a 16% reduction in thermal suitable habitats (TSH) in the B1 scenario (low GEE emissions) coupled with forest loss and a 30% reduction in TSH in an A1B scenario (moderate emissions) coupled with moderated change in forest cover. These effects are believed to aggravate if species live under temperatures that surpass their upper limits. When isolated, climate change had an effect on reduction of TSH 1.7 to 4.5 times greater than only the effect of vegetation cover, which suggests climate threat is more harmful than forest loss. When projecting TSH for lower thermal tolerance, authors found the complete loss of a suitable area, and under these conditions, temperatures may already exceed their thermal optimum, rendering them vulnerable to the combined effect of climate change and vegetation loss (WHITFIELD et al., 2007).

The second study sought to investigate whether species from secondary forests would be more vulnerable to temperature increases than primary forests despite their higher CT_{max} and experiment with higher environmental temperature (GONZÁLEZ-DEL-PLIEGO et al., 2020). The study also assessed the microhabitat's potential to buffer the effect of extreme temperatures in different vegetation covers to understand whether they would be enough to protect anurans from climate change effects in the future. Sampling involved fourteen species from the *Pristimantis* genus along primary and both young and old secondary forests in the Colombian Andes and analyses involved assessing their upper thermal limits and exposure to temperature and precipitation (projections for 1970-2000 and all future RCP scenarios for 2070).

Results revealed species' CT_{max} were similar along elevation. Further, species inhabiting secondary forests are more vulnerable to climate warming than primary forests, since the latter display more complexity and forest vegetation density, which buffers the effect of temperature (GONZÁLEZ-DEL-PLIEGO et al., 2016). Authors argue that secondary forests present a lower number of available microhabitats compared to primary ones (GONZÁLEZ-DEL-PLIEGO et al., 2016). Secondary forest species exhibited a lower thermal safety margin, which, in a future scenario may render species vulnerable to warming. Authors conclude that microhabitats are important for reducing exposure to extreme temperatures in the future in case temperature increase exceeds 2°C.

Water loss is as determinant for anuran vulnerability as temperature exposure (LERTZMAN-LEPOFSKY et al., 2020) – This hypothesis postulates that water loss is as relevant as temperature when assessing anuran vulnerability to climate change, and is likely to be even more critical due to their sensitivity to desiccation (TRACY et al., 1993). Therefore, the study assessed the combined effect of both variables using *agar* models in two sub-alpine areas northeast of the USA under four different local conditions: sunny and dry, sunny and wet, shaded and dry, shaded and wet. The goal was to estimate the frequency of which climatic conditions exceed limits of water loss and T_{opt} and to compare the different treatments to discuss possible alternatives for anurans to deal with such conditions. The study used critical limits data for species *Rana cascadae* to predict daily proportion where critical evaporative loss (EWL_{crit}) or T_{opt} were exceeded in each treatment under the current patterns (2002-2012) and future projections under moderate emissions (AIB).

The study revealed a trade-off between temperature and hydration. Empirical lab and field data indicate that amphibians tend to seek moist habitats in order to maintain a suitable hydration level while, in turn, enduring sub-optimal thermal conditions (*e.g.* ANDERSON; ANDRADE, 2017). None of the treatments was capable of eliminating the risk of exceeding thermal and dehydration limits. Despite working as a refuge against thermal and hydric adverse conditions for species, none of the habitats was capable of eliminating the risk of exceeding thermal and dehydration limits. Thus, sub-alpine anurans may not be capable of using behavioral mechanisms that buffer these risks, rendering them vulnerable to climate shifts (KEARNEY, 2013).

The article also highlights the relevance of aquatic environments in avoiding anuran's water loss, especially when projections for 2080 suggested a decrease in aquatic environments because of the premature melting of the ice during winter and severely drier and longer summer (BARTELT; KLAVER; PORTER, 2010; RYAN et al., 2014; LEE et al., 2015). Authors also argue that considering EWL for analyses was essential because results might not have indicated a possibility of individuals exceeding limits in terrestrial environments. Finally, the study predicted that this species activity period could be reduced in 2080 since almost every day that limits would not be exceeded would occur only in October.

Ability of microclimate of predicting T_b better than bioclimatic variables (SANABRIA; QUIROGA, 2019). – This hypothesis explored two possibilities: (1) desert anurans' T_b suffers with change in elevation and that it is closely related to T_s; (2) microclimate data is a better predictor of T_b than macroclimatic data. Eight species and 29 populations were sampled through four ecoregions and altitude ranges, relating T_b (obtained through literature and field sampling) altitude and climate variables (WorldClim), T_s and microhabitat variables.

Species' T_b were influenced by abiotic factors, given the relationship between anuran T_b and environment (BRATTSTROM, 1968; TRACY, 1976). Authors suggest that, because body temperature and T_s were highly related and species are nocturnal, species may present thygmothermic behavior (SANABRIA; QUIROGA; ACOSTA, 2003). Furthermore, species from elevated altitudes displayed lower T_b than lowland species, in accordance with previous studies (FEDER; LYNCH, 1982).

Literature (NAVAS et al., 2013) and field data identified that T_b and T_s were closely related. From the seven selected bioclimatic variables, only BIO5 (Maximum Temperature of Warmest Month) predicted T_b, indicating that macroclimatic variables are inefficient in predicting this variable. Authors conclude that the most appropriate form of producing reliable predicting models is incorporating, along with global climate data, microhabitat data (*e.g.*, KEARNEY; ISAAC; PORTER, 2014; OYAMAGUCHI et al., 2018).

Phylogenetically-based

The one study to explore a phylogenetic hypothesis investigated whether tolerances to heat and cold would be phylogenetically conserved, whether these tolerances evolved faster among species, and whether critical limit (CT_{min} and CT_{max}) variation could be predicted through species life history or morphological characteristics. This investigation could clarify whether the 56 Amazon species assessed in the Estação Biológica Los Amigos, Peru, were vulnerable to predicted heating for lowlands in these areas (VON MAY et al. 2019).

Based on a hypothetical increase of 3°C, the study showed that species that lived in sympatry and occupied the same habitat presented great variability of critical limits (greater than 10°C). This study observed interspecific variation in critical limits for one-third of the species. There was also an interspecific variation for all species in the relationship between heat and cold tolerances, body size (positive effect for CT_{max}, negative effect for CT_{min}), and phylogenetic relationship among taxa. Individuals from the Hylidae and Microhylidae family tolerated higher temperatures while individuals from the Strabomantidae family were more vulnerable to heat.

Critical thermal limits exhibited similar rates of evolutionary change among species, although these traits have different implications for the survival of individuals in adverse climatic conditions. Organisms may become inactive if in contact with thermal conditions reach closely to CT_{min}, while those who are exposed to temperatures close to CT_{max} may face more serious consequences, such as death (BOVO et al., 2018). Only 4% of the species in this study would experience temperatures that would exceed their CT_{max}, while 25% could be affected to a lesser level while 70% would not be as affected by heat. In contrast, the study concluded that around 27% of species presented CT_{min} similar or under 8°C (study area's minimum air temperature), suggesting that 72% may have to rely on some behavioral change strategy in order to move to more suitable areas to deal with low temperatures.

Absent hypothesis

The study performed by SAUER; SPERRY; ROHR (2016) lacks a hypothesis but displays an innovative and different approach. The study reports the development of an apparatus capable of testing and monitoring thermal physiology variables and thermoregulatory behavior long-term, efficiently, financially accessible, validated, and easy to build. Additionally, this apparatus would provide a non-invasive method to measure variables besides being able to control humidity and stress conditions. The study tested individuals from two species, *Anaxyrus terrestris* and *Osteopilus septentrionalis*, and functioned satisfactorily with uniform parameters (temperature, thermal variation, and humidity) throughout test periods. The authors discuss that this apparatus may be adapted to different studies as it allows for different temperature and humidity adjustments that can be modified to accommodate animals' thermal needs. Authors also compared the apparatus to previously built ones (*e.g.*, KLEIN; CONN; KLUGER, 1992; LOURDAIS et al., 2013), and argued that the apparatus in question could provide a wide ecologically relevant thermal gradient. Thus, this proposed methodology could help future studies explore the knowledge in the thermal ecology area.

1.4 Conclusions

By exploring a wide range of hypotheses, choice of study groups, and different techniques and tests, we concluded that two of the articles explored a methodological approach highlighting possible, appropriated and satisfactory methodologies to be carried out during the thermal ecology studies with anurans. Three of the articles reported the likelihood of some species being capable of resisting adverse effects of climate change in the future either through some kind of strategy or because they are somehow adapted to more critical conditions (*e.g.,* favoring of occurrence of certain species adapted to heat in deforested areas). Finally, most studies assessed in this paper revealed some degree of risk to thermal physiology and survival of at least one of the study groups explored due to climate change.

Despite the knowledge provided by literature about climate change effects on anurans, there are still important gaps that persist regarding its effects on their thermal physiology. More studies are necessary to more properly assess the vulnerability of restricted species, especially those unable to move over long distances. Nonetheless, studies also need to explore the differences among populations of widely distributed species more deeply.

Including microclimatic data in analyses is also needed so that we may obtain more reliable and realistic predictions of how climate change will affect the thermal physiology of anurans. Factors to be considered further are thermal performance and the interaction between hydration and temperature to try to reproduce a closer scenario to what would be observed in nature.

Finally, there is a need for a methodological alignment regarding intended studied relationships and variables used for these purposes, although this task is not a simple one. Thus, this qualitative review sheds light on aspects that require attention so that future studies can better explore the impacts of climate change on anurans' thermal physiology.

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2 VULNERABILITY OF A NEOTROPICAL ANURAN ASSEMBLAGE TO CLIMATE CHANGE

(Manuscrito sob revisão de co-autores: AMARAL-ALVES, I. M.; ROCHA C. F. D.; MOTT, T.; TORQUATTO, S.; DIELE-VIEGAS, L. M. **Vulnerability of a neotropical anuran assemblage to climate change.)**

Abstract

The Atlantic Forest faces a biodiversity crisis and one of the factors posing a threat is climate change, which affect several groups of organisms, being particularly hazardous to ectotherms, such as amphibians. In this study, we employ Ecological Niche Modeling to evaluate the vulnerability of an anuran assemblage in Serra da Saudinha, in Alagoas, Brazil. We aim to assess current suitable areas for this assemblage occurrence as well as identify the species accumulation by overlapping scenarios. We performed modelling for past, present and future (SSP 2-4.5 and SSP 5-8.5) conditions. Our results show a higher suitability in the eastern portion of the state within the Atlantic Forest. Suitability seems to have decreased considering Pliocene and Present, tending to an increase in the more optimistic future scenario and significantly reducing in the more pessimistic scenario. The overlapping of past and present models recovered a reduced portion of species accumulation for the assemblage, which proved to be restricted to the central portion of the Atlantic Forest in Alagoas. Species accumulation shows an even greater reduction when considering the overlapping of both future scenarios. We hope these findings can help future studies determine priority areas for conservation also aiming to investigate climate change impacts on amphibian’s survival.

Keywords: Ecological Niche Modelling, climate shifts, Atlantic Forest, thermal suitability.

2.1 Introduction

Over the last decades, declines in anuran diversity have been mainly attributed to direct and indirect effects of habitat loss and climate change (BILLETER et al., 2008; CEBALLOS et al., 2015; GRANT; MILLER; MUTHS, 2020). Earth's temperature is expected to increase by over 1.8°C compared to the pre-industrial period, reaching up to 5.7°C in a more extreme scenario by the end of the century (IPCC, 2023). In addition to changes in global temperatures per se, interactions between climate change and other ecological factors can negatively affect anuran thermal tolerance. For example, it is known that frog thermal tolerance can be affected by anuran infection by pathogens especially fungal infection (GREENSPAN et al., 2017). The increase in the frequency and intensity of extreme temperature events can affect amphibians due to their relatively limited acclimatization capacity and low vagility (SINSCH, 1990; BLAUSTEIN; WAKE; SOUSA, 1994;

DIFFENBAUGH; FIELD, 2013; GUNDERSON; STILLMAN, 2015). Thus, while some generalist species may thrive (RAMALHO et al., 2021), specialist species may not withstand the thermal fluctuations driven by climate change, leading to retractions in their distributions when the adequate environmental conditions for their survival become available only in inaccessible areas (HUGHES, 2000; PARMESAN, 2006; SCHIVO et al., 2019).

Amphibians are a very diverse group, with over eight thousand species distributed around the world (FROST, 2023). Many species are expected to be discovered (Moura and Jetz, 2020), especially in the neotropics (PIMM et al., 2010; MOURA; JETZ, 2020). However, shortfalls such as Linnean (*i.e.*, unknown existing species; LOMOLINO, 2004) and Wallacean (*i.e.*, unknown species geographical distributions; LOMOLINO, 2004) deficits may impose barriers to these new discoveries (HORTAL et al., 2015).

Throughout the years, researchers have been using Ecological Niche Modeling in amphibian studies to investigate the relationship between species and the environment ranging from invasive species' distribution (*e.g.*, GIOVANELLI; HADDAD; ALEXANDRINO, 2007) to the suitability of an area considering different scenarios (*e.g.*, SIQUEIRA; PETERSON, 2002) and their thermal refugia (*e.g.*, CARNAVAL et al., 2009; SOUSA-GUEDES; ARENAS-CASTRO; SILLERO, 2020). Such studies have allowed to better comprehend strategies to mitigate such effects and to help decision-makers implement effective measures and management actions to minimize loss of amphibian biodiversity under climate change (SLATYER; ROSAUER; LEMCKERT, 2007; WIENS et al., 2009; SHOO et al., 2011; FERRAZ et al., 2012).

About 41% of amphibian species are currently threatened with extinction (IUCN, 2023), which characterizes them as the world's most endangered vertebrate group (STUART et al., 2004; WELLS, 2010). Therefore, understanding the impact of climate change on their potential distribution is paramount to develop strategies for their conservation and to allow mitigate the adverse effects of climate change to prevent the loss of frog populations. The present study aimed to understand the distribution patterns of a Neotropical anuran assemblage from an Atlantic Forest remnant and adjacent eucalyptus plantation, assessing its potential vulnerability to climate change through Ecological Niche Modeling. In this sense, our goals were 1) to determine the areas of species accumulation throughout time considering past, present and future climatic conditions; 2) to define thermal suitable areas for the occurrence of the frog assemblage in the present; 3) to identify potentially suitable areas for maintaining the assemblage in the future; and 4) determine priority areas for their conservation.

2.2 Material e Methods

2.2.1 *Study Area*

The state of Alagoas is located within the Pernambuco Center of Endemism (CEP), which encompasses Atlantic Forest areas located north of the São Francisco River (RIBEIRO et al., 2009). This CEP is a biogeographical region of great importance since it is home to several endemic species from different biological groups (TABARELLI; SIQUEIRA-FILHO; SANTOS, 2006; PONTES et al., 2016; FRANÇA et al., 2020; PEREIRA FILHO et al., 2023) and is characterized mainly by lowland, submontane and montane vegetation (*sensu* VELOSO; RANGEL FILHO; LIMA, 1991). Pernambuco Center of Endemism extends along the coastal region of Alagoas (10°25'S) to Rio Grande do Norte (5°18'S) states, and is currently the most devastated among the Centers of Endemism in Brazil (DA SILVA; TABARELLI, 2001; RIBEIRO et al., 2009; BERNARD; MELO; PINTO, 2011).

We defined the focal assemblage based on anuran records for 12 species from six families (Table 1) located in the Serra da Saudinha territory (09° 22' S - 09° 24' S, 35° 43' W - 35° 44' W). This area encompasses circa 1210 hectares (12.1 km^2) distributed among the municipalities of Maceió, São Luiz do Quitunde, Flexeiras, and Messias, in the state of Alagoas, in northeastern Brazil (ALVES et al., 2002). We recovered records from the Herpetological Collection of the Natural History Museum of Alagoas/UFAL. All species recorded for the Serra da Saudinha were considered as coexisting species and therefore included in the assemblage analysis. After defining the focal species, we obtained further occurrence data from the Global Biodiversity Information Facility (GBIF, 2023), the Reference Center for Environmental Information (CRIA, 2023), and the Brazilian Biodiversity Information System (SIBBR, 2022). This search resulted in 31,389 coordinate points from 12 species (Table 2).

Table 1. Anuran assemblage species and their vulnerability according to IUCN.

Species	**IUCN status**
Aromobatidae	
Allobates olfersioides (Lutz, 1925)	VU
Bufonidae	

Rhinella crucifer (Wied-Neuwid, 1821)	LC
Rhinella diptycha (Cope, 1862)	DD
Rhinella hoogmoedi (Caramaschi and Pombal, 2006)	LC
Hylidae	
Boana albomarginata (Spix, 1824)	LC
Dendropsophus minutus (Peters, 1972)	LC
Scinax nebulosus (Spix, 1824)	LC
Leptodactilydae	
Adenomera hylaedactyla (Cope, 1868)	LC
Leptodactylus natalensis (Lutz, 1930)	LC
Physalaemus cuvieri (Fitzinger, 1826)	LC
Odontophrynidae	
Proceratophrys renalis (Miranda-Ribeiro, 1920)	NE
Strabomantidae	
Pristimantis aff. *ramagii* (Boulenger, 1888)	LC

Subtitle: DD: data deficient; LC: least concern; NE – not evaluated; VU – vulnerable.

2.2.2 *Ecological Niche Modelling*

To identify potentially suitable areas for the frog assemblage occurrence, we performed a stacked Ecological Niche Modeling (CALABRESE et al., 2014; ZURELL et al., 2020). The area of interest was delimited for the Atlantic Forest of the Alagoas state, through *shapefiles* available on the Brazilian Institute of Geography and Statistics website (IBGE, 2022)(IBGE, 2022).

Since data available in open databases may contain incorrect records (MEYER; WEIGELT; KREFT, 2016), after downloading data from Gbif, CRIA, and SiBBr, we performed an initial filtering to exclude non-identified or missing records. Further, a more accurate filtering was performed using the "*clean_coordinates*" function of the CoordinateCleaner package (ZIZKA et al., 2019), in the R environment (R CORE TEAM, 2022). We excluded coordinates falling in the sea or in urban areas, centroids of municipalities, states and countries, duplicates, coordinates falling in the same pixel (within a radius of 10km) and those in which latitude and/or longitude were equal to zero. After this sequential filtering, 305 occurrence points for the assemblage were kept. Details on the filtering process for each species are available in Table 2.

Table 2. Occurrences points of each species prior and post filtering**.**

Species	Obtained Occurrences	Occurrences after filtering
Aromobatidae		
Allobates olfersioides	610	2
Bufonidae		
Rhinella crucifer	244	0
Rhinella diptycha	3483	9
Rhinella hoogmoedi	11665	3

Hylidae		
Boana albomarginata	657	1
Dendropsophus minutus	9667	2
Scinax nebulosus	122	1
Leptodactylidae		
Adenomera hylaedactyla	110	6
Leptodactylus natalensis	2273	8
Physalaemus cuvieri	1298	2
Odontophrynidae		
Proceratophrys renalis	488	16
Strabomantidae		
Pristimantis aff. *ramagii*	772	25

Total	31389	305

To determine the environmental space for the modeling under past conditions, bioclimatic layer data were extracted from the Paleoclim database (BROWN et al., 2018) referring to the Late Pliocene (~3.3 million years ago, HILL, 2015) at a resolution of 2.5 arcseconds (~5km²), and imported through the "*rasterstack*" function of the Raster package (HIJMANS et al., 2022). For the determined period, 14 bioclimatic variables are available (BIO1, BIO4, BIO8-BIO19, Table 3). We chose Late Pliocene due to the nearest proximity of this time to the last evolutionary distinction of the anurans that are part of this assemblage (~4 million years ago, JETZ; PYRON, 2018). We performed a Spearman correlation (WISSLER, 1905) to determine the environmental space for the area of interest through which we selected the least correlated variables considering a cutoff of 75% to avoid collinearity, since highly correlated variables would have the same effect in our analysis (DORMANN et al., 2013). The selected variables were the average temperature of the wettest quarter (BIO8), average temperature of the driest quarter (BIO9), precipitation of the driest month (BIO14) and precipitation seasonality (BIO15). The bioclimatic data were aggregated and cut for the state of Alagoas. Subsequently, the occurrence data of the component species from the assemblages were fitted into the region of interest.

Table 3: Bioclimatic variables obtained from the WorldClim database.

Variable	**Description**	**Category**
BIO 1	Annual Mean Temperature	Temperature
BIO 2	Mean Diurnal Range (Mean of monthly (max temp - min temp))	
BIO 3	Isothermality (BIO2/BIO7) (×100)	
BIO 4	Temperature Seasonality (standard deviation ×100)	
BIO 5	Max Temperature of Warmest Month	
BIO 6	Min Temperature of Coldest Month	

BIO 7	Temperature Annual Range (BIO5-BIO6)	
BIO 8	**Mean Temperature of Wettest Quarter**	
BIO 9	**Mean Temperature of Driest Quarter**	
BIO 10	Mean Temperature of Warmest Quarter	
BIO 11	Mean Temperature of Coldest Quarter	
BIO 12	Annual Precipitation	Precipitation
BIO 13	Precipitation of Wettest Month	
BIO 14	**Precipitation of Driest Month**	
BIO 15	**Precipitation Seasonality (Coefficient of Variation)**	
BIO 16	Precipitation of Wettest Quarter	
BIO 17	Precipitation of Driest Quarter	
BIO 18	Precipitation of Warmest Quarter	
BIO 19	Precipitation of Coldest Quarter	

Subtitles: Variables in bold indicate the ones used as selected by the model.

We used three different algorithms to build the models (Bioclim, GLM, and SVM), seeking a consensus of those with the best ability to predict the species' potential distributions. Bioclim is an envelope algorithm consisting only of presence and background data (HIJMANS; GRAHAM, 2006). Generalized Linear Models (GLM) is a statistical algorithm and considers points of presence and pseudo-absences (AUSTIN; MEYERS; DOHERTY, 1994). Finally, the Support Vector Machine (SVM) is a machine learning algorithm that considers presence and background data (SCHÖLKOPF et al., 2001; TAX;

DUIN, 2004). Analyses using the Bioclim model were obtained through the Dismo package (HIJMANS; PHILLIPS; ELITH, 2022); the GLM model was obtained through the "*glm*" function of the Stats package (R CORE TEAM, 2022), and the SVM model through the Kernlab package (KARATZOGLOU et al., 2022).

For modelling evaluation, we performed a training and test set from the random division of the occurrence data with 80% of the data destined for training and 20% for testing. Ten thousand pseudo-absence points were created for the GLM algorithm, and 10 background points were created for every presence point for both Bioclim and SVM, following BARBET-MASSIN et al. (2012). Ten replicas were performed for every algorithm, which were evaluated by calculating the area under the curve (AUC, ELITH et al., 2006), to validate the distribution models. AUC values equal to or less than 0.5 indicate that that replica is not a good model to predict species distribution; values from 0.5 to 0.7 indicate a poor predictive ability; and values above 0.7 are considered satisfactory to validate the models (PETERSON et al., 2001; ARAUJO et al., 2005). In this study, only models with AUC values greater than 0.75 were selected.

Consensus models (GIANNINI et al., 2012) of thermal suitability for species' occurrence were generated by combining the algorithms' selected replicas through their AUC weighted average ("*ensemble*", ARAUJO; NEW, 2007) using the biomod2 package (THUILLER et al., 2022). For suitability maps, pixel values ranged from zero to 1; the closer to 1 (one) the pixel value is, the more suitable that area will be for assemblage.

Further, we overlapped the suitable layers of each species to obtain their intersection area, *i.e.*, the area suitable for the entire assemblage. In doing so, the corresponding pixels of each species were summed up to represent the total suitability for the assemblage (CALABRESE et al., 2014), resulting in a continuous map of suitability.

For the present and future models, we obtained the variables BIO8, BIO9, BIO14 and BIO15 in the resolution of 2.5 arc-seconds through the WorldClim database, considering historical data (average of 1970-2000) and two future scenarios (SSP2-4.5 and SSP5-8.5), in a temporal projection for 2041-2060 (hereafter, 2050). The SSP2-4.5 scenario predicts an increase in global average temperature ranging from 1.6°C to 2.5°C by 2050, with the best estimate stable at 2°C, while the SSP5-8.5 scenario predicts an increase ranging from 1.9°C to 3.0 °C, with the best estimate stable at 2.4°C (IPCC, 2023). We used the global circulation models BCC-CSM2-MR (WU et al., 2018), IPSL-CM6A-LR (BOUCHER et al., 2018) and MIROC6 (SHIOGAMA; ABE; TATEBE, 2019) for future projections since these models perform well in tropical regions (CANNON, 2020).

To determine species accumulation areas, or areas that remained suitable for the assemblage over time, we overlapped past and present models through the product of suitability values. We used the raster generated from this overlapping to establish a relationship between the identified areas of species accumulation and future scenarios to identify potentially conservation areas for the assemblage in a climate change context.

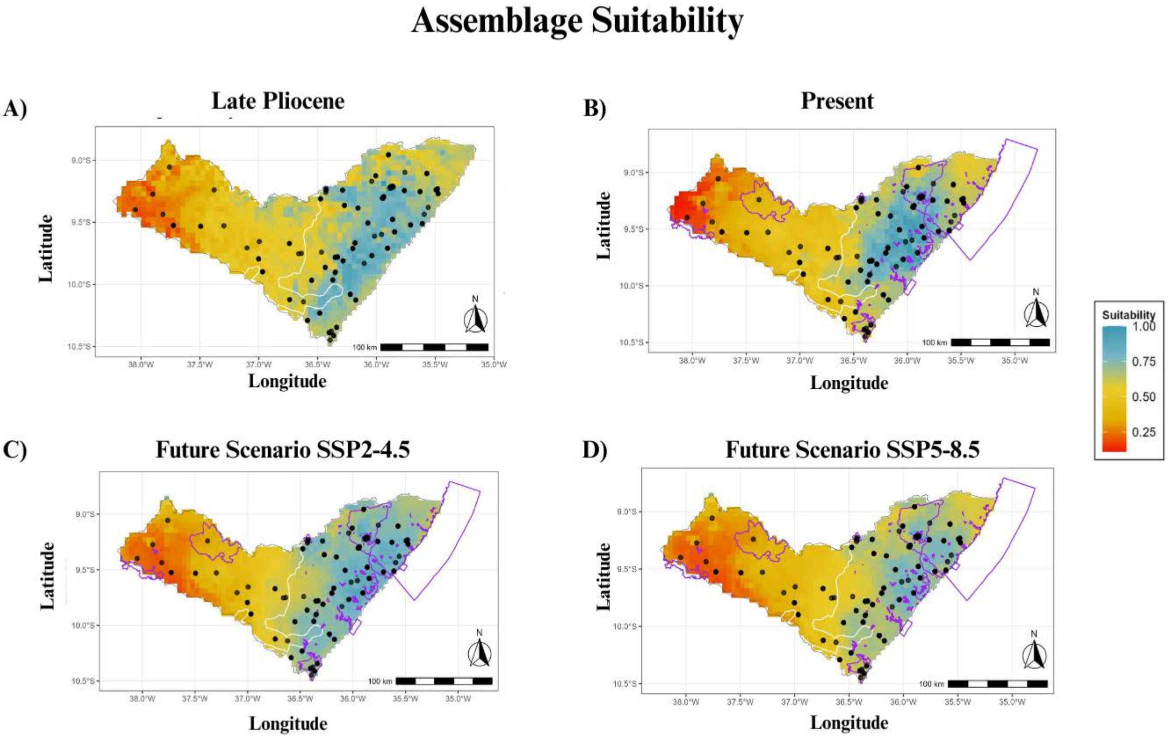
2.3 Results

2.3.1 *Thermal suitability*

Suitable areas for assemblage occurrence are concentrated in Alagoas' eastern portion, predominantly in the Atlantic Forest area. In contrast, suitability tends to decrease in the Caatinga region from the central to the western portion of the state.

Thermal suitability for the frog assemblage seems to have reduced throughout time. It tends to increase in the future considering the SSP 2-4.5 scenario and decrease for SSP5-8.5 (Figure 1). Tendency in such variation is clear for the whole state, for the Atlantic Forest and considering only PAs (Figure 1). Additionally, is important to highlight that Integral Protection PAs presented higher suitability than PAs where the sustainable used is allowed.

Figure 1. - Thermal suitability for the anuran assemblage in the state of Alagoas, Brazil.



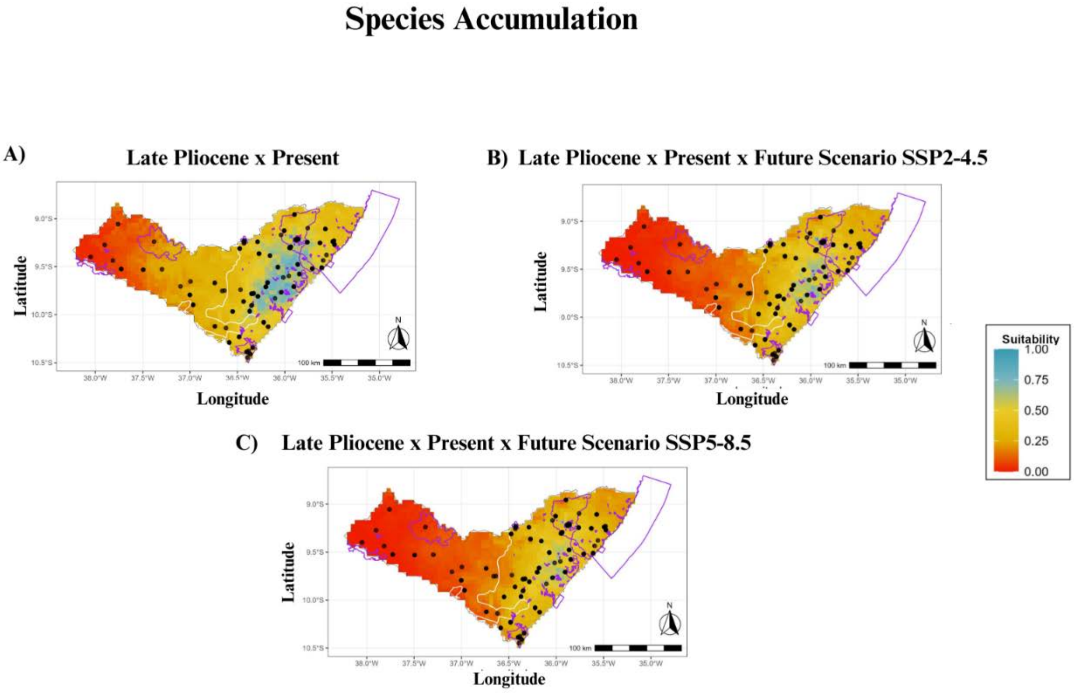
Subtitle: A) Late Pliocene. B) Present. C) Future (2050) under SSP2-4.5 scenario. D) Future (2050) under SSP5-8.5 scenario. Every scenario depicts a suitability map, followed by the variation on the suitability values for Alagoas, the Atlantic Forest and State's Protected Areas respectively. The white line delimits the

Atlantic Forest area in the state’s eastern portion, while the purple line delimits protected areas present in Alagoas.

2.3.2 *Species Accumulation*

The overlapping of the past and present models indicated a reduced area of species accumulation for this frog assemblage, restricted to the central portion of the Alagoas’ Atlantic Forest. This area tends to a severe decrease in future climate change scenarios (Figure 2). This is also true when analyzing Protection Areas, as suitability lowers within these areas.

Figure 2. - Species accumulation map for the anuran assemblage in the state of Alagoas, Brazil.



Subtitle: A) Retained species accumulation in the present. B) Projected species accumulation for the SSP2-4.5 scenario. C) Projected species accumulation for the SSP5-8.5 scenario. The while line delimits the Atlantic Forest area in the state’s eastern portion, while the purple line delimits protected areas present in Alagoas.

2.4 Discussion

Here we evaluate the thermal suitability for an assemblage of 12 anuran species co-occurring in the northern portion of the Atlantic Forest biome, in Brazil. Species assessed differ phylogenetically (FROST, 2023), in reproductive modes (*e.g.*, HADDAD; PRADO, 2005; FORTI, 2009; NUNES-DE-ALMEIDA; HADDAD; TOLEDO, 2021), habits and activity period (*e.g.*, HADDAD et al., 2013; BORGES-LEITE et al., 2015; FREITAS et al., 2022) and thus, present different ecological niches in spite of their co-occurrence verified through previous field sampling. These aspects reinforce the validation for our data.

2.4.1 *Model Limitations*

Ecological Niche Modelling techniques have been increasingly explored due to the need of understanding more on the hazardous effects of climate change throughout time as well as for future projections in different scenarios (*e.g.*, ESCOBAR; CRAFT, 2016; LOMBARDI et al., 2022). Despite widely applied (WIENS et al., 2009), the correlative approach may exhibit some limitations. Species occurrence data may contain spatial biases and errors in species identification or occurrence data (ELITH et al., 2011). Moreover, depending on the algorithms used, modelling may provide the study with over or underestimated predictions of the species' potential distribution (SINCLAIR; WHITE; NEWELL, 2010).

Another possible limiting factor is the scarcity of data on the biology of the species that constitute the anuran assemblage in the Atlantic Forest region of Serra da Saudinha in Alagoas, especially regarding their interspecific interactions. Literature displays knowledge on Atlantic Forest species regarding their habitats (*e.g.*, BARBOSA et al., 2020; DUBEUX et al., 2020; SOUZA et al., 2021), known distribution (*e.g.*, FROST, 2023), diet (*e.g.*, FERREIRA; DANTAS; TEIXEIRA, 2007; COSTA; BRITO, 2022), reproductive modes (*e.g.*, HADDAD; PRADO, 2005; SANTOS; AMORIM, 2005; FORTI, 2009) and vulnerability status (*e.g.*, IUCN, 2023). Nevertheless, information regarding these species ecophysiology and their interactions between species and with the environment are still lacking.

Despite these limitations, ENMs play a crucial role in several areas of biodiversity conservation, climate change impact assessment, species distribution prediction, and potential occurrence areas. Indeed, ENMs may provide an over or underestimation of species distribution. However, these approaches provide a powerful tool for decision-making in environmental management. Our study highlights the relevance of the Atlantic Forest for this anuran assemblage's conservation, especially when considering that this portion is located within the country's most devastated Endemism Center (DA SILVA; TABARELLI, 2001)

2.4.2 *Thermal suitability*

Our models indicated that suitable areas for the Alagoas anuran assemblage are mainly concentrated within the Atlantic Forest area. The opposite portion of the state, less suitable for the assemblage, encompasses the Caatinga biome, which extends over about 826,411 km^2, covering all Brazilian Northeast states and the northern portion of Minas Gerais (PORTAL EMBRAPA, 2022). It displays semi-arid climate, predominantly hot and dry, with irregular rainfall and usually small-sized dry forest vegetation (PORTAL EMBRAPA, 2022). Such conditions make this biome less favorable for the anurans' occurrence (PORTAL EMBRAPA, 2022), since these animals are susceptible to desiccation due to their thin and permeable skin, and are generally associated with more humid environments, such as tropical forests (WINTER et al., 2016). In this sense, the Atlantic Forest has more favorable climatic and environmental conditions for the survival of these individuals, allowing this biome to harbor a great diversity of amphibians (HADDAD et al., 2013).

We predicted a reduction of potentially suitable areas for the occurrence of the frog assemblage in the present when compared to the past. Pliocene paleoclimate reconstructions indicate relative stability, with no abrupt changes and reduced variability in sea surface temperature (DRAUT et al., 2003). These past conditions may have favored greater thermal suitability for anurans, since these individuals are ectothermic thermoconformers and may not withstand large temperature variations depending on their thermal tolerance ranges (NAVAS, 2002; LAMBRINOS; KLEIER, 2003; TAYLOR et al., 2020).

Future forecasts have shown that the Atlantic Forest would suffer the most amongst biomes from climate risk effects by 2050 in the SSP 2-4.5 scenario, which would be aggravated in the SSP 5-8.5 scenario, with high rates of vegetation loss (DIELE-VIEGAS et al., 2022). However, another forecast for the Atlantic Forest depicted two contradictory results (REBOITA et al., 2022). While modelling based on Global Circulation Models (GCMs) predicts an increase in precipitation and temperature in the Atlantic Forest region for the period from 2050 to 2080 for intermediate and pessimistic future scenarios, regional atmospheric modeling (Eta model) displays a decrease in both conditions (REBOITA et al., 2022). A possible increase in future precipitation could explain the subtle increase in potential frog assemblage distribution observed in our prediction for the SSP2-4.5 scenario. Such increase could favor the survival of anurans, since water relations are extremely important for these organisms' physiology (TITON et al., 2010). If precipitation does increase in a more pessimistic scenario (SSP5-8.5), however, temperature may play a bigger role on reducing the frog assemblage's potential distribution. Furthermore, reduction in precipitation along with temperature increases may render the environment unsuitable for the species, so it is possible that these factors will act synergistically to cause a reduction in the frog assemblage's distribution (LOYOLA et al., 2014).

2.4.3 *Species accumulation*

Our analyses indicate a current area of species accumulation restricted to the central portion of the Alagoas' Atlantic Forest, which tends to decrease when overlapping past and present with both future climate change scenarios assessed. Previous refugia analyses considering current and past climates have depicted small refugia for three hylid species in the northeasternmost region of the Atlantic Forest (CARNAVAL et al., 2009). A forest inference through ecological paleomodelling identified these refugia as a high stability area in the Atlantic Forest during the Last Glacial Maximum (~20 ka, CARNAVAL; MORITZ, 2008; COSTA et al., 2018).

2.4.4 *Implications for anuran conservation*

Currently, the International Union for Conservation of Nature (IUCN) classifies most of this frog assemblage species as Least Concern (IUCN, 2023). *Allobates olfersioides* falls under the Vulnerable category, while *Rhinella diptycha* is Data Deficient, and *Proceratophrys renalis* remains not evaluated (NE; IUCN, 2023). The IUCN classifies taxa at risk according to five different criteria that consider several aspects on their risk analysis, and geographical distribution and population size at different periods in time considering decline, fragmentations and fluctuation (COMITÊ DE PADRÕES E PETIÇÕES DA IUCN, 2022). The Not Evaluated category comprises taxa that are yet to be evaluated according to these criteria, while Data Deficient taxa may be broadly researched as to their biology, but are, however lacking in details concerning their distribution and/or abundance (COMITÊ DE PADRÕES E PETIÇÕES DA IUCN, 2022). The Vulnerable category is one of the three "threatened" categories. Taxa are Vulnerable when any of the risk evaluation criteria surpasses the "vulnerable" limit (COMITÊ DE PADRÕES E PETIÇÕES DA IUCN, 2022). Finally, although Least Concern category includes taxa that, are not at immediate or predicted forthcoming risk, it does not mean such taxa should be overlooked (COMITÊ DE PADRÕES E PETIÇÕES DA IUCN, 2022). In addition, half of the species that compose the assessed assemblage display a relatively broad distribution throughout the Atlantic Forest and territories within other biomes (*e.g.* PRAMUK, 2006; GAZONI et al., 2021; FROST, 2023). The remaining half, however, are endemic to the Atlantic Forest, and present more restricted distributions going from the northeasternmost portion of Brazil, reaching either only southeast states or both southeast and south states (*e.g.* MÂNGIA; GUIMARÃES; FEIO, 2012; DUBEUX et al., 2020; TUPY et al., 2021; FROST, 2023). Out of all 12 species from the assessed assemblage, *Pristimantis* aff. *ramagii* is the only one restricted solely to states of the northeast region, from Paraiba to Bahia (ROSSA-FERES et al., 2017; FROST, 2023). Although our study did not account for interspecific interaction, our ENM approach allows for an estimation of the thermal suitability and species accumulation throughout different time periods of a diverse assemblage composed of species with different distribution ranges. Broad-distribution species may inhabit sites where they could experience a wider range of climatic conditions and whereas restricted ones may be more specialized (ISAAC et al., 2009). Therefore, our study may offer helpful information on priority areas for conservation measures that may benefit this assemblage as a whole.

The extent of the original territory occupied by the Atlantic Forest in Alagoas is unknown, although data estimates that the biome has occupied around 52% of the state's territory (MOURA, 2006). Currently, only a small portion of the biome is left at the state (~6%), with most of its forests found in altitude areas, with few fragments present in lowland areas (MOURA, 2006). The state of Alagoas has 96 Protected Areas (PAs) divided into *Área de Proteção Ambiental (APA), Estação Ecológica, Monumento Natural, Reserva Biológica, Parque Municipal, Reserva Ecológica, Reserva Extrativista, Refúgio de Vida Silvestre,* and *Reserva Particular do Patrimônio Natural* (ALAGOAS EM DADOS, 2023). It is possible that the fact that PAs are subjected to more strict regulations and norms than other forest areas favors conservation in the area to make it suitable for species (LEI 9985, 2000). When we crossed the data on the PAs map with current suitability, we observed a portion of suitable areas for the assemblage within the Estação Ecológica de Murici. This Integral Protection PA covers the municipalities of Murici, Flexeiras and Messias (BRASIL, 2001), being comprised within the territory of Área de Proteção Ambiental de Murici, and contains a vast portion of the original biome remnants (DUDLEY, 2008). Therefore, this station should be considered as an important priority area for the conservation of the assemblage. The species accumulation identification in this central portion of Alagoas' Atlantic Forest may assist on the determination of future studies sampling areas aiming at collecting ecophysiological data in order to increase knowledge about species and their interactions with each other and with the environment and thus support future conservation policies.

2.5 Conclusions

We conclude that thermal suitability of the environment where the studied anuran assemblage is located is decreasing throughout time, reducing their occurrence likelihood. In the future, we predict thermal suitability will reduce even further, harming the prevalence of these species in these areas, and, potentially, their survival. Furthermore, the current thermal species accumulation in the central region of the Atlantic Forest in the state of Alagoas is likely to present a reduced suitability in both analyzed scenarios of future climate change. We recommend that more studies be developed in these areas in order to reduce possible Linnean and Wallacean shortfalls, as well as to broaden the knowledge regarding the relationship between species and between them and the environment, so conservation public policies may be supported.

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Author contributions

IMAA played a central role in this project as the first author by conceiving the initial idea, collecting data, and writing the manuscript's first draft. TM contributed to the project by providing feedback, and assisting with logistical aspects such as organizing data and coordinating project activities. ST also made important contributions to the project through revision and logistic support. CFD made substantial contributions by critically revising the project, specifically focusing on aspects related to species’ ecological aspects. Finally, LMDV contributed significantly by designing the project, providing support in data analysis and interpretation of the results, and critically reviewing the manuscript, offering insightful suggestions and feedback. All authors commented on previous versions of the manuscript and have read and approved the final version of the manuscript.

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3 - IMPACTS OF CLIMATE CHANGE ON THE DISTRIBUTION OF A FROG ASSEMBLAGE IN AN ATLANTIC FOREST REMNANT REGARDING THERMAL PHYSIOLOGY

(Manuscrito sob revisão de co-autores: AMARAL-ALVES, I. M.; ROCHA C. F. D.; MOTT, T.; TORQUATTO, S.; CAETANO, G. H. O.; DIELE-VIEGAS, L. M. **Impacts of climate change on the distribution of a frog assemblage in an Atlantic Forest remnant regarding thermal physiology.**)

Abstract

Anurans are highly vulnerable to climate change, since their behavior and physiological processes are closely related to environmental conditions. As ectotherms and mostly unable to actively thermorregulate, their body temperature must vary within a range that enables a satisfactory performance and survival. Ecological Niche Modelling (ENM) may contribute to the study of climate change impacts on anurans, being able to make predictions of their potential distribution in a particular area under different scenarios. In this study, we model a distribution of an anuran assemblage from an Atlantic Forest remnant in Alagoas. In order to find thermally suitable areas for the assemblage, we performed a mixed ENM, combining a correlative and a mechanistic approach. Thus, we considered macro and microclimate variables as well as thermal traits in our analysis. Our mechanistic approach consisted in the use of NicheMapR where we coupled microclimatic conditions with thermal traits achieved through laboratory tests. Subsequently, with the outputs provided, we performed a correlative modelling where we obtained macroclimate variables the WorldClim database. Results found that highly suitable areas were mostly concentrated along the eastern portion of the state, specifically within the Atlantic Forest. This pattern tends to worsen for the future with expressive decreases for both future scenarios. We suggest that thermal traits analyzed may play an important role in suitability, rendering species to be more vulnerable to climate change. In contrast, the high suitable areas concentrated in a portion of the Atlantic Forest suggest that this biome may provide enough microclimate conditions to buffer adverse effects of climate change for the frog assemblage.

Keywords: thermal physiology, Ecological Niche Modelling, NicheMapR

3.1 Introduction

Anuran’s behavior and physiological functions, such as reproduction and growth, may be influenced by climatic fluctuations due to their strong relationship with environmental temperature (HUEY; STEVENSON, 1979; LAMBRINOS; KLEIER, 2003; ZUO et al., 2012;

KHATIWADA; ZHAO; JIANG, 2020). Thus, their body temperature (T_b) must vary within a suitable temperature range so animals perform satisfactorily to guarantee their survival (HUEY; STEVENSON, 1979). For some species, even minor changes in their body temperature may represent a major threat (NAVAS, 2002; ALGIRIYAGE; JAYAWEERA; WIJESINGHE, 2020).

Endemic species with restricted distributions are usually adapted to specific environmental conditions and potentially are more sensitive to cope with changes in their local conditions (MITTERMEIER et al., 1998). The Atlantic Forest is one of the most important biodiversity hotpots in the world, housing over eight thousand endemic species (MYERS et al., 2000; FUNDAÇÃO SOS MATA ATLÂNTICA & INSTITUTO NACIONAL DE PESQUISAS ESPACIAIS, 2009). Over 540 amphibian species occur in this biome, of which around 529 are anurans and 85% are endemic (HADDAD et al., 2013).

Originally, the Atlantic Forest covered approximately 150 million hectares, ranging from Rio Grande do Norte to the Southern portion of Brazil (RIBEIRO et al., 2009). However, only 11.7% of this forest's original extent left across 17 states (RIBEIRO et al., 2009). This remnant houses over 100 million Brazilian residents in large cities or small villages (RODRIGUES et al., 2009). The biome's main vegetation are seasonal semideciduous forest and dense coastal Ombrophylous dense forest (MORELLATO; HADDAD, 2000), characterized by a warm and humid climate without a marked dry season (OLIVEIRA-FILHO; FONTES, 2000). Moreover, it is associated to other biomes, such as the Bolivian Chaco, Pampas, Pantanal, Cerrado, and Caatinga (RIBEIRO et al., 2009).

The state of Alagoas is located in the Pernambuco Endemism Center (CEP), which encompasses the Atlantic Forest portions located north of the São Francisco River (PRANCE 1982; SANTOS AND CARNAVAL, 2002). This center is a highly relevant biogeographical region for harboring several endemic species from different biological groups (*e.g.*, (RODRIGUES; BORGES, 1997; PEIXOTO; CARAMASCHI; FREIRE, 2003) and is characterized mainly by lowland, submontane, and montane vegetation (VELOSO; RANGEL FILHO; LIMA, 1991). The CEP extends along the coastal region of the State of Alagoas to Rio Grande do Norte, and is currently the most devastated endemism center in the Atlantic Forest (DA SILVA; TABARELLI, 2001). The Atlantic Forest's degradation is mainly caused by deforestation, changes in land use, and climate change (PEREIRA et al., 2010; JOLY; METZGER; TABARELLI, 2014) and may affect several groups of organisms, including amphibians (CORDIER et al., 2021)

While many studies assess the effect of climate change on biological aspects of anurans, including reproduction, growth, distribution, and susceptibility to pathogens among others (*e.g.*, BEEBEE; GRIFFITHS, 2005; FISHER; GARNER; WALKER, 2009; SCHIVO et al., 2019), information regarding its effects on this group's thermal physiology are still limited. Therefore, it is essential to develop studies that assess species' current and future vulnerability in a climate change scenario and to understand how species respond to these changes through their physiological strategies.

Ecological niche modelling (ENM) has gained significant importance over the years for several purposes, such as predicting species distributions under historical, current or future climate (HIJMANS; GRAHAM, 2006), wildfire occurrences (BATLLORI et al., 2013), species richness (*e.g.* MARTÍNEZ-FREIRÍA et al., 2013), and more. Essentially, ENMs can predict whether a species (or a specific group) may occur in a given area of interest by using their geographic coordinates, climate data, environmental and physiological data, amongst other possible variables (LOZIER; ANIELLO; HICKERSON, 2009). Correlative models encompasses the relationship between species distribution and environmental variables that may not have direct effects on distribution but can synthesize these effects by approximating the fundamental to the realized niche (SILLERO, 2011). Mechanistic modelling, in contrast, assesses processes that may limit organisms' distribution, displaying a cause and effect relationship among variables that affect directly on species survival and distribution (KEARNEY; PORTER, 2009; SILLERO, 2011). In this sense, it is possible to estimate the fundamental niche by incorporating physiological, morphological, and behavioral data, and relate them to climate (KEARNEY; PORTER, 2009; SILLERO, 2011).

In this paper, we modelled the potential distribution of species of an anuran assemblage from the Atlantic Forest at Serra da Saudinha, in Alagoas, assessing the relationship between their thermal ecology and climate change through an Ecological Niche Modelling. This approach encompasses a mechanistic modelling step (KEARNEY; PORTER, 2017) and a following correlative modelling step using bioclimatic data (WORLDCLIM DATABASE, 2023) and the geographical coordinates of the assemblage species. By integrating both approaches, we aimed to understand how climate change might affect this assemblage's distribution when accounting for their physiology.

3.2 Material and methods

3.2.1 *Study Area and field sampling*

Vegetation cover in CEP's southern portion, where Serra da Saudinha is located, features an area covered by dense Ombrophilous Forest and adjacent areas with eucalyptus plantations, with a warm and humid climate (ASSIS, 2000; VIEIRA et al., 2002; THOMAS; BARBOSA, 2008; NEMÉSIO; SANTOS JUNIOR, 2014). Around 396 hectares of native forest were transformed into the protected area Reserva Particular do Patrimônio Natural (Private Natural Heritage Reserve; RPPN) Senador Carlos Lyra (IMA - INSTITUTO DO MEIO AMBIENTE, 2023). To identify the composition of the anuran's assemblage, we sampled an Atlantic Forest remnant (*Fazenda Riachão*) and eucalyptus vegetation area in Serra da Saudinha between February and October 2022, under permits granted by the System of Authorization and Information on Biodiversity (SISBIO – ICMBio), number 77134-1, and the Ethical Commission on the Use of Animals (CEUA) number 07/2021.

In the field, we resorted to active search and pitfall traps to capture individuals. Active searches took place during anurans' activity period, from 9 a.m. to 11p.m. Pitfalls consisted of 120 units of 60L-buckets buried in the ground up to its edge with drift fence approximately 50cm high, arranged in a "Y" shape. The traps were installed at six different points with two transects each: eucalyptus vegetation area (E1 and E2), areas at the edge of forest (B1 and B2), and forest interior area (M1 and M2). Each transect had 20 buckets distributed in five sets. Each pair of transects was installed at a distance of 200m from each other, with buckets apart in four meters from each other. Monitoring of the buckets took place once a day, for 22 days. We captured 159 individuals from eight species of anurans belonging to five families (Bufonidae, Hylidae, Leptodactylidae, Odontophrinidae and Strabomantidae - Table 1), and they were posteriorly transported to the laboratory of *Setor de Herpetologia,* Museu de História Natural de Alagoas, Universidade Federal de Alagoas, to be incorporated in its Herpetological collection.

Table 1. Anuran assemblage species, their vulnerability according to IUCN and occurrence points after filtering.

Species	IUCN status	Occurrences after filtering
Bufonidae		
Rhinella crucifer	LC	28

Hylidae		
Boana albomarginata	LC	19
Dendropsophus minutus	LC	23
Leptodactylidae		
Adenomera hylaedactyla	LC	12
Leptodactylus natalensis	LC	41
Physalaemus cuvieri	LC	52
Odontophrynidae		
Proceratophrys renalis	NE	26
Strabomantidae		
Pristimantis aff. *ramagii*	LC	21
Total		222

Subtitle: DD: data deficient; LC: least concern; NE – not evaluated; VU – vulnerable.

3.2.2 *Thermal physiology experiments*

After determining the assemblage, we performed thermal physiology experiments with the identified species. We evaluated their thermal preferences and limits by measuring their preferred temperature in laboratory (T_{set}) and their critical thermal limits (CT).

Preferred temperature (T_{set})

We estimated T_{set} by creating a temperature gradient ranging from 10 to 40°C, using a standard laboratory heater at one end and ice packs positioned at the other. Gradients were built from cardboard boxes varying in size according to the tested animals' size. Animals were positioned inside the gradient for 30 minutes for acclimation. Then, we measured their body temperature (T_b) every 1 to 2 minutes, always totaling 60 measurements per individual.

We defined T_{set} as the average of the recorded body temperatures, and the voluntary minimum and maximum temperatures (VT_{min} and VT_{max}, respectively) as the first and third quartile of the recorded temperatures. Voluntary temperatures are described as lower or upper thermal threshold at which animals tend to seek shelter to avoid stressful temperatures (TAYLOR et al., 2020), this representing the limits of the T_{set} range (HUEY, 1982; HERTZ; HUEY; NEVO, 1983; CHRISTIAN; WEAVERS, 1996).

Critical thermal minimum and maximum (CT_{min} and CT_{max})

We considered CT_{min} and CT_{max} as the extreme temperatures at which the animal presents loss of righting response, *i.e.*, when it loses locomotor capacity when positioned on its back and it is not able to straighten up in a prone position after a stimulus (TAYLOR et al., 2020). In order to measure CT_{min}, we placed individuals in a cooling chamber, measuring their T_b every 30 seconds until they reached the critical temperature. The procedure for measuring CT_{max} was similar, where animals were placed in a heating chamber and their T_b was measured every 20 seconds. In this case, animal were carefully positioned at a distance of at least 10cm of the heating source to avoid overheating. The summary of the ecophysiological data can be found in Table 2.

Table 2. Averaged ecophysiological data for each species.

Species	N	T_{set}	VT_{min}	VT_{max}	CT_{min}	CT_{max}
Rhinella crucifer	4	22.76	21.4	23.86	8,275	38.2

Boana albomarginata	10	27.05	26.6	27.7	12.54	30.9
Dendropsophus minutus	9	27.4	25.37	29.33	19.74	29.87
Adenomera hylaedactyla	12	24.2	22.57	25.39	15,775	31.87
Leptodactylus natalensis	10	27.42	25.36	27.94	16.94	34.19
Physalaemus cuvieri	19	24.68	23	25.9	12.32	30.76
Proceratophrys renalis	7	26.66	23.62	30.26	8.2	34.24
Pristimantis ramagii	7	23.02	20.44	25	8.11	31.67

Subtitles: N = number of individuals tested; T_{set} = preferred temperature; VT_{min} = minimum voluntary temperature; VT_{max} = maximum voluntary temperature; CT_{min} = critical thermal minimum; CT_{max} = critical thermal maximum.

3.2.3 *Mechanistic modelling*

We performed a mechanistic model through the package “NicheMapR 3.2.1” (KEARNEY; PORTER, 2017), in a two-step procedure. First, we ran a microclimate model to simulate microclimatic condition to which the anuran assemblage experiences at Serra da Saudinha. Then, we ran an ectotherm animal model (hereafter ectotherm model) by using microclimatic result, thermal physiology data obtained through laboratory experiments, and activity period of the assemblage’s species as input data, in order to simulate physiological conditions. Finally, we used both microclimate and ectotherm models to build a raster considered as a proxy for the species’ thermal stress.

Microclimatic modelling

We obtained microclimatic data from Serra da Saudinha through a microclimate model included in the NicheMapR package. This model communicates with the FORTRAN library, which comprises several subroutines and functions to obtain microclimate conditions that an animal would experience at specific location (KEARNEY; PORTER, 2017). First, we loaded a climate raster through the *get.global.climate* (NEW et al., 2002) and obtained coordinates of each pixel for the state of Alagoas. Then we ran the *micro_global* function, which is connected to global monthly data from 1960 to 1990 on a ~10x10 grid (NEW et al., 2002),to download microclimatic data for the specific area based on the coordinates. We ran the model for areas under default settings, except for shading levels, where we set a minimum shade as 0% (full sun) and maximum shade as 90% for a 12-day interval.

The model generated an output containing microclimatic variables such as air temperature at different heights and depths, relative humidity, presence of dew and frost and many other microclimate variables at a specific day and time (hourly) for the state of Alagoas.

Ectotherm modelling

The ectotherm model considers morphological, physiological and behavioral aspects to depict ectotherms' thermal and water interactions with the environment (KEARNEY; PORTER, 2020). For this model, we used the output of the microclimate model combined with the thermal data (T_{set}, VT_{min}, VT_{max}, CT_{min}, and CT_{max}) of the assemblage species as input to the modelling approach.We also considered body mass, activity period (diurnal or nocturnal) and body shape (= 4, default shape for amphibians).

The ectotherm model output embraces variables regarding energy, mass balance and environment. Energy balance results from variables related to animal's heat gains and losses (*i. e.,* heat budget), whereas mass balance accounts for oxygen consumption and water loss (mass budget). Environment variables embrace activity and thermal conditions experienced by the animals. We also performed two distinct calculations of warming tolerance by subtracting T_b from CT_{max} (*i.e.,* $CT_{max} – T_b$, hereafter WT_{tb}) and air temperature (T_a) from CT_{max} (*i.e.,* $CT_{max} – T_a$, here after WT_{ta}). The first may reflect mostly microclimatic conditions, while the second is related to macroclimatic conditions (ANDERSON; MEIRI; CHAPPLE, 2022). In both cases, when values approach zero, it suggests that frogs are likely to reach their limits (ANDERSON; MEIRI; CHAPPLE, 2022). Each form of calculation might produce different outcomes (KEARNEY; MATZELLE; HELMUTH, 2012). To understand how warming tolerances relate, we performed a Pearson's correlation between WT_{tb} and WT_{ta}. We also performed linear regressions to verify the influence of WTs on ENB.

Following this step, we created a new raster where we replaced coordinate values for corresponding ENB values, including it as a layer for the correlative modelling along with climate variables (detailed in the following next section). We performed the same steps with WTs values. At the end, for each variable (ENB and both WTs), the outcome produced one raster per species.

3.2.4 *Correlative modelling*

We performed a stacked Ecological Niche Modelling (CALABRESE et al., 2014; ZURELL et al., 2020) aiming to identify areas potentially suitable for the anuran assemblage occurrence. We downloaded *shapefiles* for Alagoas as well as for the Atlantic Forest and the states' Protected Areas to delimit interest areas through Brazilian Institute of Geography and Statistics website (IBGE, 2022).

Following this step, we downloaded the assemblage species' occurrence data from Gbif, CRIA and SiBBr. We also retrieved coordinates from the record in the registry book of the Herpetological Collection of the Museu de História Natural de Alagoas/UFAL. We filtered data eliminating non-identified or missing records, since those are common errors when using data from open databases (MEYER; WEIGELT; KREFT, 2016). Further, we submitted data through an additional filtering by using the "*clean_coordinates*" function of the CoordinateCleaner package (ZIZKA et al., 2019) through the R environment (R CORE TEAM, 2022). This filtering intended to exclude data that could possibly bias our results, such as coordinates in the same pixel (within a radius of 10km), in the sea or urban areas, centroids of municipalities, states or countries, duplicates and those where latitude and/or longitude were equivalent to zero. We obtained 305 occurrences points for the assemblage after filtering.

We modelled assemblage under present and two future scenarios of climatic conditions. We determined environmental space under current climate through WorldClim database, considering historical data (average of 1970-2000). In order to do so, we imported data from 19 bioclimatic variables (BIO1-BIO19) by running the "*rasterstack*" function of the Raster package (HIJMANS et al., 2022) as well as ENB and both WTs variables obtained from the ectotherm model since we wanted to include them as layers to understand the effects of climate and physiology in species distribution.

We determined the least correlated variables for one species at a time through a Spearman correlation (WISSLER, 1905), with a cutoff of 75%. Variables selected for each species are displayed in Table 3.

Table 3. Variables selected from Spearman correlation for each species.

Species	**Least correlated variables**
Rhinella crucifer	BIO2, BIO3, BIO 8, BIO 9, BIO 15, ENB, WT_{ta}

Boana albomarginata	BIO3, BIO7, BIO 8, BIO 9, BIO 15, ENB, WT_{ta}
Dendropsophus minutus	BIO3, BIO7, BIO 8, BIO 9, BIO 15, ENB, WT_{tb}
Adenomera hylaedactyla	BIO3, BIO7, BIO 8, BIO 9, BIO 15, ENB, WT_{ta}
Leptodactylus natalensis	BIO8, BIO9, BIO 15, BIO 19, ENB, WT_{tb}
Physalaemus cuvieri	BIO2, BIO3, BIO 8, BIO 9, BIO 15, ENB, WT_{ta}
Proceratophrys renalis	BIO2, BIO3, BIO 8, BIO 9, BIO 15, ENB, WT_{ta}
Pristimantis ramagii	BIO2, BIO3, BIO 8, BIO 9, BIO 15, ENB, WT_{ta}

For a more robust result, we resorted an ensemble of models from three different algorithms to build the models: Bioclim, GLM and SVM. Bioclim is an envelope algorithm that considers presence and background data (HIJMANS; GRAHAM, 2006). Generalized Linear Models (GLM) is a statistical algorithm that considers presence and pseudo-absence data (AUSTIN; MEYERS; DOHERTY, 1994). Finally, Support Vector Machine (SVM) is a machine learning algorithm that consists on presence and background data (SCHÖLKOPF et al., 2001; TAX; DUIN, 2004). We ran models through the Dismo (HIJMANS; PHILLIPS; ELITH, 2022), Stats (R CORE TEAM, 2022) and Kernlab (KARATZOGLOU et al., 2022) packages (Bioclim, GLM and SVM models, respectively).

The next step on our analyses was assessing the models' performance. We conducted a random division where 80% of the data was selected for training, while the remaining 20% was reserved for testing. We created 10,000 pseudo-absence points for the GLM algorithm and 10 background points for every presence point for Bioclim and SVM, following (BARBET-MASSIN et al., 2012). We generated ten replicas per algorithm, which were evaluated based on the area under the curve (AUC, ELITH et al., 2006). We kept in our next steps only replicates presenting AUC values over 0.75, which are considered satisfactory to validate models (PETERSON et al., 2001; ARAUJO et al., 2005).

We created consensus models (GIANNINI et al., 2012) for the assemblage occurrence by employing an ensemble approach based on the algorithms' AUC weighed average (ARAUJO; NEW, 2007). Combination of algorithms' selected replicas was performed using package biomod2 (THUILLER et al., 2022). In the maps depicting thermal suitability, pixel values vary from zero to one, where the closer the pixel value is to one, the more suitable that pixel will be for the species occurrence.

Following the consensus, we stacked all the suitability maps generated for the assemblage's species into one by summing up the corresponding pixels of every species to represent total suitability for the assemblage (CALABRESE et al., 2014).

Future models used same variables of the present modelling for each species in the same resolution for the SSP2-4.5 and SSP5-8.5 for the 2041-2060 period (hereafter 2050). Projected global thermal average for the SSP2-4.5 scenario spans between 1.6°-2.5°C (IPCC, 2021). Moreover, SSP5-8.5 scenario foresees an increase varying between 1.9°C to 3.0 °C (IPCC, 2021). Global circulation models selected for future forecast were BCC-CSM2-MR (WU et al., 2018), IPSL-CM6A-LR (BOUCHER et al., 2018) and MIROC6 (SHIOGAMA; ABE; TATEBE, 2019) due to their satisfactory performance in tropical areas (CANNON, 2020).

3.3 Results

We assessed 78 individuals from five families (Bufonidae, Hylidae, Leptodactylidae, Odontophrynidae and Strabomantidae, Table 2).

3.3.1 *Relationship between mechanistic variables*

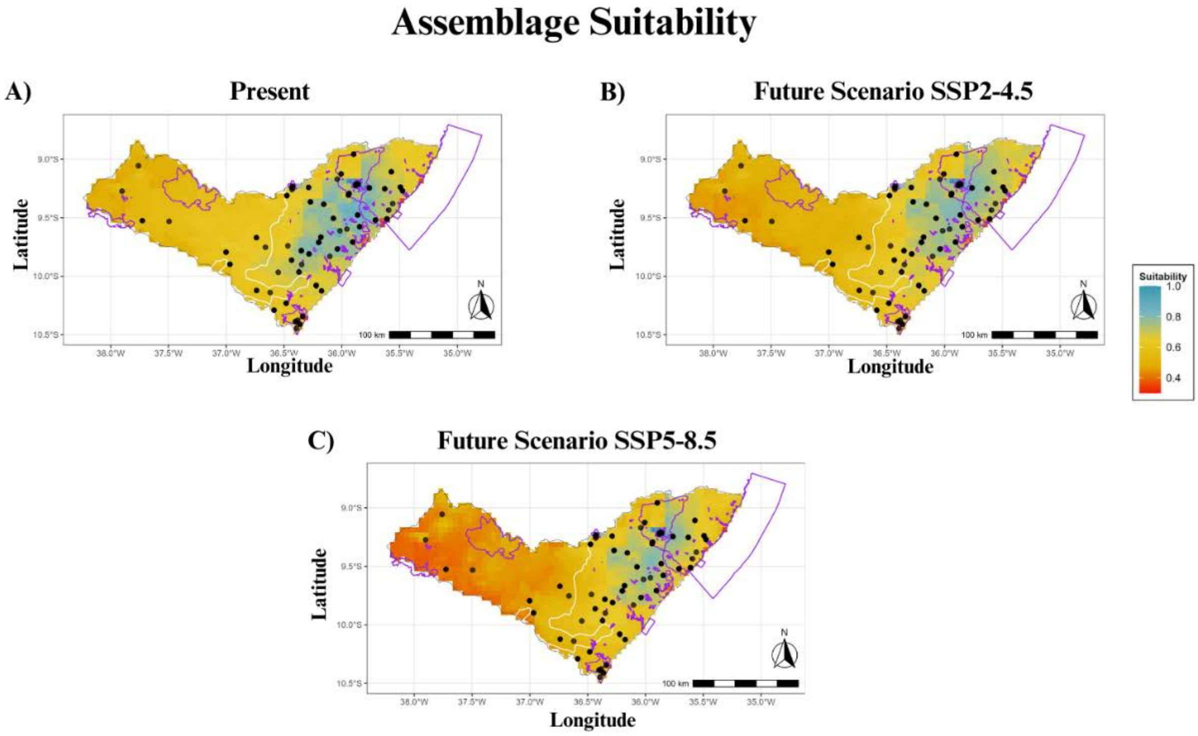
We obtained higher WT from the calculations based on T_b ($9.02 \pm 1.06^{\circ}C$) when compared to T_a ($8.27 \pm 0.94^{\circ}C$), although they do not differ greatly. These two variables are highly correlated (cor = 0.988, df = 8758, P < 0.001). Morevoer, ENB ($9.504922e^{-05\circ}C$) varied significantly with both WT_{tb} (LR: $F = 482.1$, df = 1, 8758, $R^2 = 0.52$, P < 0.001) and WTta (LR: $F = 398.5$, df = 1, 8758, $R^2 = 0.043$, P < 0.001).

3.3.2 *Thermal suitability*

Highly suitable areas for assemblage occurrence are mostly distributed along the state's eastern portion, specifically in the Atlantic Forest area (darker blue pixels, Figure 1). Some less suitable areas (faded blue pixels) are subtle, but present in the central portion of Alagoas, reaching Atlantic Forest's border with Caatinga. In contrast, some unsuitable isolated areas (orange-red pixels) occur on the coast of the Atlantic Forest. Caatinga areas in the western portion of the state are not entirely unsuitable; but do not exhibit high levels of suitability.

The moderate emissions' scenario (SSP 2-4.5) displays a decrease of thermal suitability in both central and the southwestern most portion of the Atlantic Forest. However, this scenario also depicts new slightly suitable areas at the northeastern portion of Alagoas that were not as suitable in the present. Isolated areas on the coast remain unsuitable for assemblage occurrence. A more substantial decrease in suitability occurred for the high emissions’ scenario (SSP5-8.5) where the highest suitability occurs at a small portion within Estação Ecológica de Murici, a Protected Area (PA) in Alagoas. In addition, areas of Caatinga become highly unsuitable for the assemblage and isolated coastal areas observed in the previous scenarios remain unsuitable.

Figure 1. Thermal suitability for the anuran assemblage in the state of Alagoas, Brazil accounting for macroclimatic, microclimatic and thermal physiology variables.



Subtitles: A) Present B) Future under (2050) SSP2-4.5 scenario. C) Future (2050) under SSP5-8.5 scenario. Darker blue pixels depict highly suitable areas and it decreases as it becomes lighter. Yellow to red pixels indicate areas becoming more unsuitable. The white line delimits the Atlantic Forest area in the state’s eastern portion, while the purple line delimits protected areas present in Alagoas.

3.4 Discussion

In the present study, we assessed thermal suitability for an assemblage of eight species that co-occur in Serra da Saudinha, a remnant of Atlantic Forest in Alagoas, Brazil. We assessed suitability not only by considering macroclimatic variables (WORLDCLIM DATABASE, 2023), but also considering microclimate conditions (NEW et al., 2002) and thermal physiology data obtained through laboratory tests.

3.4.1 *Model limitations*

Aside from some limitations mentioned in chapter II that may still persist despite the use of an additional approach (mechanistic modelling), such as spatial biases, mistakes on species identification, or occurrence, the main limitation for our analysis was not accounting for mass balance variables when considering the ectotherm modelling. Such variables include mainly respiratory and cutaneous water loss (KEARNEY; PORTER, 2020), since anurans highly depend on water (TITON et al., 2010). Nonetheless, we believe this study provides relevant information on the influence of climate in anurans' physiology and how important it is to account for thermal physiology and macroecological data to obtain results that are more closely related to what occurs in nature.

3.4.2 *Mechanistic variables*

We found a strong correlation between WT_{tb} and WT_{ta}, which is expected due to the great influence of air temperature on anuran's T_b (NAVAS, 1996; DUARTE et al., 2012; NAVAS et al., 2013). Accordingly, because air temperature influences anuran's body temperature, it would influence in the individuals' heat intake and therefore on their energy balance.

Warming tolerance corresponds to the temperature increase an ectotherm can withstand before it exceeds its CT_{max}. Although the frog assemblage's mean WTs obtained from T_b and T_a displayed different results, they were highly correlated. This is an interesting finding because it has been argued that warming tolerances deriving from air temperatures may result in overestimating measurements such as thermal safety margins and warming tolerances (*e.g.*, Sunday et al., 2014).

3.4.3 *Thermal suitability*

By using both mechanistic and correlative approaches, our models suggest that anuran assemblage suitable areas are distributed in the central portion of the Atlantic Forest in the state of Alagoas. This result is somewhat in accordance with results obtained in Chapter II of this thesis; highly suitable areas, however, are scarce. It is possible that thermal traits analyzed (warming tolerances and energy balance) play a crucial role for the assemblage suitability in this area. However, suitability in similar areas indicate that this portion of Atlantic Forest may provide microclimate conditions to support the occurrence of the assemblage by buffering adverse thermal effects caused by climate. Compared to Caatinga, the Atlantic Forest displays better climate and vegetation cover for these animals (HADDAD et al., 2013). Precipitation exerts a significant influence on climate regulation within the Atlantic Forest (SCARANO; CEOTTO, 2015), which may also favor the survival of amphibians, which are highly dependent on humidity (WINTER et al., 2016). While a variety of factors (*e.g.* deforestation for agriculture expansion) have led to habitat fragmentation in the Atlantic Forest (LAURANCE, 2009), it remains a resilient biome, providing habitat for numerous animal and plant species (MITTERMEIER et al., 2011).

Potentially suitable areas for the frog assemblage tend to reduce in the SSP2-4.5 future scenario (moderate emissions) in the central portion of the state and in some previously suitable areas in the present scenario. Moreover, some suitability seems to move towards the coastal area at the northeastern portion of the state. In the more pessimistic scenario, SSP5-8.5, suitable areas decrease drastically. Although both scenarios present similar tendencies when compared to results from correlative modelling alone (see Chapter II), decrease in suitable areas are expressive when applying both mechanistic and correlative approaches. Future forecasts for the Atlantic Forest predict high rates of vegetation loss for the Atlantic Forest by 2050 (DIELE-VIEGAS et al., 2022), which may render species more vulnerable to temperatures in these scenarios, since vegetation cover may buffer adverse effects of temperature in forested areas (GONZÁLEZ-DEL-PLIEGO et al., 2016; NOWAKOWSKI et al., 2017; CAETANO et al., 2020).

Although some species of the assemblage may be found active during the day (*Adenomera hylaedactyla* and *Pristimantis* aff. *ramagii),* species that compose this assemblage are predominantly nocturnal (*e.g.* HADDAD et al., 2013; LEITE FILHO, 2013; BORGES-LEITE et al., 2015). As such, nocturnal species experience comparatively lower colder temperatures because they are not exposed to solar radiation, so they may face relatively less thermal stress (NAVAS, 1997). On the other hand, despite displaying WTs not necessarily low, it is possible that for this assemblage, this trait’ plasticity is not enough to endure future climatic shifts. With few exceptions, frogs usually display limited plasticity of thermal traits so, in a future projection where climate gets hotter, species living closer to their upper limit are expected to live under severe risk (DUARTE et al., 2012; ENRIQUEZ-URZELAI et al., 2018). Therefore, with climate’s tendency to worsen for future projections, our results suggest that these frogs may not be able to display strategies to tolerate further warming.

3.5 Conclusion

From our results, we gather that thermal suitability for this assemblage is likely to decrease severely as time progresses. In a more pessimistic scenario, a severe reduction of suitable areas is expected. We suggest that the assemblage's species may not be able to adapt fast enough to endure changes predicted for the future. However, we strongly believe that, within a reduced portion, the Atlantic Forest is able to buffer effects of climate change enough to preserve some suitability that allow for species' survival. Further, we encourage the development of studies that account for both thermal and hydric conditions, so that the relationship between the assemblage and the environment are better understood and decision makers are better oriented in order to employ conservation policies.

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