JESUS EDUARDO ORTEGA CHINCHILLA

Inferindo a desidratação de anuros terrestres e arbóreos na Mata Atlântica do sul do Brasil a partir do microclima, tamanho corporal e permeabilidade de modelos de ágar

Inferring dehydration in terrestrial and arboreal anurans in the Atlantic Forest of southern Brazil from microclimate, body size and permeability of agar models

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Tese apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção de Título de Doutor em Ciências Biológicas, na Área de Fisiologia Geral.

Orientador(a):
Prof. Dr. Carlos Arturo Navas Iannini

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especialmente a meus pais: Maria[†] e Jesus;
à minha esposa: Laura Camila;
meus irmãos: Yaneth, Fernando, Myriam e Nubia;
meus sobrinhos e sogros
pelo amor, exemplo e suporte.

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“La calle te da lo que un libro no te enseña
Y un libro te enseña lo que la calle no te da
La dignidad por el oro no se empeña
Por eso me mantengo firme frente a la sociedad”

D. Y.

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Resumo geral

O aquecimento climático apresenta um impacto sem precedentes na biodiversidade, afetando o desempenho fisiológico e a tolerância dos organismos, particularmente para ectotermos terrestres, que funcionam perto dos seus limites fisiológicos superiores. No entanto, estas relações entre clima e biodiversidade têm sido estudadas principalmente em grandes escalas que não explicam o microclima, entendido como clima numa escala individual. Atualmente o microclima é considerado essencial para compreender o impacto de suas alterações sobre a diversidade biológica com interesses focais sobre dado táxon. A Mata Atlântica do Brasil apresenta uma diversidade fisionômica e biológica que são causa e efeito do clima. Essas relações amplas não são suficientes para explicar as opções de termo e hidro regulação disponíveis para pequenos animais, que é particularmente importante para anfíbios anuros, um táxon particularmente diversificado. Portanto, o objetivo deste projeto foi investigar os microclimas que os anuros da Mata Atlântica podem experienciar em diferentes fisionomias, estratos e habitats fragmentados (áreas abertas, borda e interior da floresta). Todos esses fatores devem influenciar variação hidrotermal numa escala espaço temporal, compatível com o tamanho do corpo dos adultos. Para definir estas relações, usamos um conjunto de indicadores (modelos de ágar com sensores e imagens infravermelhas) que estimam a temperatura operativa e disponibilidade de água no ambiente. Este estudo foi baseado em dados de tamanho corporal real que incluem a maioria das gamas de tamanhos e permeabilidade de pele de anuros presentes no Parque Estadual Intervales. Usamos como unidade amostral pares de modelos de ágar que simulam a forma e extremos de tamanho corpóreo (pequena vs. grande) e tipos de

permeabilidade (totalmente permeáveis vs. impermeáveis). Em geral, modelos permeáveis, especialmente de tamanhos menores, apresentam maior perda de água em áreas de vegetação mais baixa, especialmente no dossel, áreas abertas e em mata de altitude. Os dados obtidos permitiram propor hipóteses de impacto diferencial de altas e baixas temperaturas e baixa umidade relativa, de acordo com a faixa microambiental ocupada pelos anuros. Estas informações ajudam no entendimento da paisagem hidrotermal e do impacto das mudanças climáticas sobre a história natural, em interpretação de dados eco-fisiológicos, modelagem climática e previsão do impacto do clima sobre anuros, particularmente no contexto da dinâmica térmica e do balanço hídrico em escalas espaciais e temporais.

Abstract

Climate warming presents an unprecedented impact to global biodiversity, affecting physiological performance and tolerance of organisms, particularly for terrestrial ectotherms, which function near their upper physiological limits. However, the relationships between climate and biodiversity have been studied mainly at large scales that do not explain microclimate, understood as climate at individual scale. Microclimate is currently considered essential to understand the impact of climate on biological diversity with focal interests on given taxa. Atlantic Forest of Brazil presents physiognomic and biological diversity that are cause and effect of climate. These relationships are not enough to explain options of thermo and hydro-regulation available for small animals, particularly important for anuran amphibians, a particularly diverse taxon. Therefore, the aim of this project was to investigate the microclimates from Atlantic Forest experimented by anurans in different physiognomies, stratum and fragmented habitats (open areas, edge and inside forest). All these factors should influence hydrothermal variation at temporal-space scale compatible with the body size of adults. To define this diversity, we used a set of indicators (agar models with sensors and infrared images) that estimate the operative temperature and water availability. This study was based on actual body size data to include most ranges of size and of skin's permeability. We used as sampling unit; pairs of agar's models that simulate shape and two body size extremes (small vs. large) and two types of permeability (fully permeable vs. impermeable). In general, permeable models especially of smaller sizes have a higher water loss in lower vegetation areas, especially in Canopy, Open Area and High-Altitude Forest. The data collected allowed to propose hypotheses regarding

differential impact of high and low temperatures, and low relative humidity, according to the micro-environmental range occupied by anurans. This information will help to understand hydrothermal landscapes and impact of climate on natural history, interpretation of eco-physiological data, climate modeling and prediction of the impact of climate change on anurans, particularly in the context of thermal dynamics and water balance in spatial and temporal scales.

Introdução Geral

O microhabitat é entendido como a soma dos recursos disponíveis e as condições ambientais presentes, compatíveis com o tamanho corporal dos indivíduos (Johnson, 1980). Estes recursos incluem alimentos, refúgios, e o conjunto de fatores climáticos relacionados com sobrevivência e êxito reprodutivo (Leopold, 1933). Assim como existe uma relação entre habitat e clima, o microhabitat possui relação com o conceito de microclima, entendido como o clima na escala individual de um organismo alvo. O clima em geral é um conjunto de fatores ambientais que influenciam a estrutura e funcionamento dos ecossistemas, com impactos nos processos biológicos, hidrológicos, edáficos e geomorfológicos (Zheng e col., 2000), e frequentemente é discutido numa escala espacial definido por grandes domínios morfoclimáticos (Ab'saber, 1997; 2005). Os ecossistemas também são influenciados pelas suas variações fitofisionômicas e florísticas, que, por sua vez, são influenciadas pelas variações climáticas produzidas por gradientes da costa para o interior do continente. Também, podem ser influenciados por elementos de variação sazonal ou diária, como o aumento da variação térmica ao longo do dia ou estacional, na medida em que a latitude aumenta (Oliveira-Filho e Fontes, 2000).

Entretanto, estes grandes padrões climáticos não são ideais para explicar as opções de termo e hidrorregulação disponíveis para vertebrados de pequeno porte, especialmente em animais de pele permeável, como é o caso dos anfíbios. Na ecofisiologia, é importante que as escalas climáticas sejam compatíveis com o tamanho dos indivíduos, e o termo “microclima” é usado nesse contexto. Essas considerações numa escala individual demonstram porque o tamanho corporal dos organismos determina o microclima que eles vivenciam (Potter e col., 2013). Além

disso, estudos na escala individual fornecem informação para analisar compreensivamente a exposição à variação climática em animais de pequeno porte (Barnes e col., 1998), incluindo as opções para regular sua temperatura e água corporal. Por exemplo, no caso dos anuros na Mata Atlântica, a escala climática mais importante é medida em milímetros ou decímetros. Portanto, informações sobre microclima, interessam para investigar a real exposição a flutuações de insolação, temperatura, umidade e disponibilidade de água, e assim entender o potencial de respostas comportamentais e fisiológicas à variação climática.

Sobre o exposto acima, é claro que compreender o microclima numa escala adequada para linhagens específicas é fundamental na conservação das espécies com relação às mudanças climáticas, especialmente em bosques com grandes transformações estruturais de habitat (Selbach e col., 2010). Entretanto, aplica-se a premissa de que, para anuros, não existe um “microclima da Mata Atlântica”, sequer ao se falar da mesma área contínua. Isso acontece porque existem variações micro geográficas relacionadas, por exemplo, com a altitude, estratificação e dimensão vertical do bosque, que impactam o microclima na mata e elementos da paisagem, como fragmentação, que podem influenciar os microclimas. Além disso, existe variação entre espécies no uso de micro-habitat e, portanto, do microclima; e existe uma variação no tamanho corpóreo e a permeabilidade da pele, que afetam a temperatura e o balanço hídrico. Portanto, diferenças importantes no impacto do microclima vivenciado podem ser evidentes mesmo entre espécies sintópicas que usem um habitat similar.

A continuação se detalha brevemente as transformações estruturais que podem se apresentar ao interior da Mata Atlântica e sua influencia no microclima e na diversidade de anfíbios.

Microclima em anuros da Mata Atlântica: Ecologia

Microgeografia e altitude

Em paisagens topograficamente complexas a distribuição da água e da umidade atmosférica pode ser altamente variável (Bennie e col., 2008), e, na medida em que se ascende altitudinalmente, o dossel diminui, as árvores passam a ser de pequeno porte, mais densas e com folhas coriáceas (Frahm e Gradstein, 1991; Stadtmüller, 1997; Bruijnzeel e Hamilton, 2000), favorecendo uma diversidade microclimática em termos de condições locais, com um potencial para influenciar a distribuição e abundância de organismos através da paisagem (Scherrer e Korner, 2011; Long e Prepas, 2012).

Estratificação

No interior destas formações florestais, Rudolf Geiger (1965) identifica três estratos microclimáticos principais: 1) o sub-bosque localizado no solo da floresta, ocupado por plantas hidrófilas e plântulas de pequeno porte, onde a matéria orgânica é decomposta, sendo reintegrado ao solo e novamente consumido pelas plantas, com grande diversidade de pequenos vertebrados como anuros, este estrato permanece fresco e com uma grande umidade, pois, ao longo do dia a radiação que chega é bloqueada pelo dossel, evitando a elevação da temperatura e o ressecamento do ar; 2) o espaço dos fustes, caracterizado por ser uma camada de transição ocupada pelos troncos das árvores emergentes e pelas copas das árvores de altura; e 3) o dossel ocupado por diversos tipos de plantas como epífitas e lianas. Este último estrato sob o ponto de vista microclimatológico, se caracteriza por uma grande instabilidade microclimática, influenciada diretamente pela radiação solar e pelo vento, com variações diárias de temperatura e umidade tão altas, que se assemelha àquela encontrada num terreno ausente de vegetação arbórea (Geiger, 1965). No

caso dos anfíbios o dossel é um importante direcionador da distribuição de espécies, onde se experimentam menores taxas de crescimento e sobrevivência por causa da desidratação (Skelly e col., 2005). Dado que anfíbios da Mata Atlântica ocupam habitats ao longo desses estratos (Haddad e col., 2013), com uma variação microclimática (Oliveira-Filho e Fontes, 2000), esperamos reportar uma variabilidade no uso de micro habitats por parte das diferentes espécies de anuros dependendo das escalas climáticas compatíveis com o tamanho dos indivíduos.

Fragmentação

Dentro da paisagem também existem importantes variações microclimáticas que podem ser consequência da alteração da fragmentação, nestas, os efeitos da área e de borda podem alterar as condições microclimáticas florestais (Murcia, 1995), inclusive desencadeando respostas estruturais como redução da diversidade e riqueza de espécies, que podem levar à extinção das mesmas (Hill e Curran, 2003). Além disso, fragmentos pequenos desconectados da floresta exibem mudanças na estrutura da vegetação e do microclima, bem como alterações na temperatura, velocidade do vento, umidade e insolação (Haddad e col., 2013). Esse conjunto de fatores pode influenciar a dinâmica de populações de anfíbios fragmentadas ou, numa escala individual, reduzir a disponibilidade de abrigos, alimento e ambientes adequados para a reprodução (Haddad e col., 2013). Anuros de floresta associados a corpos de água no sub-bosque tendem a ser afetados em curto prazo pela fragmentação, em razão de uma maior exposição à desidratação atrelada à sua necessidade de atravessar ambientes modificados na estrutura vegetal e no microclima, especialmente com mudanças na temperatura (Cushman, 2006; Becker e col., 2007).

Mata Atlântica como foco de alta diversidade de anuros

No caso da diversidade, a Mata Atlântica é um dos biomas mais diversos e prioritários para a conservação biológica dentro do continente americano (Morellatto e Haddad, 2000), apresentando afinidade com a Floresta Amazônica e a Mata de Planalto (Leitão Filho, 1987). Alguns fatores como latitude e altitude fazem da Mata Atlântica um bioma com alta diversidade climática, fisionômica e biológica (Oliveira-Filho e Fontes, 2000; Almeida, 2000; MMA, 2002; Scudeller, 2002). Dentre esta grande biodiversidade se destaca a anuro-fauna com mais de 500 espécies, sendo 88% destas endêmicas (Duellman, 1999; Haddad e col., 2008; Haddad e col., 2013; Loyola e col., 2014). Além disso, existe uma enorme diversidade no comportamento e ecologia entre as espécies de anuros, com, por exemplo, representantes de 27 dos 39 modos reprodutivos conhecidos (Haddad e Prado, 2005).

Além das influências relatadas anteriormente, os anuros presentes na Mata Atlântica diferem no uso e grau de especialização no micro-habitat, há espécies de anuros presentes principalmente do solo, na serapilheira, na superfície, parcialmente arbóreas, de riachos e de dossel (Haddad e col., 2013). Além disso, há espécies aparentemente generalistas (por exemplo, no uso genérico de subsolo, como em *Rhinella crucifer*, *R. icterica* e *R. margaritifera*) e especialistas, por exemplo no contexto de associação com bromélias em *Dendrophryniscus brevipollicatu* e *Scinax perpusillus* (Haddad e col., 2013). A história natural e a evolução atrelada ao uso prioritário de certos microhabitats associado a adaptações morfológicas, comportamentais e fisiológicas da espécie influenciam o microclima vivenciado pelos indivíduos (Crump, 1971; Pough e col., 1977; Cardoso e col., 1989) e deve existir então uma variação microclimática em decorrência da diversidade dos micro-habitats.

Ecofisiologia dos anuros em função da variação microclimática

A história natural dos anuros sugere que a manutenção do balanço hidrotermal tem um impacto importante sobre sua fisiologia e seus processos comportamentais (Lillywhite, 1971; Lillywhite e col., 1973), que se reflete em parâmetros relacionados à flexibilidade e adaptações da fisiologia. Entre os anuros o desafio da hidrorregulação é alcançado mediante estratégias fisiológicas dependendo da história de vida das espécies como: impermeabilização da pele, capacidade de baixar a taxa da filtração glomerular, e mecanismos para aumentar a capacidade da bexiga de armazenamento da água e tolerância à desidratação (Shoemaker e col., 1992), o incremento nas taxas de reabsorção de água (Titon e Gomes, 2015), a diminuição nas taxas metabólicas (Whithers e col., 1982) e excreção de resíduos nitrogenados (Shoemaker e McClanahan, 1975). Existem também estratégias comportamentais para reduzir a perda da água nos diferentes microhábitats resultantes da fragmentação, fundamentadas no mecanismo de detectar e encontrar água (Bentley e col., 1958; Seebacker e Alford, 2002; Hillyard e col., 2007; Maia, 2014).

Neste contexto, alguns grupos de anfíbios têm uma grande capacidade de manter o equilíbrio entre a temperatura corporal e a dinâmica hídrica durante as mudanças do ambiente térmico via exploração da diversidade termal da paisagem (Hock, 1967; Tracy, 1976; Brattstrom, 1979). Estes mecanismos comportamentais podem estar relacionados às preferências térmicas dependendo do estado nutricional e reprodutivo dos indivíduos (Angilleta e col., 2002) e envolve a alteração temporal do padrão de atividade (Dullman e Trueb, 1986), exposição ao sol (Valdivieso e Tamsitt, 1974; Huey, 1982), alternância periódica entre tigmotermia (ganho de energia por contato direto com o substrato) e heliotermia (Brattstrom, 1979; Sinchs, 1989;

Navas, 2006), e seleção de diferentes microhábitats com pouca variação termica, como rochas e vegetação (Navas, 1996), que também permitem evitar a desidratação (Thorson e Svihla, 1943). Além disso, os anuros têm outros mecanismos comportamentais, como a agregação com outros indivíduos (Johnson, 1969), posturas de conservação de água, pressionando a superfície ventral do corpo contra o substrato (Heatwole e col., 1969; Pough e col., 1983), e uso de hábitos frágmticos (Navas e col., 2002).

Dentro das adaptações fisiológicas nos anuros, existem diferenças tanto na tolerância à perda da água (Pough e col., 1977), quanto na variabilidade termal que pode influenciar a escolha de microhábitats (Hutchison e Dupre', 1992). Além disso, estudos ecofisiológicos têm mostrado uma importância diferenciada no tamanho (massa) corporal (Wygoda, 1988). Em anuros que não possuem mecanismos fisiológicos para a regulação da perda de água, um tamanho corporal maior pode representar uma menor perda de água por evapotranspiração, devido a uma menor relação superfície-volume (Olalla-Tárraga e col., 2009), como por exemplo, anuros do gênero *Acris*, *Hyla*, *Lithobates*, *Pseudacris* (Schmid, 1965; Nevo, 1973) e *Spea* (Thorson, 1955); assim como ocorre para anuros em condições de maior sazonalidade, como os que habitam o cerrado brasileiro (Titon e Gomes, 2015).

Tracy e col. (2010) sugerem um tamanho corporal maior como uma das características que podem ser seletivamente favorecidas em espécies de anuros arbóreos, quando apresentam um tempo de desidratação mais longo comparado aos de tamanho menor. Portanto, a presença de um tamanho corporal maior pode representar uma das características morfológicas favorecidas em espécies de anfíbios que habitam regiões de baixa disponibilidade e imprevisibilidade de água, ou com hábitos como o arbóreo, que apresentam maiores riscos de desidratação.

Assim, sendo constantes todas as outras variáveis, a massa corpórea deve influenciar principalmente a variância termal e a capacidade de retenção de água dos anuros.

Ao longo da última década a ecosiologia tem expressado grande interesse no impacto das mudanças climáticas sobre a biodiversidade, sendo um importante objetivo de a biologia moderna entendê-los (Parmesan, 2006; Tylianakis e col., 2008; Schwenk e col., 2009; Walther, 2010; Somero, 2012). Centenas de trabalhos recentes têm como objetivo entender e, inclusive, antecipar, alterações induzidas pelo clima em parâmetros tais como resiliência e distribuição das espécies em resposta a mudanças no clima. Isso envolve modelos estatísticos de distribuição (MED) (Elith e Leathwick, 2009) que confiam frequentemente em dados climáticos fornecidos por sistemas de análise como Worldclim, e que visam escalas de Km². Portanto, no caso dos anuros, parte substancial da pesquisa contemporânea se fundamenta em dados obtidos em uma escala espacial maior do que aquela compatível com o tamanho corporal dos indivíduos. Por essa razão diversos autores defendem enfaticamente a necessidade de estudos em escala adequada para entender com detalhe o impacto da variação microclimática sobre a fauna (Bakken, 1992; Kearney e Porter, 2009; Sears e col., 2011; Navas e col., 2013; Potter e col., 2013).

Esta situação requer estudos novos, de alta resolução e em escalas espaciais compatíveis com o tamanho corporal dos indivíduos, tanto para entender a variação de microclimas em ambientes específicos, quanto para criar bases de relacionamento entre microclima e clima em escalas maiores. Este desafio é enorme e admite diversas abordagens. Uma é a modelagem microclimática, fundamentada no entendimento dos animais como corpos físicos, acrescentando o maior número

possível de elementos da fisiologia para obter modelos mais precisos, tanto no tempo quanto no espaço (Bakken, 1992; Kearney e Porter, 2009; Sears e col., 2011), e que contribuem para diferenciar macroclima e microclima (Bartholomew, 1966; Willmer, 1982; Oke, 1987; Geiger e col., 2009). Os modelos físicos, por exemplo, podem mapear a heterogeneidade térmica em escalas pequenas (Bakken e Gates, 1975), mimetizando as propriedades da transferência de energia como radiação, convecção e condutividade dos organismos vivos na ausência de função fisiológica. Como tal, a temperatura do modelo no seu estado estacionário é igual à temperatura operativa (Bakken, 1992).

Estes modelos físicos requerem pouco tempo e dinheiro para sua construção, e podem ser usados para estimar a frequência das temperaturas operativas em pontos aleatoriamente determinados (Grant e Dunham, 1990) ou em intervalos ao longo de transeptos (Grant, 1990). Alternativamente, podem-se colocar em microambientes específicos e usar a área de cada microambiente para inferir a frequência das temperaturas operativas (Grant e Dunham, 1988), dependem do tipo de substrato (Adolph, 1990), a inclinação do solo (Helmuth e Hofmann, 2001; Sartorius e col., 2002), a quantidade de sombra (Christian and Weavers, 1996; Hertz, 1992) e a postura e orientação do organismo (Bakken 1989; Christian e Bedford 1996; Grant e Dunham, 1988). Modelos de ágar, assim como outros tipos de modelos físicos úmidos (esponjas, formas de cobre cobertas por tecido, modelos de gesso, etc; Bradford, 1984; Hasegawa e col., 2005; Tracy e col., 2007; Peterman e col., 2013) são importantes para estudar vários aspectos das relações térmicas e hídricas dos anfíbios (Spotila, 1972; Wygoda, 1984; Schwarzkopf e Alford, 1996; Navas e Araujo, 2000; Navas et al., 2002; Young e col., 2005). Os modelos de ágar podem ilustrar as condições ambientais experimentadas por animais reais, para algumas espécies de

anuros, as taxas de evaporação entre os indivíduos e os modelos de água são comparáveis sob condições testadas, o que significa que animais reais podem se comportar como superfícies de água livre (Spotila, 1972; Wygoda, 1984).

Outra abordagem é a obtenção de dados empíricos numa escala adequada (Wilby e col., 1998), com excelentes tentativas de síntese na modelagem mecanicista fundamentada em dados microclimáticos (Porter e col., 1973; Gates, 1980; Helmuth, 1998; Pincebourde e Casas, 2006; Kearney e Porter, 2009; Sinervo e col., 2010; Sautreau e col., 2013). Nós podemos usar a modelagem mecanicista para descrever uma relação precisa entre muitas variáveis independentes e a temperatura operativa. No entanto, precisamos conhecer os valores dessas variáveis numa escala espacial e temporal muito alta para mapear as temperaturas operativas e assim, obter uma grande quantidade de informações sobre ambientes térmicos operativos a partir de um modelo estatístico (Hertz, 1992; Howe e col., 2007; Sears e col., 2004). O poder deste modelo vai depender do grau em que as variáveis independentes como a temperatura do ar, velocidade do vento, declive, elevação, e variáveis categóricas, como o tipo de substrato ou vegetação se relacionam mecanisticamente à temperatura operativa (Angilleta Jr, 2009).

Estas duas vertentes são complementares e não excludentes, porque na medida em que dados empíricos possam ser usados para avaliar modelos mecanicistas, a qualidade e capacidade de predição desses modelos poderão ser aprimoradas. Tendo em vista o que foi colocado anteriormente, um estudo detalhado das consequências no microclima a partir da ecologia, uso do habitat, e ecofisiologia são essenciais para entender a diversidade microclimática experimentada por anuros na Mata Atlântica. Especificamente, conhecer o impacto dessas dimensões em variáveis como temperatura e disponibilidade de água se faz necessário para

entender o grau de exposição à variação microclimática, e, portanto, saber as opções para regular o balanço hidrotermal nos diferentes microhábitats usados.

Deste modo, este projeto admite hipóteses estatísticas (por exemplo, que existem diferenças entre o microclima em diferentes microhábitats), mas envolve tanto iniciativas indutivas, quanto hipotético-dedutivas. Tal situação se explica porque o estado atual do conhecimento, em muitos casos, não permite postular mecanismos que levem a predições teóricas sobre diferenças entre microhábitats. No entanto, o trabalho pode confirmar algumas tendências que parecem válidas segundo o conhecimento atual, como por exemplo: 1) que as taxas de perda de água por evaporação devem ser maiores na parte alta das montanhas, onde a exposição solar diária deve ser maior; 2) a variação termal e a variação na perda de umidade por evaporação devem ser menores dentro do sub-bosque comparadas com o espaço dos fustes e o dossel que estão expostos a variações diárias do vento e da radiação solar e maior convecção; 3) a tendência central, variância, ou extremos do universo de temperaturas operativas e da perda da água por evaporação devem ser menores em áreas com maior cobertura vegetal (interior da mata), em comparação com os ambientes expostos com menor cobertura vegetal (áreas abertas e borda da mata); 4) o efeito diferencial no potencial do ambiente para promover desidratação por evaporação dos animais medida nos modelos de ágar com menor tamanho, comparada os modelos de ágar com maior tamanho; 5) a variação na permeabilidade dos modelos de ágar, que poderia apresentar uma menor perda de água por evaporação nos modelos menos permeáveis, mas apresentaram uma maior temperatura comparada com aqueles modelos de ágar permeáveis, refletindo o compromisso entre a variação termal e perda de água por evaporação.

Capítulo 1

Inferring potencial for dehydration in terrestrial and arboreal anurans: Lessons from an agar model study in the Atlantic forest of southern Brazil

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Abstract

Climates are influenced by the structure and function of ecosystems, and, in turn, influence biological, hydrological, edaphic, and geomorphological processes. The relationships between climate and biodiversity, for example, have been studied primarily at large scales that do not explain the influence of climate, namely microclimate, at the level of individual organisms. Currently, microclimate is essential to understand the impact of climate change on biological diversity. In this context, Atlantic Forest ecosystems of Brazil exhibit unique physiognomy, biological diversity, and climate conditions that provide options of thermoregulation and hydoregulation for small animals, particularly the diverse taxa of anurans of the Atlantic Forest. The aim of this study was to investigate the temporal variation of hydration potential in terrestrial and arboreal anurans; within five environment types (high altitude forest,

wet forest, transitional forest, canopy, and open area) with three levels of vegetation cover (low, medium, high) in three seasons (interface spring/summer 2015; winter 2016; and summer 2017) of the South Atlantic Forest of Brazil. This study was conducted at Parque Estadual Intervales, São Paulo. From our sampling unit composed of four agar models that differed in size and permeability we registered operational temperatures, rates of warming, and dehydration potential, all of them analyzed from perspectives of central tendency, dispersion, and ranges. We used infrared images also to infer differences among environments in the thermal landscape experienced by terrestrial and arboreal anurans. In general, permeable models, especially of smaller sizes, have a higher rate of water loss in areas with less vegetation, especially in high altitude forest, open area and canopy. Our study also corroborated differentiated importance of body size and permeability of the anurans, in both the tolerance to the loss of water, and thermal variability that can influence the choice of microhabitats. This information will help to understand hydrothermal landscapes and impact of climate change on anurans, particularly in the context of thermal dynamics and water balance at spatial and temporal scales.

Key Words: Tetrad components, operational temperatures, hydrothermal potential, permeability

1. Introduction

Climate is composed of a set of environmental factors that interact with dynamic and structure of ecosystems (Zheng et al. 2000) and is frequently defined in the context of large-scale morph-climatic domains (Ab'saber 1997, 2005). Tropical forests, for example, are typically characterized by hundreds of thousands of square kilometers (km²) of integrated areas varying in geological relief, soil types, vegetation

forms, hydrological cycles, and climatic conditions (Ab'saber 1997, 2003). This broad-scale view of climate can hardly be related to individual-scale phenomena because, at small scales, organisms live in microclimates in which the temperatures can deviate substantially from macroclimate temperatures (Gates 1980, Angilletta 2009) affecting physiological performance and tolerance of organisms (Huey and Kingsolver 1993, Huey et al. 2012, Pincebourde et al. 2016). Data on climate at scales consistent with the body size of individuals (microclimate) is relevant to investigations of individual exposure to temperature and moisture fluctuations, including, for example, the mechanisms for thermoregulation and hydoregulation in small animals (Donnelly and Crump 1998) and, in turn, allow understanding the behavioral and physiological responses available to free-ranging animals (Dodd 2009). In the case of anurans, studies of microclimate linked with ecology, habitat use, and ecophysiology, are essential to understand the impact of exposure to climate changes and potential for response across the natural microclimatic landscape animals explore.

One context in which the above considerations are particularly critical is the Atlantic Forest, one of the world's most diverse biomes with a faunal affinity with the Amazonian Rainforest and High-Plateau Forest (Leitão Filho 1987), and that is particularly rich in terms of anuran species (Duellman 1988, Haddad et al. 2013). This biome presents a microclimatic diversity that varies with latitude, altitude, physiognomy (Oliveira-Filho and Fontes 2000, Almeida 2000, MMA 2002, Scudeller, 2002), topography, landscape, vertical stratifications (Rudolf Geiger 1965) and fragmentation (Bennie et al. 2008). Besides, changes in the structure of vegetation, changes in temperature, humidity, and precipitation in time and space can influence the pool of microclimates available for anurans, promoting shifts in community

structure across gradients and, in some contexts, decreasing species richness (Crump et al. 1992, Pounds and Crump 1994, Hill and Curran 2003).

This ecological variation may relate to morphology, physiology, behavior, systematic position and natural history of anurans (Pough et al. 1977; Cardoso et al. 1989). Also, given their typical high skin permeability, amphibians exposed to high temperatures tend to be susceptible to desiccation when compared to other tetrapods (Wygoda 1984, 1988). Terrestrial amphibians, for example, display rates of evaporative water loss (EWL) determined primarily by habitat selection, activity periods and body posture (Donnelly and Crump 1998). In other words, such rates tend not to be physiologically modulated. By contrast, in other anurans species, hydroregulation may involve skin waterproofing, ability to regulate glomerular filtration rate, water storage capacity in the urinary bladder, and a family of physiological functions integrated into what could be called “tolerance to dehydration” (Shoemaker et al. 1992). Despite important differences in natural history, hydrothermal balance is related to both physiological and behavioral processes in all anurans (Lillywhite 1971, Lillywhite et al. 1973). However, the strategies and relative importance of ecology and physiology may differ significantly even among syntopic species. Behavioral mechanisms related to search for and detection of water bodies may be crucial for water balance in some species (Bentley et al. 1958; Seebacker and Alford, 2002; Hillyard et al. 2007; Maia 2014).

This diversity in the ecology and physiology of water balance in anurans has been determined through careful observation and investigation; nonetheless, little is known about how amphibians explore and use hydrothermal landscapes in the field. One problem is that “hydrothermal landscapes” in the field are likely variable, complex and non-linear, and contrast with the carefully established gradients that

characterize experimental work in the laboratory (e.g., Hock 1967, Spotila 1972, Tracy 1976, Hutchison and Hill 1978, Brattstrom 1963, 1979). For example, field studies with agar models seem appropriate to estimate thermal ranges for amphibians because they approach a free surface in terms of skin permeability (Spotila and Berman 1976, Wygoda 1984) and convey excellent information about at least temporal gradients. Furthermore, when applying agar model techniques, it is important to consider different key aspects of amphibian natural history, skin permeability and body size (Feder and Burggren 1992). Differences in body size affect rates of EWL, heating, and cooling because, for a given radiation level, larger bodies can absorb more energy per unit of relative radiation to body mass than small bodies (Stevenson, 1985). Thus, body size relates to specific rates of EWL in anurans because the surface area is related allometrically with body mass, as has been corroborated in ecophysiology studies (Thorson and Svihla 1943, Wygoda 1988). Even if all other variables are constant, the body mass should influence primarily the thermal variance and water retention capacity of frogs.

Regarding the intrinsic potential for dehydration, amphibians evaporate water according to the resistance of the laminar boundary layer of moist air surrounding the animal, which confers greater or lesser degrees of permeability (Shoemaker et al. 1992). High rates of evaporation water loss (EWL) occur in many amphibians, however terrestrial and aquatic anurans generally exhibit lower resistance to water loss (RWL) than treefrogs (Young et al. 2005, Withers et al. 1984, Wygoda 1984, 1988). This ecological diversity and the need to incorporate EWL into analysis have hindered the use of physical models in amphibian thermal biology (Bartelt and Peterson 2005, Lillywhyte 2009). However, the physical models provide a good estimation that allows for at least general inferences on thermal and hydric

relationships (Bakken, 1992) under specific conditions, but depend on information on biophysical and physiological properties (which may be regulated). Modified agar models, for example, can mimic from zero to total permeability when using pairs of models that are identical except for an impermeable coating (Navas et al. 2002).

On the other hand, initiatives related to Worldclim or similar databases offer broad spatial coverage, but do not apply at individual scale yet, so further inference or modelling is required (Kearney e Porter, 2009; Sears et al. 2011, Navas et al. 2013, Potter et al. 2013, Pincebourde et al. 2016). Some methods such as infrared thermography allow noninvasive and real-time visualization of fixed or transient changes in the long-wave radiant energy emanating from an object, hence allowing for the estimation of the surface temperature of various substrate and organisms (Seuront et al. 2018). This technology is particularly appealing in the research fields of thermal biology, physiology, landscape, and behavioral ecology, as it allows measurements of surface temperature patterns over a very broad range of scales, from ocean basins and continents (Kerr and Ostrovsky 2003), to landscapes (Scherrer and Körner, 2010, Faye et al. 2016) and individual organisms (Tattersall and Cadena 2010, Pincebourde et al. 2013, Tattersall 2016). Nevertheless, the technique does not convey information about water availability in spatial-temporal gradients in the field. In summary, no ideal method exists to address the questions discussed above but combining methods at same or similar locations offers valuable detail on the hydrothermal gradients experienced by anurans in the field and may consider both time and space. These studies are necessary to promote physiological research, to understand whether regulation by anurans occurs in the field, and to formulate hypotheses regarding behavioral drives and neural perception of the environment.

Given the above context, we conducted a study reporting the temporal variation of hydration potential in terrestrial and arboreal anurans; within five environment types (high altitude forest, wet forest, transitional forest, canopy, and open area) with three levels of vegetation cover (low, medium, high) in three seasons (interface spring/summer 2015; winter 2016; and summer 2017) of the South Atlantic Forest of Brazil, as well as the effects of microclimatic diversity, body size and permeability.

The main question of this paper is to learn about the extent to which the structural complexity of a tropical forest influences hydrothermal balance in anurans of different ecologies. To answer this question; we compared indicators of thermal ecology and hydration potential in terrestrial and arboreal environments. We targeted microclimates exploited by actual species and looked for similarities and differences that could be relevant in the context above. Thus, we paid attention to indicators of body temperature and water balance of anurans, which we quantified in the context of the following five families of hypotheses: 1) thermal variation and evaporative water loss are enhanced in higher altitude regions of the forest. Within low elevations, these parameters should be also enhanced in open areas, forest edge and on the canopy, relative to closed areas and ground; 2) thermal variation and evaporative water loss variation are enhanced in summer, relative to spring/summer and winter. The above hypotheses relate to the central tendency, variability, and extremes of the variables studied. 3) The diverse effects pointed out in the above items should be exaggerated in anurans (or their models) of small body size, compared to larger animals; 4) Anurans of permeable skin (or models mimicking this) would be more exposed to dehydration but less exposed to thermal variation towards

the high end of the range. In contrast, waterproof anurans (impermeable models) shall present an opposed pattern.

We focus on variables representing temperature regime and water availability, which we considered particularly relevant and may be linked to potential restriction hours for amphibians (Donnelly and Crump 1998, *sensu* Sinervo 2010). The variables we have chosen are evaluated in agar models conforming tetrads (four units of measurement integrated in one system), as detailed in the Methods. Tetrads consisting of models contrasting in size and evaporation rate were considered the best option in our survey, for all terrestrial and arboreal frog species in the southern Atlantic Forest fall somewhere along the spectrum of a free water surface and an impermeable body. They also vary in size according to a known distribution. So, our tetrads are composed of four agar models, contrasting in size and permeability. The variables collected with these tetrads were operational temperatures, rates of warming, and dehydration potential, all of them analyzed from perspectives of central tendency, dispersion, and ranges.

2. Materials and methods

2.1 Study location

The Parque Estadual Intervales (PEI) is an Integral Conservation Unit with 48,000 ha situated in the Atlantic Forest (Bononi et al., 2005), and located between the municipality of Iporanga, Ribeirão Grande, and Sete Barras, in the coastal mountain range of Southeastern Brazil (24°12' - 24°25' S; 48°03' - 48°30' W; altitude between 800 and 1010 m). This conservation unit is dominated by 20 to 30 m trees in valley bottoms, changing to dense, low forest at the more elevated areas; depending

on the soil depth and fertility (Barbosa et al., 1990). It receives average annual rainfall of 4216.2 ± 245.5 mm (Pizo 2000) and includes diverse bodies of water such as lakes, ponds, rivulets, and streams. Fieldwork was carried in ~ 5 ha near to PEI administrative base.

2.2 Sampled environments

Hydrothermal variation was studied in five environments with different physiognomies and was characterized for different microhabitats used by anuran assemblages. Environment descriptions are derived from Bertoluci and Trefaut (2002), who described microhabitat use by 47 anuran species at PEI: 1. High altitude forest (1005m) (Morro da Anta) is in the highest part of PEI ($24^{\circ} 16' 20.43''$ S $48^{\circ} 24' 19.6''$ W). It is characterized by small trees (~ 3m) and bromeliads located on the floor of the understory, generally is drier but exposed to light rainfall and to the presence of mist throughout the day. 2. Wet forest (900m) is located along the east path of Morro da Anta ($24^{\circ} 27' 20.43''$ S $48^{\circ} 40' 6''$ W). This site is humid and includes larger trees ~ 40 meters in height with a variety of epiphytes and bromeliads in the canopy. 3. Transitional forest (772m) (Cachoeira do Mirante) includes medium-sized trees (~ 7m) adjacent to an open area ($24^{\circ} 27' 29.4''$ S $48^{\circ} 41' 30''$ W). 4. Open area (780m) is a large open wetland, formerly used for rice cultivation ($24^{\circ} 16' 57.3''$ S $48^{\circ} 24' 92.1''$ W), including a semi-permanent lagoon dominated by *Heteranthera zosterifolia* and *Juncus* sp. 5. Canopy habitat is present in the wet forest (Fig. 1).

2.3 Sampling unit (Tetrads)

Using agar models as a null model of free water evaporation, we provide a common point for comparison among anuran species despite potential variation in

physicochemical contexts among species. This comparison requires four cautions: 1) the models do not display identical physicochemical properties as frogs, 2) a shape and size similar to frogs is necessary, 3) the agar models must have a water-conserving posture similar to live frogs, and 4) permeable and impermeable agar models are necessary to represent degrees of permeability of frogs. The use of agar models has been discussed in the literature (see for example Bakken, 1992, Navas and Araujo 2000, Bartelt and Peterson 2005, Lillywhyte 2009, Riddell et al. 2017), and it requires a number of careful considerations. Our study is based on the assumptions that agar models are good null models for this analysis despite their lack of physiology, for we focus on the potential impacts of the environment. However, we acknowledge that our analysis is designed to compare null models of static animals and that moving animals may depart heavily from the trends here reported.

Our sampling unit, the tetrad, consisted of four agar models shaped as anurans but differing in size (*Rhinella icterica* SVL~ 15 cm for “large model versus *Physalaemus olfersii* SVL ~ 6 cm for “small model”) and permeability (fully permeable to waterproofed). The terms “large” and “small” derive from calculations of body mass frequency distributions of anurans at PEI using published literature (Haddad et al. 2013) and field data. The two sizes of agar models were derived from a distribution of mean snout-vent lengths ($8.92 \text{ cm} \pm 4.71\text{SD}$). The largest sized frog reported was 11.81cm and 121.72 g, which corresponds to the “large” models and encompasses 95% of the distribution. The minimum size was 4.27cm with 10g of mass; that corresponds with “small” models and encompasses 5% of this same distribution (Fig. 2A). With this range, we aimed to include 90% of the size range of anurans in the Atlantic Forest, avoiding both miniaturized species, for which conventional methods

may not apply, and the largest species, which are not practical to simulate using agar models. Each tetrad included 1) large permeable (LP), 2) small permeable (SP) 3) large impermeable (LI) and 4) small impermeable (SI) (Fig. 2B). All agar models were made from alginate casts (22 g/l; modified of Navas and Araujo 2000). Waterproofing for models was achieved using a layer of varnish (SIPA®). The maximum linear distance between models within a tetrad was ~ 10 cm.

2.4 Microclimate monitoring

We conducted our research in three seasons: 1) Interface spring/summer 2015 (16 to 31 October); 2) winter 2016 (23 to 30 June and 1 to 7 July); and 3) summer 2017 (6 to 21 February). We identified 12 study sites along defined transects in each environment (four in each vegetation cover level) and two study sites in the canopy. We recorded the operational temperature using twelve tetrads distributed throughout each environment and surrounding the twelve study sites and two tetrads for the canopy. Each model in the tetrad was fitted with a HOBO® Data Logger (U12-008) sensor programmed to record temperature every 15 min for three days. Based on these data, the lower and the higher rates of warming were calculated. In parallel to operational temperature recording, we recorded air temperature (mean, maxima, and minima) and relative humidity (mean, maxima, minima) continuously over three days in each environment with a HOBO® Data Logger (U10-003) programmed to record every 15 minutes. Additionally, we characterized the thermal landscape surrounding the models with infrared images (thermography) in winter 2016 and summer 2017 and measured both model surface temperature and environmental temperature using a FLIR C2 Compact Thermal Imaging System Camera $\pm 1^{\circ}\text{C}$ (Fig 3).

2.5 Hydration potential monitoring

The hydration potential of the environment was measured using tetrads without sensors. Each model was weighed every two hours between 06:00 to 17:00h and every three hours between 17:00 to 23:00h for three days in each of the five environments. Water loss rate was estimated from the difference between initial weight and the final weight of each agar model from each tetrad, divided by the exposure time.

2.6 Statistical analysis

We compared mean air temperature of each environment using a one-way ANOVA followed by an *a posteriori* test (Tukey) to detect significant differences in these five environments with three levels of vegetation cover (L, M, and H). To compare the dehydration potential of each environment, we used a one-way ANOVA followed by an *a posteriori* test (Tukey) to detect significant differences in these five environments with three levels of vegetation cover (L, M, and H). To determine patterns of variability in the hydration rate obtained from our tetrads and from environmental variables, we used a Principal Component Analysis (PCA) applied to the following variables: hydration potential from the agar models (LP, SP, LI, SI); operational and air temperature (mean, max, min); relative humidity (mean, max, min); low and high heating rate; vegetation cover level (L, M, and H), and time of day (05:00; 09:00; 11:00; 13:00; 15:00, 17:00, 20:00, 23:00h). Additionally, we included five variables derived from infrared images of the thermal landscape (mean, maximum, minimum, standard deviation, and model temperature) for winter 2016 and summer 2017. Finally, we find the most parsimonious generalized linear mixed model

(GLMM) that includes the minimum number of parameters estimated by Akaike information criterion (AIC) to explain the behavior of our response variable (hydration rate).

3. Results

3.1 Thermal variation across environments

Mean air temperature differed among the five environments (high altitude forest, wet forest, transitional forest, canopy, and open area) and among each season studied (interface spring/summer 2015 ANOVA $F_{(4; 2587)} = 237.41$ $P < 0.000001$; Winter 2016 ANOVA $F_{(4; 4771)} = 355.34$ $P < 0.00001$ and Summer 2017 ANOVA $F_{(4; 4795)} = 127.19$ $P < 0.000001$) (Fig. 4). Overall, the highest temperatures were recorded in summer 2017 (between 22° to 27°C), followed by interface spring/summer 2015 (18° to 26°C) and winter 2016 (13° to 22°C). Independently of the time of year, the open area had the highest temperatures recorded followed by transitional forest, canopy, high altitude forest, and finally wet forest. Wet forest and high altitude forest recorded the lowest temperatures.

Within each season, the variance and extreme values of temperature were recorded in the open area followed by the transitional forest and canopy (Table 1). The temperature variation differed among each season studied (*Levene's test* $F_{(2; 12149)} = 239.43$ $P < 0.0001$) and among the five environments. Overall, within each environment, the highest temperature was recorded in areas of low vegetation cover (Tables 2, 3). Among the five environments, the wet forest exhibits a temperature stability, the ideal climatic parameter to keep the potential of hydration constant in all the models (Fig. 5A, B and C). Relative to the time of day, the greatest variation and

extreme values in temperature were recorded between 11:00 at 15:00h in all environments (Fig. 6A, B and C).

3.2 Evaporative water loss across environments

In general, the mean percentual hydration measured from the agar models differed among the five environments (high altitude forest, wet forest, transitional forest, canopy, and flooded open area) and among each season studied (interface spring/summer 2015 ANOVA $F_{(12; 2571)} = 4.9827$ $P < 0.000001$; Winter 2016 ANOVA $F_{(12; 4756)} = 3.4518$ $P < 0.00004$ and Summer 2017 ANOVA $F_{(12; 4779)} = 3.9618$ $P < 0.000001$) (Fig. 7). Overall, tetrads experiencing the highest dehydration potential were recorded in the open area and canopy, followed by high altitude forest principally in the interface spring/summer 2015 and summer 2017. Tetrads from the wet forest and transitional forest recorded the lowest dehydration potential, and values were similar among tetrad units.

The variance and extreme values along of time in the hydration potential were recorded for the open area followed by the high altitude forest and canopy (Table 4). The hydration variation differed among the five environments and among each season studied (*Levene's test* $F_{(14; 12153)} = 135.21$ $P < 0.000001$). Overall, within each environment, the highest percentage of dehydration was recorded for the pair of permeable models of each tetrad, and greatest for the small model in areas of low vegetation cover (Tables 5, 6). Among the five environments, the wet forest exhibits a microclimatic stability with a temperature and humidity homogenous, ideal climatic parameters to keep the potential of hydration constant all the models (Fig. 8A, B and C). Relative to the time of day, the greatest variation and extreme values in hydration were recorded between 13:00 at 15:00h in all environments (Fig. 9A, B and C).

3.3 Evaporative water loss patterns within environments and among seasons

We observed patterns of distribution of the agar models derived from the linear composite variables (PCA factors) that are related to microclimate variation (temperature and humidity), vegetation cover levels, and time of day in each environment and in each season independently (Figures 10 to 24). In all cases, these patterns were generated from two factors with Eigenvalues higher than one and with a percentage of total variance higher than 15 %. Nevertheless, these factors are neither similar across environments nor do they have similar values for the same variables (Factors are reported in figures 10 to 24). In general, we recorded a negative effect of temperature on the percentage of hydration for the permeable models, especially for the small models located in areas with exposed environments and between 13:00 to 17:00h. Among seasons, we recorded a negative stronger effect of temperature on the percentage of hydration in summer 2017 and the interface spring/summer 2015, relative to winter 2016. Nevertheless, for winter 2016, we observed a negative effect of temperature in the permeable models within high altitude forest, similar to that of other seasons. Below, we present the patterns of distribution of agar models present in each environment and in each sampling season.

3.3.1 Interface spring/summer 2015

High altitude forest showed that the hydration was affected negatively by the temperature variation (Factor 1), especially registered in the permeable models (Fig. 10A), among 13:00 to 17:00h (Fig. 10B) located in areas with lower vegetation cover

(Fig. 10C). The second Factor showed that the higher rate of warming negatively influenced the hydration variation registered on permeable models as well.

PCA analysis in the wet forest reflects a homogeneity at the hydration variation present in the agar model distributions (Fig. 11A), nonetheless, the models distribution is affected by time of day among 11:00 to 17:00h (Fig. 11B) and areas with lower vegetation cover (Fig. 11C). However, this water loss is lower than in high altitude forest. The second Factor, based on the higher rate of warming, affects negatively the hydration variation present in all agar models.

Transitional forest pattern reflects a partial homogeneity in the hydration variation present in the agar model distribution (Fig. 12A), nonetheless, the model distributions in the PCA is affected by time of day among 13:00 to 15:00h (Fig. 12B) and in areas with lower vegetation cover (Fig. 12C). The second Factor, based on the maxima relative humidity, does not affect the hydration variation present in all agar models.

Open Area does not reflect a tendency to differentiate the hydration variation registered in the agar models (Fig. 13A). Additionally, the vegetation cover is scarce and does not allow a heterogeneous landscape to differentiate the variation in this environment (Fig. 13C) independent of the time of day (Fig. 13B).

Canopy patterns reflect a differentiated tendency for hydration variation in the agar models (Fig. 14A). There is a greater loss of water, especially among 11:00 to 13:00h (Fig. 14B), independently of the vegetation cover (Fig. 14C).

3.3.2 Winter 2016

PCA data showed in the high altitude forest a distribution differentiates in the hydration variation affected by the temperature variation (Fig. 15A), especially among

11:00 to 17:00h (Fig. 15B) in areas with lower vegetation cover (Fig. 15C). The second Factor showed that the relative humidity positively influenced the hydration registered on medium and high vegetation cover.

Wet forest showed a homogeneous distribution in the hydration variation (Factor 1) registered in all models (Fig. 16A) independently of the time of day (Fig. 16B) and the vegetation level (Fig. 16C). However, the second Factor based on the minimum and mean relative humidity affects the distribution of models; in areas with lower vegetation (Fig. 16C).

This analysis in the transitional forest reflects homogeneity in the hydration variation present in the agar model distribution (Fig. 17A), nonetheless, this distribution within PCA is affected by time of day (Fig. 17B) and vegetation cover level (Fig. 17C). The second Factor based on the maximum temperature of the thermal landscape affects slightly the distribution of models; in areas with lower vegetation (Fig. 17C).

Open area PCA does not reflect a tendency to differentiate the hydration variation registered in the agar models (Fig. 18A). The low vegetation cover present in this environment does not allow a heterogeneous landscape to differentiate the variation (Fig. 18C). In this environment, the significant differences of the hydration variation depend on weather conditions, especially by the maximum temperature of thermal landscape among 11:00 to 15:00h registered in the lower quadrants (Fig. 18B).

In the canopy area reflects a tendency differentiated in the hydration variation in the agar models related to the relative humidity (Fig. 19A). Nevertheless, this distribution does not show differences for both the weather conditions along of day (Fig. 19B) and vegetation cover (Fig. 19C).

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PCA analysis reflects a homogeneity in the hydration variation within of high altitude forest (Factor 1) present among the agar model distributions (Fig. 20A), nevertheless, there is an influence in this distribution among 11:00 to 15:00h (Fig. 20B) and areas with lower vegetation (Fig. 20C). The parameters of the second Factor did not influence hydration variation registered on all models.

The Factor 1 showed that within wet forest the hydration variation was affected negatively all models by the air temperature variation (mean and maxima, Fig. 21A) especially among 13:00 to 17:00h (Fig. 21B) located in areas with lower vegetation cover (Fig. 21C). The second factor based on the maximum relative humidity affected in the same way this distribution of models as well as the temperature variation present in the first factor.

This analysis registered in the transitional forest showed the agar model distribution (Fig. 22A) based in the air temperature variation, among 11:00 to 17:00h (Fig. 22B) in lower vegetation cover (Fig. 22C). Similarly, this distribution of models is affected by mean operational temperature (Factor 2).

Open area does not reflect a tendency differentiated in the hydration variation registered in the models agar (Fig. 23A) In this environment the significant differences from the distribution depend of the weather conditions among 09:00 to 17:00h (Fig. 23B), but is independent of the vegetation cover (Fig. 23C).

PCA does not reflect a tendency differentiated within canopy in the hydration variation in the agar models related to air temperature and maximum operational temperature (Fig. 24A, B, C).

3.4 Differential effect on the evaporative water loss in relation to size and permeability

Based on published literature and previous work about hydration in anurans, we built 14 linear models that included different combinations and interactions of parameters such as size and permeability of agar models, temperature, humidity, environment type and season. These interactions include different microclimatic changes along the day (e. g. temperature and humidity) within environments, which can trigger behavioral responses in the use of different microhabitats for hydrothermal balance in anurans in a temporal scale attributed to altitude and structural differences such as levels of vegetation cover and fragmentation. Additionally, size and permeability associated with the use of habitat affect rates of EWL in anurans.

The most parsimonious linear mixed model called: Warming presented a value $AIC = 57137.71$; with $wAIC = 1$ (Table 7). This model incorporated the next parameters: size and permeability of models, maximum air temperature, maximum relative humidity, vegetation cover level, environment type, and interactions among environment type and the vegetation cover level. The most significant interactions of this linear mixed model are presented in Table 8.

4. Discussion

This research offers a general diagnostic of how microclimate responds to the structural complexity of a tropical forest. The study, contextualized in the ecology of anuran amphibians, indicates that microclimate selection, body size, and seasonal variation influence, by themselves and through synergisms, influence body

temperature and potential for hydrothermal balance. The challenges imposed by this balance varies among terrestrial and arboreal anurans and is affected by diverse details of ecology in the Southern Atlantic Forest.

4.1 Thermal variation and evaporative water loss across environments

Our results on operational temperatures and water loss in agar models support the hypotheses that thermal variation and evaporative water loss for terrestrial and arboreal anurans are greater in higher altitude regions of the Atlantic Forest, and that elevational gradients, particularly above of 1000 m, are of consequence. Although each environment exhibits daily cycles in microclimatic conditions throughout the day, the most pronounced variation occurs in altitude forest compared with other environments, for example, wet areas and transitional forest, open area and canopy. So, we assume that, in the context of hydrothermal balance, behavioral responses and association with microhabitats may be more evident in anurans from a higher elevation, and that these responses may be more prone to natural selection. This is a strong pattern permeating measures of central tendency, dispersion, and full ranges of operational temperature. This pattern likely has underlying mechanisms in changes of solar radiation, convection and wind speed during the day, like in the density of air layers and air temperature, and vegetal cover that decreases with increasing altitude (Geiger 1965). For example, maximum air temperature occurs when the sun is perpendicular to the top of the mountain surface and the minimum air temperature occurs with the sunrise. So, high elevations in the Atlantic Forest studied, although technically a forested environment, display an overall microclimatic

profile resembling that of open environments, and that increases the potential for dehydration particularly for small permeable frogs. Among the species observed in the field, perhaps *Scinax perpusillus*, which lives and breeds in bromeliads on the mountaintop, may be particularly affected in the sense that this microhabitat association may be essential for survival. This may not be a general trend for anurans, since we also observed *Rhinella icterica* foraging between 10:00 and 12:00h, when operational temperatures are highest. These focal observations, together with the overall data, suggest that both strong microhabitat associations and large body size become permissive conditions that allow for the occupancy of these areas among anurans.

Temporal and spatial variation of microclimate in low elevation environments (wet and transitional forest, open area and canopy) emerged also from *stratification*, a term we will use only when referring to the vertical arrangement layering of a habitat, and which interacts with other structural factors such as forest fragment type and vegetation cover level (Root and Scheider 1995). When considered low altitude forest, thermal variation and evaporative water loss for terrestrial and arboreal anurans is greater in open areas, forest edge, and in near areas to the canopy, relative to the understory. Overall, open areas display the highest operational temperatures and the greatest evaporative water loss. This feature is tied to the direct incidence of solar radiation over the soil surface according to Daubenmire (1979). Frogs in these environments are exposed to drastic shifts in temperature and humidity. Such rapid transitions likely affect terrestrial anurans such as *Rhinella crucifer*, *R. ornata*, *R. margaritifera*, *Leptodactylus fuscus*, and *L. ocellatus*, perhaps typically intolerant to high temperatures and dehydrating conditions (Churchill and Storey 1995, Shoemaker and Naggy 1977, Shoemaker et al. 1992, Tracy et al. 2010,

Tracy et al. 2013, Tracy et al. 2014). Nevertheless, near to these areas, there are water bodies may favor the presence of semi-aquatic anurans such as *Physalaemus cuvieri* and treefrog species such as *Dendropsophus microps*, *D. sanborni*, *D. werneri*, *Hyla albosignata*, *Scinax crospedospilus*, and *S. hayii*. Given trends in the data and field observations, individuals of these and other species with similar ecologies may depend on such water bodies. Also, these species may benefit from gradients and thermally variable microhabitats under the influence of herbaceous and shrub layers present in this area, and from water conservation strategies (Heatwole et al. 1969, Pough et al. 1983).

The fragmentation of forest also has implications for anurans. For example, at the forest edge, variation in microclimate compares to that experienced by anurans in open environments. We observed anurans both at the forest edge and inside the forest, but our study suggests that potential for dehydration would be higher in the former, particularly for small permeable frogs including *Bokermannohyla circumdata*, *Dendropsophus minutus*, *Hypsiboas bischoffi*, *Scinax hayii*, *Physalaemus olfersii* and *Proceratophrys boiei*.

The structure and density of canopy affect the vertical stratification of microclimate within a forest, apparently tied to the amount of solar radiation received (Pezzopane et al. 2002). These climatic parameters associated with cover are relevant in studies related to the abundance and diversity of animals, especially anurans (Skelly et al., 1999, Werner and Glennemeier 1999, Skelly and Freidenburg 2000, Skelly et al., 2002, Halverson et al., 2003, Skelly and Golon 2003, Skelly et al., 2005). Our data support that, at PEI, frogs living in the canopy experience a microclimatic profile resembling that experienced by terrestrial and arboreal anurans in partially open environments, which are characterized by high potential for

dehydration. Canopy microclimate is influenced directly by the high variation in temperature and humidity, and these characteristics affect particularly evaporative water loss for small permeable frogs (Pezzopane et al. 2002). Overall, body size seems important because larger frogs absorb more energy per unit of body mass and have higher thermal capacity than small counterparts. In the canopy, we find larger arboreal anurans like *Gastrotheca microdiscus*, *Phyllomedusa distincta*, and *Trachycephalus imitatrix*, which display specialization to avoid water loss such as mucus and/or lipids secretion on skin surfaces. So, these data support a view in which such reduction of permeability emerges in the context of dehydrating environments (canopy) with sources of water nearby (floor) (Tracy et al. 2010, Tracy et al. 2014)

According to our study, factors reducing microclimatic variation in the understory of the closed forested environment (wet forest) include high vegetation and dense canopy (Geiger 1965, Hidore and Oliver 1993; Hupfer and Kuttler 1998). During the day, solar radiation is blocked by the canopy, thus temperature increases are minimal (Geiger 1965, Cunningham et al. 2008, Renaud and Rebetez 2009). These factors tend to enhance overall climatic stability and create vertical thermal flows between the soil and different substrates (Hidore and Oliver 1993), increasing the humidity inside the forest (Walsh 1996A). Under these conditions, a large number of arboreal and terrestrial anuran species tend to experience similar temperature and hydric conditions even on cloudy days, a factor that may promote less costly hydrothermal regulation, particularly for small species. More implications of body size are presented in section 4.3.

4.2 Evaporative water loss patterns of each environment and among seasons

Our conclusion on the microclimatic variation of each environment relies on a PCA analysis applied to variables associated with anuran distribution in the habitat. This distribution was represented by the spatial position of agar models in a coordinate system related to climatic parameters, such as operational and air temperature, and relative humidity. These results also inform on the scope of variation in microclimatic conditions along of day and among levels of cover vegetation, in a scale compatible with the typical size of Atlantic forest frogs, within of each environment. We observed that the microclimate parameters, especially temperature at highest altitude may be challenging for small frog species with permeable skin because they are at higher risk of dehydration (Churchill and Storey 1995, Shoemaker and Naggy 1977, Shoemaker et al. 1992, Tracy et al. 2010, Tracy et al. 2013, Tracy et al. 2014).

In Transitional forest, the microclimates resemble those of a wet forest in the sense that both are strongly influenced by thermal and humidity factors. However, important differences may develop even within very short distances, and potential for water loss variation depends on whether frogs are in the adjacent open area or inside the forest. These differences are large enough, and possibly may be sensed by at least some frogs. We suppose that some anuran species may, through environmental sensing, be less prone to leave a forested environment.

Open areas in the Southern Atlantic forest seem characterized by large thermal variation, a higher rate of warming, and influenced by a high solar radiation rate due to low or non-existent vegetation cover. The microclimatic conditions here

are strongly related to thermal and humidity factors influenced by rain patterns. This variation reaches drastic conditions occasionally, some possibly fatal for small permeable terrestrial amphibians, typically intolerant to high temperatures and dehydrating conditions. Finally, Canopy is characterized by large thermal variation and influenced by a high solar radiation, like open areas. This variation may have drastic consequences for small permeable treefrog species. Overall these results show that the potential for dehydration and magnitude of thermal variance shifts mark the possible passage from fully covered areas to open areas within wet, low-altitude ranges of the Atlantic Forest.

Finally, some microclimatic differences found among study periods are related to the macroclimatic characteristics specific of each season and to daily cycles. Below, we present the macroclimatic characteristics in each season studied. Interface spring/summer 2015 was characterized by a climatic irregularity, because is a period of transition between winter (dry) and summer (rainy). During this season, we recorded a strong daytime heating that may be challenging for small frog species. Winter 2016 presented the driest and coldest period of the year, with the lowest rainfall and temperature index. In this season humid mist is common early in the morning, mainly in the high-altitude forest, but also so in some low elevation areas where temperatures below zero are reached, and frosts occur. This characteristics and parameters may be explanatory when analyzing presence/absence of anuran species of Atlantic forest. During the summer of 2017, enhanced solar radiation favored higher temperatures, increased precipitation and relative humidity. In this context, with intense rains along most of the day, electric discharges and strong winds, we observed the highest number of anuran species from PEI. To finish, independently of the season, the time of day with higher temperatures was typically

13:00 to 15:00h, a period that, under a different rain pattern, could be particularly severe for small species due to their high risk of dehydration. As a case, we registered individuals of *Rhinella icterica* actively foraging during this time. This observation, together with other reported in this manuscript, support the notion that larger size confers some independence from climatic variables.

4.3 Differential effect on the evaporative water loss in relation to size and permeability

According to our study, smaller frogs have greater thermal variability and higher rates of dehydration in high altitude forest independent of season and in low vegetation cover areas within low elevation environments (wet and transitional forest, open area and canopy) especially in spring and summer, relative to large frogs. Geise and Linsenmair (1988) reported that individuals of smaller species tend to have higher rates of dehydration and are more vulnerable to reach near-lethal levels and proposed a relationship between body size and life in the tropics. The rationale for this hypothesis is that the reduced surface-to-volume ratio of larger species makes them less prone to water loss, conferring a stronger desiccation tolerance in drier areas (Nevo 1973, Ashton 2002). This hypothesis may be relevant in tropical regions with a pronounced dry season, such as the Cerrado and Caatinga biomes. Olalla-Tárraga et al. (2009) identified a trend of increasing anuran body size with decreasing available environmental water in Cerrado. According to Navas et al. (2004), ~ 40 anuran species in the Caatinga have developed physiological, behavioral, reproductive or morphological adaptations to survive dry seasons, including aestivation, phragmotic behavior and changes in skin morphology to improve water balance and thermal tolerance. Although Atlantic forest does not have

dry areas in the sense of “arid”, or a pronounced dry season, the high altitude forest and open areas has characteristics resembling those of a dry tropical area. In this context, the hypothesis of surface-to-volume ratio in the evaporative water loss, would seem relevant, with adjustments.

In our study area, most small anurans species inhabit the open area with highest temperatures and variable humidity, conditions that make small individuals susceptible to desiccation; nevertheless, the tolerance capacity of these species to desiccation possibly is associated with the use of habitat. For example, semiaquatic/arboreal species such as *Aplastodiscus perviridis*, *Bokermannohyla circumdata*, *Dendropsophus microps*, *D. sanborni*, *D. weneri* and other species of the family Hylidae registered in our open area that exhibit a greater tolerance to dehydration and use a great variety of substrates thermally less variable of the herbaceous layer such as various herbaceous plants, grasses, moss, dwarf shrubs such as hemicryptophytes, therophytes and chamaephytes, young shrubs and rocks. Other behavioral mechanisms observed in these species were the exploration of landscape thermal diversity, like those reported in twelve species of hylid frogs from northern Australian in laboratory conditions (Tracy 2005). Species of the Bufonidae family (*Rhinella icterica*, *R. ornata*) are also present in this open area, nevertheless, they use less the variety of substrates available, however, have larger sizes that may represent a lower water loss by evapotranspiration due to a lower surface-volume ratio (Olalla-Tárraga et al., 2009). In addition, these species may have a specialized bladder, where urine retention could help maintain a constant internal solute concentration by reabsorbing water from body fluids such as other species of Bufonidae family (Ruibal, 1962).

Regarding permeability, the most notorious result is permeable frogs had higher water loss in all five environments, nevertheless, high altitude forest independent of season and in low vegetation cover areas within low elevation environments presented the highest rates of dehydration. In these environments with highly variable climates, the structure, function, and variation of skin resistance in amphibians are important, especially with temperature regulation, water relation, and gas exchange (Lillywhite 2009). However, the need to keep the integument permeable to respiratory gases leads to high rates of water loss through evaporation in most amphibians. Some studies have suggested the importance of the relationship between skin thickness in anurans and the environment (Toledo and Jared 1993); however, skin thickness alone is not the main mechanism to reduce water evaporative loss (Lillywhite, 2006). In this way, our permeability treatment simulated the variation in skin resistance to moist air surrounding the animal, based in current theory (Shoemaker et al. 1992), and with the expectation that arboreal species tend to be less permeable (Young et al. 2005; Tracy et al. 2008, Tracy et al. 2014). In this sense, arboreal anuran species such as *Phyllomedusa distincta* present in the wet and transitional forest, open areas and canopy; as well as *Gastrotheca microdiscus* and *Trachycephalus imitatrix* presents in the canopy can secrete lipids from specialized cutaneous glands create an external impermeable barrier to minimize trans-epidermal water loss (TEWL) (Lillywhite and Mittal 1999; Lillywhite 2006).

In the above context, anurans with arboreal habits are generally more resistant to water loss when compared to terrestrial and aquatic species (Whithers et al., 1984, Wygoda 1984). We registered; species with impermeable skin have higher resistance to water evaporative loss in areas with high thermal variability as the high-altitude forest and in low vegetation cover areas or a season as summer. Young et al. (2005)

demonstrated this influence of habit on the resistance to water evaporative loss, which remains significant even after correcting for phylogenetic relationships of the 25 species included. Titon (2010) also recorded high resistance to water evaporative loss in two anurans species with specialized cutaneous glands as *Phyllomedusa distincta* and *P. tetraploidea* in arboreal habitats. This habitat relationship was also recorded by Titon (2012), who compared species of anurans from the Atlantic Forest and Cerrado Biome with species from dry environments of Costa Rica and Brazil (Wygoda, 1984) and with species from Africa and Australia (Whiters et al. 1984). Cruz-Piedrahita et al. (2018) compared 17 species of anurans, including arboreal and terrestrial, in a semi-arid environment of Colombia, and recorded that arboreal species can maintain and absorb water more efficiently from a standing source and, perhaps, are more efficient in detecting water than terrestrial species. The above results suggest that the increase in resistance appeared multiple times throughout the evolutionary history of this group and hydric characteristics of altered and/or open areas and forested environments associated with microhabitat use may constitute ideal systems for comparative studies of the relationship between physiological aspects of water balance and anuran habitat distribution.

5. Conclusions

Our study has presented the implications of microhabitat associations for hydrothermal balance in anurans from the Atlantic Forest. By using agar casts as null models, we have shown that not only broad physiognomy patterns matter, but also that variation within these physiognomies may be substantial, and highly dependent on weather and local factors. Our results also show a complex network of relationships among operational temperature, air temperature, and that the use of different microhabitats across the Atlantic forest. These relationships provide insights

into habitat selection requirements for anuran species and distribution within a general habitat. The thermal landscape in each environment analyzed has implications given habitat selection and ecological associations of species: high altitude forest, canopy, and the open area exhibit high thermal instability caused by high levels of radiation received during the day. These areas include many species from the Hylidae and Leptodactylidae families, most of them nocturnal.

Wet and transitional forest exhibits greater availability of habitats in the sense of continuity and extension, because of climate homogeneity. In these environments, most anuran species are nocturnal in terrestrial and arboreal habitats, selecting substrates that minimize water loss throughout the day to remain hydrated. In amphibians, as corroborated by our study, spatial and temporal temperature variation and hydration variation depend on microhabitat selection and likely play an important role in anuran evolution (Lehtinen et al. 2004).

Our study also corroborated the importance of body size (mass) and permeability of anurans, both parameters related to tolerance to hydric challenges, and thermal variability. So, size and permeability likely relate to microhabitat choice or occupancy. A larger body size and impermeable skin may be relevant for anuran species in habitats promoting longer exposure to dehydration, relative to smaller, more permeable species. The possibilities to buffer climatic challenges and extremes are influenced by these parameters and is a key aspect of conservation in a changing environment.

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7. References

- AB'SABER, A.N. Os domínios morfoclimáticos na América do Sul. Primeira aproximação. Geomorfologia, Instituto de Geografia, Universidade de São Paulo. 53: 1–23, 1997.
- AB'SABER, A.N. Os domínios de natureza no Brasil: potencialidade paisagística. Ateliê Editorial, São Paulo. 163 pp, 2003.
- ALMEIDA, D.S. Recuperação ambiental da Mata Atlântica. Ilhéus: Editus, p 130, 2000.
- ANGILLET, Jr. M.J. Thermal adaptation: a theoretical and empirical synthesis. Oxford University Press, Oxford. 2009.
- ASHTON, K.G. Do amphibians follow Bergmann's rule? - Canadian Journal Zoology 80: 708–716, 2002.
- BAKKEN, G.S. Measurement, and application of operative and standard operative temperatures in ecology. American Zoologist 32: 194–216, 1992.

- BARBOSA, L.M.; BONONI, V.L.; MANTOVANI, W. Zoneamento Agroecológico da Fazenda Intervales. Relatório Técnico, IBt/ IBUSP, Sao Paulo, Brazil, 1990.
- BARTELT, P.E.; PETERSON, C.R. Physically modeling operative temperatures and evaporation rates in amphibians. *Journal of Thermal Biology* 30: 93–102, 2005.
- BENNIE, J.; HUNTLEY, B.; WILTSHIRE, A.; HILL, M.O., BAXTER, R. Slope, aspect and climate: spatially explicit and implicit models of topographic microclimate in chalk grassland. *Ecological Modelling* 216(1): 47–59, 2008.
- BENTLEY, P.J.; LEE, A.K.; MAIN, A.R. Comparison of dehydration and hydration of two genera of frogs (*Heleioporus* and *Neobatrachus*) that live in areas of varying aridity. *Journal Experimental Biology* 35: 667–684, 1958.
- BERTOLUCI, J.; TREFAUT-RODRIGUES, M. Utilização de habitats reprodutivos e micro-habitats de vocalização em uma taxocenose de anuros (AMPHIBIA) da Mata Atlântica do sudeste do Brasil. *Papéis Avulsos de Zoologia* 42 (11): 287–297, 2002.
- BONONI, V.; GUILHERME, F.A.G.; ALMEIDA-SCABBIA, R.J.; MORELLATO, L.P.C. Levantamento florístico de floresta Atlântica no sul do estado de São Paulo, Parque Estadual Intervales, base Saibadela. *Biota Neotropica* 5(1): 147–170, 2005.
- BRATTSTROM, B.H. A preliminary review of the thermal requirements of amphibians. *Ecology* 44(2): 238–255, 1963.
- BRATTSTROM, B.H. Amphibian temperature regulation studies in the field and in the laboratory. *American Zoologist* 19: 345–356, 1979.

- CAMARGO, J.L.C.; KAPOS, V. Complex edge effects on soil moisture and microclimates in Central Amazonia Forest. *Journal of Tropical Ecology* 11: 205–221, 1995. <http://dx.doi.org/10.1017/S026646740000866X>
- CARDOSO, A.J.; ANDRADE, G.V.; HADDAD, C.F.B. Distribuição espacial em comunidades de anfíbios (Anura) no sudeste do Brasil. *Revista Brasileira de Biologia* 49: 241–249, 1989.
- CHURCHILL, T.A.; STOREY, K.B. Metabolic effects of dehydration on an aquatic frog, *Rana pipiens*. *The Journal of Experimental Biology* 198: 147–154, 1995.
- CRUMP, M.L.; HENSLEY, F.R.; CLARK, K.I. Apparent decline of the golden toad: underground or extinct? *Copeia* 1992: 413–420, 1992.
- CRUZ-PIEDRAHITA, C.; NAVAS, C.A.; CRAWFORD, A.J. Life on the edge: A comparative study of ecophysiological adaptations of frogs to tropical semiarid environments. *Physiological and Biochemical Zoology* 91 (1): 740–756, 2018.
- CUNNINGHAM, J.D. Aspects of the ecology of the Pacific slender salamander, *Batrachoseps pacificus*, in southern California. *Ecology* 41(6): 88–99, 1960.
- CUNNINGTON, G.M.; SCHAEFER, J.; CEBEK, J.E.; MURRAY, D. Correlations of biotic and abiotic variables with ground surface temperature: An ectothermic perspective. *Ecoscience* 15(4): 472–477, 2008.
- DAUBENMIRE, R.F. *Ecología Vegetal: Tratado de Autoecología de plantas*. Limusa, Mexico. 1979.
- DODD, C.K. Jr. *Amphibian Ecology and Conservation*. Oxford University Press. 2009.
- DONN, W. *Meteorología*. Reverte. Barcelona. 1978.
- DONNELLY, M.A.; CRUMP, M.L. Potential effects of climate change on two neotropical amphibian assemblages. *Climate Change*. 39: 541–561, 1998.

- DUELLMAN, W.E. Distribution patterns of amphibians in South America. In: Patterns of Distribution of Amphibians: a Global Perspective. Duellman W.E. (ed.). The John Hopkins University Press, Baltimore. pp. 255–328, 1998.
- FAYE, E.; DANGLES, O.; PINCEBOURDE, S. Distance makes the difference in thermography for ecological studies. *Journal of Thermal Biology* 56: 1–9, 2016.
- FAYE, E.; REBAUDO, F.; YANEZ-CAJO, D.; CAUVY-FRAUNIÉ, S.; DANGLES, O. A toolbox for studying thermal heterogeneity across spatial scales: from unmanned aerial vehicle imagery to landscape metrics. *Methods in Ecology and Evolution* 7: 437–446, 2016.
- FEDER, M.E.; BURGGREN, M.W. *Environmental Physiology of the Amphibians*. The University of Chicago Press. 1992.
- FIEBERG, J.; MATTHIOPOULOS, J.; HEBBLEWHITE, M.; BOYCE, M.S.; FRAIR, J.L. Correlation and studies of habitat selection: problem, red herring or opportunity? *Philosophical Transactions of The Royal Society of London, Series B, Biological Sciences* 365: 2233–2244, 2010.
- GEIGER, R. *The climate near the ground*. Cambridge: Harvard University Press. 611 p. 1965.
- GEISE, W.; LINSSENMAIR, K.E. Adaptations of the reed frog *Hyperolius viridiflavus* (Amphibia, Anura, Hyperoliidae) to its arid environment. IV. Ecological significance of water economy with comments on thermoregulation and energy allocation. *Oecologia* 77: 327–338, 1988.
- HADDAD, C.F.B.; TOLEDO, L.F.; PRADO, C.P.A.; LOEBMANN, D.; GASPARINI, J.L.; SAZIMA, I. *Guia dos Anfíbios da Mata Atlântica – Diversidade e Biologia*. São Paulo; Anolis Books Editora, 1ª edição. 544 p. 2013.

- HALVERSON, M.A.; SWELLY, D.K.; KIESECKER J.M.; FREIDENBURG, L.K. Forest mediated light regime linked to amphibian distribution and performance. *Oecologia* 134: 360–364, 2003.
- HEATWOLE, H.; TORRES, F.; DE AUSTIN, S.B.; HEATWOLE, A. Studies on anuran water balance-I. Dynamics of evaporative water loss by the coqui, *Eleutherodactylus portoricensis*. *Comparative Biochemistry and Physiology* 28: 245–269, 1969.
- HIDORE, J.; OLIVER, J. *Climatology*. Mac Millan, New York. 1993.
- HILL, J.L.; CURRAN, P.J. Area, shape and isolation of tropical forest fragments: effects on tree species diversity and implications for conservation. *Journal of Biogeography* 30: 1391–1403, 2003.
- HILLMAN, S.S. The effect of anemia on metabolic performance in the frog, *Rana pipiens*. *Journal of Experimental Zoology* 211: 107–111, 1980.
- HILLYARD, S.D.; VIBORG, A.; NAGAI, T. Chemosensory function of salt and water transport by the amphibian skin. *Comparative Biochemistry and Physiology-Part A: Molecular & Integrative Physiology* 148(1): 44–54, 2007.
- HOCK, R.J. Temperature effect on breeding of the toad, *Bufo variegatus*, in southern Chile. *Copeia* (1967): 227–330, 1967.
- HUEY, R.B. Physiological consequences of habitat selection. *American Naturalist* 137: 91–115, 1991.
- HUEY, R.B.; KEARNEY, M.R.; KROCKENBERGER, A.; HOLTUM, J.A.M.; JESS, M.; WILLIAMS, S.E. Predicting organismal vulnerability to climate warming: roles of behavior, physiology, and adaptation. *Philosophical Transactions of the Royal Society B* 367(1596): 1665–1679, 2012.

- HUTCHISON, V.H.; HILL, L.G. Thermal selection of bullfrog (*Rana catesbeiana*) at different stages of development and acclimation temperatures. *Journal of Thermal Biology* 3(2): 57–60, 1978.
- HUTCHISON, V.H.; DUPRE', R.K. Thermoregulation. In: *Environmental Physiology of the Amphibians* (Edited by Feder, M. E., Burggren, W. W.) pp. 206-249. University of Chicago Press, Chicago. 1992.
- HUPFER, P.; KUTTLER, W. *Witterung und Klima – eine Einführung in die meteorology und climatologie*. 10. Auflage, B. G. Teubner, Stuttgart. 1998.
- JAEGER, R.G. Plant climbing by salamanders: Periodic availability of plant-dwelling prey. *Copeia* 1978: 686–691, 1978.
- JAEGER, R.G. Microhabitats of a terrestrial forest salamander. *Copeia* 1980: 265–268, 1980.
- KEARNEY, M.; PORTER W. Mechanistic niche modeling: combining physiological and spatial data to predict species' ranges. *Ecology Letters*. 12: 334–350, 2009.
- KERR, J.T.; STROVSKY, M. From space to species: ecological applications for remote sensing. *Trends in Ecology and Evolution* 18: 299–305, 2003.
- LAUCK, B. Can life history studies contribute to understanding the impacts of clearfell logging on pond-breeding anurans? A review. *Applied Herpetology* 2: 125–137, 2005.
- LEITÃO FILHO, H.F. Consideracoes sobre a florística de florestas tropicais e subtropicais do Brasil. *IPEF*, 35: 41–46, 1987.
- LEHTINEN, R.M.; LANNOO, M.J.; WASSERSUG, R.J. Phytotelm-breeding anurans: past, present, and future research. *Miscellaneous Publications Museum of Zoology University of Michigan* 193: 1–9, 2004.

- LILLYWHITE, H.B. Thermal modulation of cutaneous mucus discharge as a determinant of evaporative water loss in the frog, *Rana catesbeiana*. Zeitschrift für vergleichende Physiologie 73: 84–104, 1971.
- LILLYWHITE, H.B. Water relations of tetrapod integument. Journal of Experimental Biology 209: 202–226, 2006.
- LILLYWHITE, H.B. Physiological ecology: field methods and perspective. In: Amphibian Ecology and Conservation. Dodd, C. K. 2009. Oxford University Press. 2009.
- LILLYWHITE, H.B.; LICHT, P.; CHELGREN, P. The role of behavioral thermoregulation in the growth energetics of the toad, *Bufo boreas*. Ecology 54: (2) 375–383, 1973.
- LILLYWHITE, H.B.; MITTAL, A.K. Amphibian skin and the aquatic-terrestrial transition: constraints and compromises. In: Mittal, A. K., Eddy, F. B., Datta, J. S. Munshi (eds), Water/Air Transition in Biology. Oxford and IBH Publishing, New Delhi. 1999.
- MAIA, M.R. Variação intra e interespecífica na escolha de microambientes e sua relevância para a manutenção do balanço hídrico em anuros. São Paulo. Instituto de Biociências, Universidade de São Paulo. Dissertação de Mestrado em Fisiologia geral. 2014.
- MMA. Biodiversidade Brasileira. Avaliação e identificação de áreas e ações prioritárias para a conservação, utilização sustentável e repartição dos benefícios da biodiversidade nos biomas brasileiros. Brasília. 404 p. 2002.
- MORRIS, D.W. Toward an ecological synthesis: a case for habitat selection. Oecologia 136: 1–13, 2003.

- NAVAS, C.A. Thermal dependency of field locomotor and vocal performance of high-elevation anuran in the tropical Andes. *Journal of Herpetology* 30: 478–487, 1996.
- NAVAS, C.A. Patterns of distribution of anurans in high Andean tropical elevations: Insights from integrating biogeography and evolutionary physiology. *Integrative and Comparative Biology* 46: 82–91, 2006.
- NAVAS, C.A.; ANTONIAZZI, M.M.; JARED, C.A. preliminary assessment of anuran physiology and morphological adaptation to the Caatinga, a Brazilian semi-arid environment. *International Congress Series* 1275: 298–305, 2004.
- NAVAS, C.A.; ARAUJO, C. The use of agar models to study amphibian thermal ecology. *Journal of Herpetology* 32 (2): 330–334, 2000.
- NAVAS, C.A.; CARVAJALINO-FERNÁNDEZ, J.M.; SABOYÁ-ACOSTA, L.P.; RUEDA-SOLANO, L.A.; CARVAJALINO-FERNÁNDEZ, M.A. The body temperature of active amphibians along a tropical elevation gradient: patterns of mean and variance and inference from environmental data. *Functional Ecology* 27: 1145–1154, 2013.
- NAVAS, C.A.; JARED, C.; ANTONIAZZI, M.M. Water economy in the casque-headed tree-frog *Corythomantis greeningi* (Hylidae): role of behavior, skin, and skull skin co-ossification. *Journal of Zoology* 257: 525–532, 2002.
- NEVO, E. Adaptive variation in size of cricket frogs. *Ecology* 54: 1271–1281, 1973.
- OLIVEIRA-FILHO, O.; FONTES, M.A. L. Patterns of floristic differentiation among Atlantic forests in southeastern Brazil, and the influence of climate. *Biotropica* 32: 793–810, 2000.
- PEZZOPANE, J.E.M.; DOS REIS, G.G.; REIS, M.G.F.; DA COSTA, J.M.N.; CHAVES, J.H. Temperatura do solo no interior de um fragmento de floresta

- secundária semidecidual. *Revista Brasileira de Agrometereologia*. 10(1): 1–8, 2002.
- PINCEBOURDE, S.; MURDOCK, C.C.; VICKERS, M.; SEARS, M.W. Fine-scale microclimatic variation can shape the responses of organisms to global change in both natural and urban environments. *Integrative and Comparative Biology* 1–17, 2016.
- PINCEBOURDE, S.; SANFORD, E.; HELMUTH, B. Survival, and arm abscission are linked to regional heterothermy in an intertidal sea star. *Journal of Experimental Biology* 216: 2183–2191, 2013.
- PIPER, W.H. Making habitat selection more “familiar”: a review. *Behavioral Ecology and Sociobiology* 65: 1329–1351, 2011.
- PIZO, M.A.; OLIVEIRA, P.S. The use of fruits and seeds by ants in the Atlantic Forest of Southeast Brazil. *Biotropica* 32(4b): 851–861, 2000.
- POTTER, K.A.; WOODS, H.A.; PINCEBOURDE, S. Microclimatic challenges in global change biology. *Global Change Biology* 19: 2932–2939, 2013.
- POUHG, F.H.; STEWART M.M.; THOMAS, R.G. Physiological basis of habitat partitioning in Jamaican *Eleutherodactylus*. *Oecologia* 27. 285–293, 1977.
- POUGH, F.H.; TAIGEN, T.L.; STEWART, M.; BRUSSARD, P.F. Behavioural modification of evaporative water loss by a Puerto Rican frog. *Ecology* 64: 244–252, 1983.
- POUNDS, J.A.; CRUMP, M.L. Amphibian declines, and climate disturbance: the case of the Golden toad and the harlequin frog. *Conservation Biology* 8: 72–85, 1994.

- RENAUD, V.; REBETEZ, M. Comparison between open-site and below-canopy climatic conditions in Switzerland during the exceptionally hot summer of 2003. *Agricultural and Forest Meteorology*, Amsterdam 149: 873–880, 2009.
- RIDDELL, E.A.; APANOVITCH, E.K.; ODOM, J.P.; SEARS, M.W. Physical calculations of resistance to water loss improve predictions of species range models. *Ecology Monographs* 87: 21–33, 2017.
- Root, T. L., Schneider, S. H. 1995. Ecology and climate: research strategies and implications. *Science* 269: 334–340.
- ROZNIK, E.A.; ALFORD, R.A. Using pairs of physiological models to estimate temporal variation in amphibian body temperature. *Journal of Thermal Biology* 45: 22–29, 2014.
- RUIBAL, R. The adaptative value of bladder water in the toad *Bufo cognatus*. *Physiological Zoology* 35: 218–223, 1962.
- SCHERRER, D.; KÖRNER, C. Infrared thermometry of alpine landscapes challenges climatic warming projections. *Global Change Biology* 16: 2602–2613, 2010.
- SCUDELLER, V.V. Análise fitogeográfico da Mata Atlântica – Brasil. Tese de Doutorado. Universidade Estadual de Campinas. Campinas, SP. 2002.
- SEARS, M.W.; RASKIN, E.; ANGILLETA, M.J.Jr. The world is not flat: defining relevant thermal landscapes in the context of climate change. *Integrative and Comparative Biology* 51: 666–675, 2011.
- SELBACH, G.; HASENACK, H.; BARBOSA, L.F. Microclima e a estrutura de formações vegetais. In: O clima na Reserva Particular do Patrimônio Natural SESC Pantanal. Rio de Janeiro: SESC, Departamento Nacional, 2010.

- SEEBACHER, F.; ALFORD, R.A. Movement and microhabitat use of a terrestrial amphibian (*Bufo marinus*) on a tropical island: seasonal variation and environmental correlates. *Journal of Herpetology* 33: 208–214, 1999.
- SEEBACHER, F.; ALFORD, R.A. Shelter microhabitats determine body temperature and dehydration rates of a terrestrial amphibian (*Bufo marinus*). *Journal of Herpetology* 36(1): 69–75, 2002.
- SEURONT, L.; TERENCE, P.T.; LATHLEAN, J.A. A review on the thermal biology and ecology of mollusks, and of the use of infrared thermography in molluscan research. *Journal of Molluscan Studies* 1–30, 2018.
- SCHEFFERS, B.R.; EDWARDS, D.P.; DIESMOS, A.; WILLIAMS, S.E.; EVANS, T.A. Microhabitats reduce animal's exposure to climate extremes. *Global Change Biology*. 20: 495–503, 2014.
- SCHMID, W.D. Some aspects of the water economies of nine species of amphibians. *Ecology*. 46: 262–269, 1965.
- SHOEMAKER, V.H.; HILLMAN, S.S.; HILLYARD, S.D.; JACKSON, D.C.; MCCLANAHAN, L.L.; WITHERS, P.C.; WYGODA, M.L. Exchange of water, ions, and respiratory gases in terrestrial amphibians. In M. E. Feder and W. W. Burggren eds. 1992.
- SHOEMAKER, V.H.; NAGY, K.A. Osmoregulation in amphibians and reptiles. *Annual Review of Physiology*. 39: 449–471, 1977.
- SINERVO, B.; MÉNDEZ DE LA CRUZ, F.; MILES, D.B.; HEULIN, B.; BASTIAANS, E.; VILLAGRÁN-SANTA CRUZ, M.; LARA-RESENDIZ, R.; MARTÍNEZ-MENDEZ, N.; CALDERÓN-ESPINOSA, M.L.; MEZA-LÁZARO R.N.; GADSDEN, H.; AVILA, L.J.; MORANDO, M.; DE LA RIVA, I.J.; SEPULVEDA, P.V.; DUARTE, C.F.; IBARGUENGOYTÍA, N.; AGUILAR, C.; MASSOT, M.;

- LEPETZ, V.; OKSANEN, T.A.; CHAPPLE, D.G.; BAUER, A.M.; BRANCH, W. R.; CLOBERT, J.; SITES JR, J.W. 2010. Erosion of lizard diversity by climate change and altered thermal niches. *Science*. 328: 894–899, 2010.
- SINSCH, U. Behavioural thermoregulation of the Andean Toad (*Bufo spinulosus*) at high altitudes. *Oecologia*. 80: 32–38, 1989.
- SKELLY, D.K.; FREIDENBURG, L.K. Effects of beaver on the thermal biology of an amphibian. *Ecology Letters*. 3: 483–486, 2000.
- SKELLY, D.K.; FREIDENBURG, L.K.; KIESECKER, J.M. Forest canopy and the performance of larval amphibians. *Ecology* 83: 983–992, 2002.
- SKELLY, D.K.; GOLON, J. Digestive assimilation in tadpoles: the role of forest canopy cover. *Herpetologica* 59: 37–42, 2003.
- SKELLY, D.K.; HALVERSON, M.A.; FREIDENBURG, L.K.; URBAN, M.C. Canopy closure and amphibian diversity in forested wetlands. *Wetlands Ecology and Management* 13: 261–268, 2005.
- SKELLY, D.K.; WERNER, E.E.; CORTWRIGHT, S.A. Long-term distributional dynamics of a Michigan amphibian assemblage. *Ecology* 80: 2326–2337, 1999.
- SPOTILA, J.R. Role of temperature and water in the ecology of lungless salamanders. *Ecological Monographs*. 42(1): 95–125, 1972.
- SPOTILA, J.R.; BERMAN, E.N. Determination of skin resistance and the role of the skin in controlling water loss in amphibians and reptiles. *Comparative Biochemistry and Physiology*. 55A: 407–411, 1976.
- STEVENSON, R.D. Body size and limits to the daily range of body temperature in terrestrial ectotherms. *American Naturalist*. 125: 102–112, 1985.

- TATTERSALL, G.J. Infrared thermography: a non-invasive window into thermal physiology. *Comparative Biochemistry and Physiology A*, 202: 78–98, 2016.
- TATTERSALL, G.J.; CADENA, V. Insights into animal temperature adaptations revealed through thermal imaging. *Imaging Science Journal*, 58: 261–268, 2010.
- THORSON, T.B. The relationship of water economy to terrestriality in amphibians. *Ecology*. 36: 100–116, 1955.
- THORSON, T.B.; SVIHILA, A. Correlation of the habitats of amphibians with their ability to survive the loss of body water. *Ecology*. 24:374–381, 1943.
- TITON, Jr. B. Balanço hídrico e desempenho locomotorem espécies de anuros de Mata Atlântica e Cerrado. Dissertação de Mestrado. Instituto de Biociências, Universidade Estadual Paulista, Botucatu, S.P., Brasil. 2010.
- TITON, Jr. B.; GOMES, F.R. Balanço hídrico e a distribuição geográfica dos anfíbios. *Revista da Biologia* (2012)8: 49–57, 2012.
- TOLEDO, R.C.; JARED, C. Cutaneous adaptations to water balance in amphibians. *Comparative Biochemistry and Physiology* 105A: 593–608, 1993.
- TRACY, C.R. A model of the dynamic exchanges of water and energy between a terrestrial amphibian and its environment. *Ecological Monographs* 46: 293–326, 1976.
- TRACY, C.R.; CHRISTIAN, K.A. Preferred temperature correlates with evaporative water loss in Hylid frogs from Northern Australia. *Physiological and Biochemical Zoology* 78: 839–846, 2005.
- TRACY, C.R.; CHRISTIAN, K.A.; BETTS, G.; TRACY, C.R. Body temperature and resistance to evaporative water loss in tropical Australian frogs. *Comparative Biochemistry and Physiology* 150: 102–108, 2008.

- TRACY, C.R.; CHRISTIAN, K.A.; BURNIP, N.; AUSTIN, B.J.; CORNAL, A.; IGLESIAS, S.; REYNOLDS, S.J.; TIXIER, T.; LE NÖENE, C. Thermal and hydric implications of diurnal activity by a small tropical frog during the dry season. *Australian Journal of Ecology* 38: 476–483, 2013.
- TRACY, C.R.; CHRISTIAN, K.A.; TRACY, C.R. Not just small, wet, and cold: effects of the body size and skin resistance on thermoregulation and arboreality of frogs. *Ecology* 91. 1477, 2010.
- TRACY, C.R.; TIXIER, T.; NÖENE, C.; CHRISTIAN, K. Field hydration state varies among tropical frog species with different habitat use. *Physiological and Biochemical Zoology* 87(2): 197–202, 2014.
- WALSH, R.P.D. The Climate. In P. W. Richards (Ed.). *The tropical rain forest: an ecological study*. Pp. 159 – 205. Cambridge University Press, Cambridge, England. 1996.
- WERNER, E.E.; GLENNEMEIER, K.S. Influence of forest canopy cover on breeding pond distributions of several amphibian species. *Copeia* 1999: 1–12, 1999.
- WITHERS, P.C.; HILLMAN, S.S.; DREWES, R.C. Evaporative water loss and skin lipids of anurans amphibians. *Journal of Experimental Zoology* 232(1): 11–17, 1984.
- WYGODA, M.L. Low cutaneous evaporative water loss in arboreal frogs. *Physiological Zoology* 57(3): 329–337, 1984.
- WYGODA, M.L. Adaptive control of water loss resistance in an arboreal frog. *Herpetological* 44: 251–257, 1988.
- YOUNG, J.E.; CHRISTIAN, K.A.; DONNELLAN, S.; TRACY, C.R.; Parry, D. Comparative analysis of cutaneous evaporative water loss in frogs

demonstrate correlation with ecological habits. *Physiological and Biochemical Zoology* 78(5): 847–856, 2005.

ZHENG, D.; CHEN, J.; SONG, B., XU, M.; SNEED, P.; JENSEN, R. Effects of silvicultura treatments on summer forest microclimate in southeastern Missouri Ozarks. *Climate Research* 15: 45–59, 2000.

ZUG, G.R.; ZUG, P.B. The marine toad *Bufo marinus*: A natural history resume of native populations. *Smithsonian Contributions to Zoology* 284: 1–58, 1979.

8. Tables and Figures

Table 1. Mean, σ^2 , maximum and minimum values from air temperature within each environment and in each sampling season. The highest variance and extreme values along of time at the temperature were recorded for the flooded open area followed by the high altitude forest and canopy. Mim. = Minimum air temperature; Max = Maximum air temperature.

Spring/summer 2015					Winter 2016				Summer 2017			
Environments	Mean	σ^2	Min.	Max.	Mean	σ^2	Min.	Max.	Mean	σ^2	Min.	Max.
High Altitude Forest	18.86	36.35	9.88	35.54	13.66	22.33	9.57	31.7	22.18	14.86	16.81	33.86
Wet Forest	20.03	10.89	16.13	28.5	13.75	3.52	10.27	16.9	22.67	10.89	17.19	31
Transitional Forest	19	6.29	15.09	29.12	17.09	38.84	5.63	29.51	25.61	40.27	15.37	41.53
Canopy	18.19	5.51	15.18	25.02	14.38	6.03	10.35	21.3	23.76	15.6	17.31	34.44
Open Area	25.33	14.25	18.75	32.65	22.76	83.3	6.83	34.76	25.77	33.15	18.27	36.44

Table 2. Air temperature of each environment in relation with vegetation cover level in each sampling season. The highest temperatures were registered in areas of minor vegetation cover. $P < 0,05$ represent significant differences between each tetrad component (see Fig. 5A, B and C)

	Spring/summer 2015			Winter 2016			Summer 2017		
Environments	F	N	P	F	N	P	F	N	P
High Altitude Forest	15.3	637	0,0001	100.96	1149	0,0001	39.11	1149	0,0001
Wet Forest	5.55	637	0,0001	1.725	1149	0.178	7.08	1149	0.0008
Transitional Forest	74.9	637	0,0001	8.96	1149	0,0001	69.17	1149	0,0001
Canopy	3.99	190	0.071	0.906	166	0.3425	10.32	190	<i>0.0015</i>
Open Area	1.60	477	0.2019	4.93	1149	0.0073	38.73	1149	0,0001

Table 3. Air temperature of each environment in relation to the time of day in each sampling season. The highest variation in the temperature was registered between 11:00 at 15:00 in all environments. $P < 0,05$ represent significant differences between each tetrad component (see Fig. 6A, B and C)

	Spring/summer 2015			Winter 2016			Summer 2017		
Environments	F	N	P	F	N	P	F	N	P
High Altitude Forest	9.90	635	0,0001	69.72	1144	0,0001	215.8	1144	0,0001
Wet Forest	29	635	0,0001	614.07	1144	0,0001	571.5	1144	0,0001
Transitional Forest	29.8	635	0,0001	542.48	1144	0,0001	444.5	1144	0,0001
Canopy	38.7	184	0,0001	15.77	160	0,0001	47.6	184	0,0001
Open Area	21.9	475	0,0001	2094,6	1144	0,0001	439.6	1144	0,0001

Table 4. Mean, σ^2 , maximum and minimum values from hydration potential within each environment and in each sampling season. The highest variance and extreme values along of time at the hydration potential were recorded for the flooded open area followed by the high altitude forest and canopy. Mim. = Minimum evaporation water loss; Max = Maximum evaporation water loss.

Spring/summer 2015					Winter 2016				Summer 2017			
Environments	Mean	σ^2	Min.	Max.	Mean	σ^2	Min.	Max.	Mean	σ^2	Min.	Max.
High Altitude Forest	-1.109	8.874	4.131	-24.839	-0.1890	1.467	6.193	-6.709	0.144	2.330	7.875	-6.909
Wet Forest	-0.481	5.059	13.858	-18.592	-0.0618	0.670	5.394	-5.243	-0.259	0.803	3.675	-4.049
Transitional Forest	-0.704	6.685	8.303	-20.807	-0.4554	1.151	6.452	-8.294	-0.858	2.015	4.680	-8.745
Canopy	0.173	3.343	4.875	-4.512	-0.4527	1.401	2.787	-6.038	-1.183	3.260	3.852	-8.104
Open Area	0.556	111.80	65.853	-33.030	-0.7430	1.638	3.658	-6.607	-0.714	4.053	8.980	-11.090

Table 5. Hydration potential of each environment in relation with vegetation cover level in each sampling season. The highest percentage of dehydration was registered in the pair of permeable models of each tetrad, being more significant in the small model in areas of minor vegetation cover. $P < 0,05$ represent significant differences between each tetrad component (see Fig. 8A, B and C)

	Spring/summer 2015			Winter 2016			Summer 2017		
Environments	F	N	P	F	N	P	F	N	P
High Altitude Forest	30,9	628	0,00001	6,28	1140	0,0001	0,287	1140	0,749
Wet Forest	6,24	628	0,0003	1,036	1140	0,354	1,860	1140	0,156
Transitional Forest	35,5	628	0,00001	6,252	1140	0,0001	68,52	1140	0,0001
Canopy	2,28	184	0,132	2,885	160	0,091	3,091	184	0,080
Open Area	0,44	468	0,63	2,384	1140	0,092	11,18	1140	0,0001

Table 6. Hydration potential of each environment in relation with the time of day in each sampling season. The highest variation in the hydration was registered between 13:00 at 15:00 in all environments. $P < 0,05$ represent significant differences between each tetrad component (see Fig. 9A, B and C)

	Spring/summer 2015			Winter 2016			Summer 2017		
Environments	F	N	P	F	N	P	F	N	P
High Altitude Forest	12,1	628	0,0001	14,88	1140	0,0001	26,23	1140	0,0001
Wet Forest	2,73	628	0,028	3,525	1140	0,0009	8,060	1140	0,0001
Transitional Forest	7,26	628	0,0001	23,87	1140	0,0001	42,04	1140	0,0001
Canopy	7,43	184	0,0001	3,421	160	0,0021	10,37	184	0,0001
Open Area	0,86	468	0,484	49,66	1140	0,0001	62,05	1140	0,0001

Table 7. Generalized linear mixed model (GLMM) based in different combinations and interactions of parameters such as temperature, humidity, and characteristics of each environment. The most parsimonious linear mixed model was Warming. The combinations and interactions of parameters of all models are registered in supplementary material.

Models	K	AIC	Delta_AIC	Model Lik	wAIC
1. Warming	24	57137,71	0	1	0,999999883
2. Full model- 1	17	57170,11	32,398	9,22E-08	9,22E-08
3. Full model	18	57172,71	35,002	2,51E-08	2,51E-08
4. Full model-Inter-1	25	57186,34	48,634	2,75E-11	2,75E-11
5. Full model-Inter	26	57188,94	51,235	7,49E-12	7,49E-12
6. Max	15	57197,57	59,863	1,00E-13	1,00E-13
7. Temp.	12	57212,36	74,650	6,16E-17	6,16E-17
8. Max.-Inter	24	57213,68	75,974	3,18E-17	3,18E-17
9. Temp- Inter	13	57214,03	76,320	2,67E-17	2,67E-17
10. Temp-Inter-1	19	57222,62	84,907	3,65E-19	3,65E-19
11. Temp-Inter-2	27	57231,68	93,971	3,93E-21	3,93E-21
12. RH	15	57361,37	223,665	2,70E-49	2,70E-49
13. RH-Inter	24	57373,82	236,114	5,35E-52	5,35E-52
14. Null model	4	58196,54	1058,832	1,19E-23	1,19E-230

1) Warming; 2) Full model-1; 3) Full model; 4) Full model-interactions-1; 5) Full model-interactions; 6) Maximum-temperatures-relative humidity-interactions; 7) Temperatures; 8) Maximum-temperatures-relative humidity-interactions; 9) Temperatures- interactions; 10) Temperatures-interactions-1; 11) Temperatures-interactions-2; 12) Relative humidity; 13) Relative humidity- interactions; 14) Null model

Table 8. Warming. The most important variables and the most significant interactions of this linear model include size and permeability of models, temperature, relative humidity and interactions among these variables and environments. / = interactions; $P < 0,05$ represent significant differences.

Parameters/Interactions	Estimate	Std Error	t value	P Value
Size Small	-0.160	0.064	-2.50	0.012
Permeability	-0.199	0.064	-3.10	0.002
Maximum air temperature	-0.082	0.004	-18.30	<0.001
Maximum RH	0.022	0.001	12.77	<0.001
Higher rate of warming	-0.168	0.017	-9.53	<0.001
Vegetation cover level/Low	0.096	0.243	0.39	0.692
Vegetation cover level/Medium	-0.248	0.249	-0.99	0.319
Canopy	-0.266	0.369	-0.72	0.471
Open Area	0.897	0.246	3.63	<0.001
Transitional	0.194	0.242	0.79	0.424
Wet	0.058	0.242	0.24	0.810
Size / Permeability	-0.137	0.090	-1.51	0.129
Vegetation cover level Low / Canopy	0.344	0.697	0.49	0.621
Vegetation cover level Medium / Canopy	0.084	0.577	0.14	0.883
Vegetation cover level Low / Open	0.029	0.344	0.08	0.932
Vegetation cover level Medium / Open	0.135	0.353	0.38	0.702
Vegetation cover level Low / Transitional	-0.30	0.34	-0.90	0.367

Vegetation cover level Medium / Transitional	-0.03	0.35	-0.08	0.930
Vegetation cover level Low / Wet	-0.17	0.343	-0.51	0.609
Vegetation cover level Medium / Wet	0.05	0.35	0.16	0.871

Figure 1. Parque Estadual Intervales (PEI). Categorized environments. 1) **High Altitude Forest**, 2) **Wet Forest**, 3) **Transitional Forest**, 4) **Open Area** and 5) **Canopy**.

Figure 2. Size frequency distributions of anurans at PEI using published literature and field data A). Sampling unit or “tetrad” formed by 1) large permeable, 2) small permeable 3) large impermeable and 4) small impermeable.

Figure 3. Characterization the thermal landscape surrounding the agar models with infrared images (thermography) using a FLIR C2 Compact Thermal Imaging System Camera $\pm 1^{\circ}\text{C}$.

Figure 4. Analysis of mean air temperature between environments with different vegetation cover levels in three season different showed significant differences.

Figure 5A. Analysis of mean air temperature between environments with different vegetation cover levels in spring/summer transition 2015.

Figure 5B. Analysis of mean air temperature between environments with different vegetation cover levels in winter 2016.

Figure 5C. Analysis of mean air temperature between environments with different vegetation cover levels in summer 2017.

Figure 6A. Analysis of mean air temperature between 09:00 to 17:00 h in spring/summer transition 2015.

Figure 6B. Analysis of mean air temperature between 09:00 to 23:00 h in winter 2016.

Figure 6C. Analysis of the percentage of hydration between 09:00 to 23:00 h in summer 2017.

Figure 7. Analysis of the percentage of hydration between environments with different vegetation cover levels in three season differents showed significant differences.

Figure 8A. Analysis of the percentage of hydration between environments with different vegetation cover levels in spring/summer transition 2015.

Figure 8B. Analysis of the percentage of hydration between environments with different vegetation cover levels in winter 2016.

Figure 8C. Analysis of the percentage of hydration between environments with different vegetation cover levels in summer 2017.

Figure 9A. Analysis of the percentage of hydration between 09:00 to 17:00 h in the spring/summer transition 2015.

Figure 9B. Analysis of the percentage of hydration between 09:00 to 23:00h in winter 2016.

Figure 9C. Analysis of the percentage of hydration between 09:00 to 23:00h in summer 2017.

Figure 10. PCA factors in the high altitude forest in spring/summer 2015. **A.** Patterns of distribution of the agar models (large permeable **LP**, small permeable **SP**, large impermeable **LI**, and small impermeable **SI**). **B.** Patterns according to time (09:00; 11:00; 13:00; 15:00; 17:00h) of day. **C.** Patterns according to vegetation cover degree (Low **L**, Medium **M** and High **H**). We registered the two factors with Eigenvalues.

Figure 11. PCA factors in the wet forest in spring/summer 2015. **A.** Patterns of distribution of the agar models (large permeable **LP**, small permeable **SP**, large impermeable **LI**, and small impermeable **SI**). **B.** Patterns according to time (09:00; 11:00; 13:00; 15:00; 17:00h) of day. **C.** Patterns according to vegetation cover degree (Low **L**, Medium **M** and High **H**). We registered the two factors with Eigenvalues.

Figure 12. PCA factors in the transitional forest in spring/summer 2015. **A.** Patterns of distribution of the agar models (large permeable **LP**, small permeable **SP**, large impermeable **LI**, and small impermeable **SI**). **B.** Patterns according to time (09:00; 11:00; 13:00; 15:00; 17:00h) of day. **C.** Patterns according to vegetation cover degree (Low **L**, Medium **M** and High **H**). We registered the two factors with Eigenvalues.

Figure 13. PCA factors in the open area in spring/summer 2015. **A.** Patterns of distribution of the agar models (large permeable **LP**, small permeable **SP**, large impermeable **LI**, and small impermeable **SI**). **B.** Patterns according to time (09:00; 11:00; 13:00; 15:00; 17:00h) of day. **C.** Patterns according to vegetation cover degree (Low **L**, Medium **M** and High **H**). We registered the two factors with Eigenvalues.

Figure 14. PCA factors in the canopy in spring/summer 2015. **A.** Patterns of distribution of the agar models (large permeable **LP**, small permeable **SP**, large impermeable **LI**, and small impermeable **SI**). **B.** Patterns according to time (09:00; 11:00; 13:00; 15:00; 17:00h) of day. **C.** Patterns according to vegetation cover degree (Low **L** and High **H**). We registered the two factors with Eigenvalues.

Figure 15. PCA factors in the high altitude forest in winter 2016. **A.** Patterns of distribution of the agar models (large permeable **LP**, small permeable **SP**, large impermeable **LI**, and small impermeable **SI**). **B.** Patterns according to time (05:00; 09:00; 11:00; 13:00; 15:00; 17:00; 20:00; 23:00h) of day. **C.** Patterns according to vegetation cover degree (Low **L**, Medium **M** and High **H**). We registered the two factors with Eigenvalues.

Figure 16. PCA factors in the wet forest in Winter 2016. **A.** Patterns of distribution of the agar models (large permeable **LP**, small permeable **SP**, large impermeable **LI**, and small impermeable **SI**). **B.** Patterns according to time (05:00; 09:00; 11:00; 13:00; 15:00; 17:00; 20:00; 23:00h) of day. **C.** Patterns according to vegetation cover degree (Low **L**, Medium **M** and High **H**). We registered the two factors with Eigenvalues.

Figure 17. PCA factors in the transitional forest in winter 2016. **A.** Patterns of distribution of the agar models (large permeable **LP**, small permeable **SP**, large impermeable **LI**, and small impermeable **SI**). **B.** Patterns according to time (05:00; 09:00; 11:00; 13:00; 15:00; 17:00; 20:00; 23:00h) of day. **C.** Patterns according to vegetation cover degree (Low **L**, Medium **M** and High **H**). We registered the two factors with Eigenvalues.

Figure 18. PCA factors in the open area in winter 2016. **A.** Patterns of distribution of the agar models (large permeable **LP**, small permeable **SP**, large impermeable **LI**, and small impermeable **SI**). **B.** Patterns according to time (05:00; 09:00; 11:00; 13:00; 15:00; 17:00; 20:00; 23:00h) of day. **C.** Patterns according to vegetation cover degree (Low **L**, Medium **M** and High **H**). We registered the two factors with Eigenvalues.

Figure 19. PCA factors in the canopy in winter 2016. **A.** Patterns of distribution of the agar models (large permeable **LP**, small permeable **SP**, large impermeable **LI**, and small impermeable **SI**). **B.** Patterns according to time (05:00; 09:00; 11:00; 13:00; 15:00; 17:00; 20:00; 23:00h) of day. **C.** Patterns according to vegetation cover degree (Low **L** and High **H**). We registered the two factors with Eigenvalues.

Figure 20. PCA factors in the high altitude forest in summer 2017. **A.** Patterns of distribution of the agar models (large permeable **LP**, small permeable **SP**, large impermeable **LI**, and small impermeable **SI**). **B.** Patterns according to time (05:00; 09:00; 11:00; 13:00; 15:00; 17:00; 20:00; 23:00h) of day. **C.** Patterns according to vegetation cover degree (Low **L**, Medium **M** and High **H**). We registered the two factors with Eigenvalues.

Figure 21. PCA factors in the wet forest in summer 2017. **A.** Patterns of distribution of the agar models (large permeable **LP**, small permeable **SP**, large impermeable **LI**, and small impermeable **SI**). **B.** Patterns according to time (05:00; 09:00; 11:00; 13:00; 15:00; 17:00; 20:00; 23:00h) of day. **C.** Patterns according to vegetation cover degree (Low **L**, Medium **M** and High **H**). We registered the two factors with Eigenvalues.

Figure 22. PCA factors in the transitional forest in summer 2017. **A.** Patterns of distribution of the agar models (large permeable **LP**, small permeable **SP**, large impermeable **LI**, and small impermeable **SI**). **B.** Patterns according to time (05:00; 09:00; 11:00; 13:00; 15:00; 17:00; 20:00; 23:00h) of day. **C.** Patterns according to vegetation cover degree (Low **L**, Medium **M** and High **H**). We registered the two factors with Eigenvalues.

Figure 23. PCA factors in the open area in summer 2017. **A.** Patterns of distribution of the agar models (large permeable **LP**, small permeable **SP**, large impermeable **LI**, and small impermeable **SI**). **B.** Patterns according to time (05:00; 09:00; 11:00; 13:00; 15:00; 17:00; 20:00; 23:00h) of day. **C.** Patterns according to vegetation cover degree (Low **L**, Medium **M** and High **H**). We registered the two factors with Eigenvalues.

Figure 24. PCA factors in the canopy in summer 2017. **A.** Patterns of distribution of the agar models (large permeable **LP**, small permeable **SP**, large impermeable **LI**, and small impermeable **SI**). **B.** Patterns according to time (05:00; 09:00; 11:00; 13:00; 15:00; 17:00; 20:00; 23:00h) of day. **C.** Patterns according to vegetation cover degree (Low **L**, Medium **M** and High **H**). We registered the two factors with Eigenvalues.

9. Supplementary Data

Combinations and interactions of the parameters in the Generalized linear mixed model (GLMM)

1. Warming: lmer (formula = Hydration ~ Size + Permeability + Size * Permeability + Maximum air temperature + Maximum RH + Higher rate of warming + Vegetation cover level + Environment + Vegetation cover level * Environment + (1|Tetrad number)* (1|Model number))
 2. Full model-1: lmer (formula = Hydration ~ Size + Permeability + Vegetation cover level + Environment + Mean RH + Mean air temperature + Mean operational temperature + Higher rate of warming + Lower rate of warming + (1|Tetrad number)* (1|Model number))
 3. Full model: lmer (formula = Hydration ~ Size + Permeability + Size * Permeability + Vegetation cover level + Environment + Mean RH + Mean air temperature + Mean operational temp. + Higher rate of warming + Lower rate of warming + (1|Tetrad number)* (1|Model number))
 4. Full model-interactions-1: lmer (formula = Hydration ~ Size + Permeability + Vegetation cover level + Vegetation cover level * Environment + Environment + Mean RH + Mean air temperature + Mean operational temperature + Higher rate of warming + Lower rate of warming + (1|Tetrad number)* (1|Model number))
 5. Full model-interactions: lmer (formula = Hydration ~ Size + Permeability + Size * Permeability + Vegetation cover level + Vegetation cover level * Environment + Environment + Mean RH + Mean air temperature + Mean operational temperature + Higher rate of warming + Lower rate of warming + (1|T number)* (1|Model number))
 6. Null model: lmer (formula = Hydration ~ 1 + (1|Tetrad number)* (1|Model number))
-

7. Maximum-temperatures-relative humidity-interactions: Imer (formula = Hydration ~ Size + Permeability + Size * Permeability + Maximum air temperature + Maximum RH + Maximum operational temperature + Vegetation cover level + Environment + vegetation cover level * Environment + (1|Tetrad number)* (1|Model number))
8. Temperatures: Imer (formula = Hydration ~ Size + Permeability + Mean air temperature + Maximum air temperature + Minimum air temperature + Mean operational temperature + Maximum operational temperature + Minimum operational temperature + (1|Tetrad number)* (1|Model number))
9. Maximum-temperatures-relative humidity-interactions: Imer (formula = Hydration ~ Size + Permeability + Size * Permeability + Maximum air temperature. + Maximum RH + Maximum operational temperature + Vegetation cover level + Environment + Vegetation cover level * Environment + (1|Tetrad number)* (1|Model number))
10. Temperatures- interactions: Imer (formula = Hydration ~ Size + Permeability + S * Permeability + Mean air temperature + Maximum air temperature + Minimum air temperature + Mean operational temperature + Maximum operational temperature + Minimum operational temperature + (1|Tetrad number)* (1|Model number))
11. Temperatures-interactions-1: Imer (formula = Hydration ~ Size + Permeability + Size * Permeability + Vegetation cover level + Environment + Mean air temperature + Maximum air temperature + Minimum air temperature + Mean operational temperature + Maximum operational temperature + Minimum operational temperature + (1|Tetrad number)* (1|Model number))
12. Temperatures-interactions-2: Imer (formula = Hydration ~ Size + Permeability + Size * Permeability + Vegetation cover level + Vegetation cover level * Environment + Environment + Mean air temperature + Maximum air temperature + Minimum air

temperature *+ Mean operational temperature + Maximum operational temperature +
Minimum operational temperature + (1|Tetrad number)* (1|Model number)

13. Relative humidity: lmer (formula = Hydration ~ Size + Permeability + Mean RH +
Maximum RH + Minimum RH + Vegetation cover level + Environment + (1|Tetrad
number)* (1|Model number)

14. Relative humidity-interactions: lmer (formula = Hydration ~ Size + Permeability +
Size * Permeability + Mean RH + Maximum RH + Minimum RH + Vegetation cover
level + Environment + Vegetation cover level * Environment + (1|Tetrad number)*
(1|Model number)

Discussão Geral e Conclusões

Este trabalho é um diagnóstico geral da hidratação e da distribuição do microclima nos anuros terrestres e arbóreos na Mata Atlântica do Sul do Brasil. Os resultados obtidos a partir de nossa unidade de amostragem (modelos de ágar) como modelo nulo de evaporação nos fornece um ponto comum de partida para comparar o potencial de hidratação entre diferentes espécies de anuros em diferentes ambientes dentro do Parque Estadual Intervales. No entanto, esta comparação requer modelos de ágar com pequenas variações no tamanho corporal e permeabilidade, com uma postura de conservação de água e forma semelhante aos anuros vivos que dificilmente afetaria as medidas de perda de água pela evaporação.

Outra técnica de análise é o uso da termografia como uma ferramenta não invasiva que permite a visualização em tempo real da paisagem termal e da temperatura superficial de substratos e organismos ao redor dos modelos de ágar. No entanto, estas imagens não transmitem informações sobre a disponibilidade de água. Porém, a combinação destas duas metodologias (modelos nulos e termografia) dentro da mesma área oferece detalhes extremamente valiosos sobre os gradientes hidrotermais experimentados pelos anuros no habitat natural, tanto no tempo quanto no espaço.

Deste modo, nosso trabalho fomenta a pesquisa eco-fisiológica, e o entendimento da regulação hidrotermal de anuros em campo, permitindo assim testar hipóteses como, por exemplo: 1) que as temperaturas operativas e a taxa de perda de água por evaporação são maiores na parte alta das montanhas e em áreas abertas onde a exposição solar diária e a variação termal são maiores, comparada com a variação

termal e a variação da umidade por evaporação dentro de bosques de baixa altitude e em matas fechadas e de transição; 2) que o microclima tem um efeito diferencial no potencial do ambiente para promover a desidratação por evaporação nos anuros com menor tamanho corporal comparada com anuros de maior tamanho; 3) que a variação na permeabilidade dos modelos de ágar reflete nos anuros uma menor perda de água por evaporação em espécies que têm a capacidade de impermeabilizar sua pele, de regular a taxa de filtração glomerular, de sua bexiga urinária armazenar água e de outras funções fisiológicas integradas à “tolerância à desidratação”.

Como conclusão, nossos resultados devem ser interpretados como uma ferramenta valiosa para inferir e revelar as diferenças interespecíficas na perda de água por evaporação nos anuros terrestres e arbóreos da Mata Atlântica, a partir de uma escala microclimática espacial e temporal compatível com o tamanho corporal e permeabilidade das espécies, e com inferências sobre o tipo de ambiente, a cobertura florestal e o horário do dia.

Referências Bibliográficas

- AB'SABER, A.N. Os domínios morfoclimáticos na América do Sul. Primeira aproximação. Geomorfologia, Instituto de Geografia, Universidade de São Paulo. 53: 1–23, 1997.
- AB'SABER, A.N. Os domínios de natureza no Brasil: potencialidade paisagística. Ateliê Editorial, São Paulo. 163 pp. 2005.
- ADOLPH, S.C. Influence of behavioral thermoregulation on microhabitat use by two *Sceloporus* lizards. *Ecology* 71: 315–327, 1990.
- ALMEIDA, D.S. Recuperação ambiental da Mata Atlântica. Ilhéus: Editus, p 130. 2000.
- ANGILLETTA, Jr.M.J. Thermal adaptation: a theoretical and empirical synthesis. Oxford University Press, Oxford. 2009.
- ANGILLETTA, M.; Niewiarowski, P.; Navas, C.A. The evolution of thermal physiology in ectotherms. *Journal of Thermal Biology* 27:249–268, 2002.
- BAKKEN, G.S. Arboreal perch properties and the operative temperature experienced by small animals. *Ecology* 70:922–930, 1989.
- BAKKEN, G.S. Measurement, and application of operative and standard operative temperatures in ecology. *American Zoologist* 32: 194–216, 1992.
- BAKKEN, G.S.; GATES, D.M. Heat-transfer analysis of animals: some implications for field ecology, physiology, and evolution, Pages 255–290 in D. M. Gates, and R. B. Schmerl, eds. *Perspectives of Biophysical Ecology*. New York, Springer-Verlag. 1975.

- BARNES, B.V.; ZAK, D.R.; DENTON, S.R.; SPURR, S.H. Forest ecology. 4 ed. New York, USA. John Wiley. 774p. 1998.
- BARTHOLOMEW, G.A. Interaction of physiology and behavior under natural conditions. In: The Galapagos (ed. Bowman RI). University of California Press, Berkeley. 39–45, 1966.
- BECKER, C.G.; FONSECA, C.R.; BAPTISTA, C.F.; FERNANDES, R.; PRADO, P.I. Habitat Split and the global decline of amphibians. *Science* 318: 1775–1777, 2007.
- BENNIE, J.; HUNTLEY, B.; WILTSHIRE, A.; HILL, M.O.; BAXTER, R. Slope, aspect and climate: spatially explicit and implicit models of topographic microclimate in chalk grassland. *Ecological Modelling* 216(1): 47–59, 2008.
- BENTLEY, P.J.; LEE, A.K.; MAIN, A.R. Caparison of dehydration and hydration of two genera of frogs (*Heleioporus* and *Neobatrachus*) that live in areas of varying aridity. *Journal Experimental Biology* 35: 667–684, 1958.
- BRADFORD, D.F. Temperature modulation in a high-elevation amphibian *Rana muscosa*. *Copeia* 1984: 966–976, 1984b.
- BRATTSTROM, B.H. Amphibian temperature regulation studies in the field and in the laboratory. *American Zoologist* 19: 345–356, 1979.
- BRUIJNZEEL, L.; HAMILTON, L. Bosques Nublados Tropicales Montañosos: Tiempo para la acción. UNESCO. Paris. 2000.
- CARDOSO, A.J.; ANDRADE, G.V.; HADDAD, C.F.B. Distribuição espacial em comunidades de anfíbios (*Anura*) no sudeste do Brasil. *Revista Brasileira de Biologia* 49: 241–249, 1989.

- CHRISTIAN, K.; BEDFORD, G. Thermoregulation by the spotted tree monitor, *Varanus scalaris*, in the seasonal tropics of Australia. *Journal of Thermal Biology* 21:67–73, 1996.
- CHRISTIAN, K.A.; WEAVERS, B.W. Thermoregulation of monitor lizards in Australia: an evaluation of methods in thermal biology. *Ecological Monographs* 66:139–157, 1996.
- CRUMP, M.L. Quantitative analysis of the neotropical herpetofauna. *Occasional Papers of the Museum of Natural History, the University of Kansas* 3: 1–62, 1971.
- CUSHMAN, S.A. Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biological Conservation*. 128: 231–240, 2006.
- DUELLMAN, W.E. Distribution patterns of amphibians in South America. In: *Patterns of Distribution of Amphibians: A Global Perspective*. Duellman W.E. (ed.). The John Hopkins University Press, Baltimore. pp. 255–328, 1999.
- DUELLMAN, W E.; TRUEB, L. *Biology of amphibians*. McGraw-Hill, New York, NY. 670p. 1986.
- ELITH, J.; LEATHWICK, J.R. Species distribution models: ecological explanation and prediction across space and time. *Annual Review of Ecology, Evolution and Systematics* 40: 677–697, 2009.
- FRAHM, J.P.; GRADSTEIN, S.R. An altitudinal zonation of tropical rainforests using bryophytes. *Journal of Biogeography* 18: 669–678, 1991.
- GATES, D.M. *Biophysical Ecology*. Springer-Verlag, New York. 1980.
- GEIGER, R. *The climate near the ground*. Cambridge: Harvard University Press. 611 p. 1965.

- GEIGER, R.; ARON, R.H.; TODHUNTER, P. The climate near the ground. Rowman & Littlefield Publishers, Lanham, M.D. 2009.
- GRANT, B.W. Trade-offs in activity time and physiological performance for thermoregulating desert lizards, *Sceloporus merriami*. Ecology 71: 2323–2333, 1990.
- GRANT, B.W.; DUNHAM, A.E. Thermally imposed time constraints on the activity of the desert lizard *Sceloporus merriami*. Ecology 69:167–176, 1988.
- GRANT, B.W.; DUNHAM, A.E. Elevational covariation in environmental constraints and life histories of the desert lizard *Sceloporus merriami*. Ecology 71: 1765–1776, 1990.
- HADDAD, C.F.B.; PRADO, C.P.A. Reproductive modes in frogs and their unexpected diversity in the Atlantic Forest of Brazil. BioScience 55: 207–217, 2005.
- HADDAD, C.F.B.; TOLEDO, L.F.; PRADO, C.P.A. 2008. Anfíbios da Mata Atlântica: guia dos anfíbios anuros da Mata Atlântica. São Paulo; Editora Neotropica. 185 p. 2005.
- HADDAD, C.F.B.; TOLEDO, L.F.; PRADO, C.P.A.; LOEBMANN, D.; GASPARINI, J. L.; SAZIMA, I. Guia dos Anfíbios da Mata Atlântica – Diversidade e Biologia. São Paulo; Anolis Books Editora, 1ª edição. 544 p. 2013.
- HASEGAWA, M.; SUZUKI, Y.; WADA, S. Design and performance of a wet sponge model for amphibian thermal biology. Current Herpetology 24(1): 27–32, 2005
- HEATWOLE, H.; TORRES, F.; DE AUSTIN, S.B.; HEATWOLE, A. Studies on anuran water balance-I. Dynamics of evaporative water loss by the coqui, *Eleutherodactylus portoricensis*. Comparative Biochemistry and Physiology 28: 245–269, 1969.

- HELMUTH, B.S.T. Intertidal mussel microclimates: predicting the body temperature of a sessile invertebrate. *Ecological Monographs*. 68: 51–74, 1998.
- HELMUTH, B.S.T.; HOFMANN, G.E. Microhabitats, thermal heterogeneity, and patterns of physiological stress in the rocky intertidal zone. *Biological Bulletin* 201: 374–384, 2001.
- HERTZ, P.E. Temperature regulation in Puerto Rican *Anolis* lizards: a field test using null hypotheses. *Ecology* 73: 1405–1417, 1992.
- HILL, J.L.; CURRAN, P.J. Area, shape and isolation of tropical forest fragments: effects on tree species diversity and implications for conservation. *Journal of Biogeography* 30: 1391–1403, 2003.
- HILLYARD, S.D.; VIBORG, A.; NAGAI, T. Chemosensory function of salt and water transport by the amphibian skin. *Comparative Biochemistry and Physiology-Part A: Molecular & Integrative Physiology* 148(1): 44–54, 2007.
- HOCK, R.J. Temperature effect on breeding of the toad *Bufo variegatus* in Southern Chile. *Copeia* 227–230. 1967.
- HOWE, P.D.; BRYANT, S.R.; SHREEVE, T.G. Predicting body temperature and activity of adult *Polyommatus icarus* using neural network models under current and projected climate scenarios. *Oecologia* 153: 857–869, 2007.
- HUEY, R.B. Temperature, physiology and the ecology of reptiles. In Gans, C. e high-altitude anurans: 55 years of research on altitude and oxygen. *Respire. Physiology*. 1982.
- HUTCHISON, V.H.; DUPRE', R.K. Thermoregulation. In: *Environmental Physiology of the Amphibians* (Edited by Feder, M. E., Burggren, W. W.) pp. 206-249. University of Chicago Press, Chicago. 1992.

- JOHNSON, C.R. Aggregation as a means of water conservation in juvenile *Limnodynastes* from Australia. *Herpetologica* 25: 275–276, 1969.
- KEARNEY, M.; PORTER, W. Mechanistic niche modeling: combining physiological and spatial data to predict species' ranges. *Ecology Letters* 12: 334–350, 2009.
- LEITÃO FILHO, H.F. Considerações sobre a florística de florestas tropicais e subtropicais do Brasil. *IPEF*, 35: 41–46, 1987.
- LEOPOLD, A. Game Management. Charles Scribner's Sons, New York. 1933.
- LILLYWHITE, H.B. Thermal modulation of cutaneous mucus discharge as a determinant of evaporative water loss in the frog, *Rana catesbeiana*. *Zeitschrift für vergleichende Physiologie* 73: 84–104, 1971.
- LILLYWHITE, H.B.; LICHT, P.; CHELGREN, P. The role of behavioral thermoregulation in the growth energetics of the toad, *Bufo boreas*. *Ecology* 54: (2) 375–383, 1973.
- LONG, Z.L.; PREPAS, E.E. Scale and landscape perception: the case of refuge use by Boreal Toads (*Anaxyrus boreas boreas*). *Canadian Journal of Zoology* 90(8): 1015–1022, 2012.
- LOYOLA, R.D.; LEMES, P.; BRUM, F.T.; PROVETE, D.B.; DUARTE, L.D.S. Clade-specific consequences of climate change to amphibians in Atlantic Forest protected areas. *Ecography* 37: 64–72, 2014.
- MAIA, M.R. Variação intra e interespecífica na escolha de microambientes e sua relevância para a manutenção do balanço hídrico em anuros. São Paulo. Instituto de Biociências, Universidade de São Paulo. Dissertação de Mestrado em Fisiologia geral. 2014.

- MMA. Biodiversidade Brasileira. Avaliação e identificação de áreas e ações prioritárias para a conservação, utilização sustentável e repartição dos benefícios da biodiversidade nos biomas brasileiros. Brasília. 404 p. 2002.
- MORRELLATO, L.P.C.; HADDAD, C.F.B. Introduction: The Brazilian Atlantic Forest. *Biotropica* 32(4b): 786–792, 2000.
- MURCIA, C. Edge effects in fragmented forest: implications for conservation. *Trends in Ecology and Evolution* 10 (2): 58–62, 1995.
- NAVAS, C.A. Thermal dependency of field locomotor and vocal performance of high-elevation anuran in the tropical Andes. *Journal of Herpetology* 30: 478–487, 1996.
- NAVAS, C.A. Patterns of distribution of anurans in high Andean tropical elevations: Insights from integrating biogeography and evolutionary physiology. *Integrative and Comparative Biology* 46: 82–91, 2006.
- NAVAS, C.A.; ARAUJO, C. The use of agar models to study amphibian thermal ecology. *Journal of Herpetology* 32 (2): 330–334, 2000.
- NAVAS, C.A.; CARVAJALINO-FERNÁNDEZ, J.M.; SABOYÁ-ACOSTA, L.P.; RUEDA-SOLANO, L.A.; CARVAJALINO-FERNÁNDEZ, M.A. The body temperature of active amphibians along a tropical elevation gradient: patterns of mean and variance and inference from environmental data. *Functional Ecology* 27: 1145–1154, 2013.
- NAVAS, C.A.; JARED, C.; ANTONIAZZI, M.M. Water economy in the casque-headed tree-frog *Corythomantis greeningi* (Hylidae): role of behavior, skin, and skull skin co-ossification. *Journal Zoology (Lond)* 257: 525–532, 2002.
- NEVO, E. Adaptive variation in size of cricket frogs. *Ecology* 54: 1271–12, 1973.
- OKE, T.R. Boundary Layer Climates. Methuen, London. 1987.

- OLALLA-TÁRRAGA, M.A.; DINIZ-FILHO, J.A.F.; BASTOS, R.P.; RODRÍGUEZ, M.A. Geographic body size gradients in tropical regions: Waters déficit and anuran body size in the Brazilian Cerrado. *Ecography* 32: 581–590, 2009.
- OLIVEIRA-FILHO, O.; FONTES, M.A.L. Patterns of floristic differentiation among Atlantic forests in southeastern Brazil, and the influence of climate. *Biotropica* 32: 793–810, 2000.
- PARMESAN, C. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution and Systematics* 37: 637–669, 2006.
- PETERMAN, W.E.; LOCKE, J.L.; SEMLITSCH, R.D. Spatial and temporal patterns of water loss in heterogeneous landscapes: using plaster models as amphibian analogues. *Canadian Journal of Zoology* 91: 135–140, 2013.
- PINCEBOURDE, S.; CASAS, J. Multitrophic biophysical budgets: thermal ecology of an intimate herbivore insect-plant interaction. *Ecological Monographs* 76: 175–194, 2006.
- PORTER, W.P.; MITCHELL, J.W.; BECKMAN, W.A.; DEWITT, C.B. Behavioral implications of mechanistic ecology. Thermal and behavioral modeling of desert ectotherms and their microenvironment. *Oecologia* 13: 1–54, 1973.
- POTTER, K.A.; WOODS, H.A.; PINCEBOURDE, S. Microclimatic challenges in global change biology. *Global Change Biology* 19: 2932–2939, 2013.
- POUHG, F.H.; STEWART M.M.; THOMAS, R.G. Physiological basis of habitat partitioning in Jamaican *Eleutherodactylus*. *Oecologia* 27: 285–293, 1977.
- POUGH, F.H.; TAIGEN, T.L.; STEWART, M.; BRUSSARD, P.F. Behavioural modification of evaporative water loss by a Puerto Rican frog. *Ecology* 64: 244–252, 1983.

- SARTORIUS, S.S.; DO AMARAL, J.P.S.; DURTSCHKE, R.D.; DEEN, C.M.; LUTTERSCHMIDT, D.W.I. Thermoregulatory accuracy, precision, and effectiveness in two sand-dwelling lizards under mild environmental conditions. *Canadian Journal of Zoology* 80: 1966–1976, 2002.
- SAUDREAU, M.; PINCEBOURDE, S.; DASSOT, M.; ADAM, B.; LOXDALE, H.D.; BIRON, D.G. On the canopy structure manipulation to buffer climate change effects on insect herbivore development. *Tress* 27: 239–248. 2013.
- SCHERRER, D.; KORNER, C. Topographically controlled thermal-habitat differentiation buffers alpine plant diversity against climate warming. *Journal of Biogeography* 38(2): 406–416, 2011.
- SCHMID, W.D. Some aspects of the water economies of nine species of amphibians. *Ecology* 46: 261–269, 1965.
- SCHWARZKOPF, L.; ALFORD, R.A. Desiccation and shelter-site use in a tropical amphibian: Comparing toads with physical models. *Functional Ecology* 10: 193–200, 1996.
- SCHWENK, K.; PADILLA, D.K.; BAKKEN, G.S.; FULL, R.J. Grand challenges in organismal biology. *Integrative and Comparative Biology* 49: 7–14, 2009.
- SCUDELLER, V.V. Análise fitogeográfico da Mata Atlântica – Brasil. Tese de Doutorado. Universidade Estadual de Campinas. Campinas, SP. 2002.
- SEARS, M.W.; BAKKEN, G.S.; ANGILLETTA, M.J.; FITZGERALD, L.A. Using artificial neural networks to model the operative temperatures of small animals in a spatially explicit context. *Integrative and Comparative Biology* 44: 745, 2004.

- SEARS, M.W.; RASKIN, E.; ANGILLETA, M.J.Jr. The world is not flat: defining relevant thermal landscapes in the context of climate change. *Integrative and Comparative Biology* 51: 666–675, 2011.
- SEEBACKER, F.; ALFORD, R.A. Shelter Microhabitats Determine Body Temperature and Dehydration Rates of a Terrestrial Amphibian (*Bufo marinus*). *Journal of Herpetology* 36(1): 69–75, 2002.
- SELBACH, G.; HASENACK, H.; BARBOSA, L.F. Microclima e a estrutura de formações vegetais. In: O clima na Reserva Particular do Patrimônio Natural SESC Pantanal. Rio de Janeiro: SESC, Departamento Nacional, 2010.
- SHOEMAKER, V.H.; HILLMAN, S.S.; HILLYARD, S.D.; JACKSON, D.C.; MCCLANAHAN, L.L.; WITHERS, P.C.; WYGODA, M.L. Exchange of water, ions, and respiratory gases in terrestrial amphibians. In M.E. Feder and W.W. Burggren eds. 1992.
- SHOEMAKER, V.H.; MCCLANAHAN, L.L. Evaporative water loss, nitrogen excretion, and osmoregulation in Phyllomedusine frogs. *Journal of Comparative Physiology* 100: 331–345, 1975.
- SINERVO, B.; MÉNDEZ DE LA CRUZ, F.; MILES, D.B.; HEULIN, B.; BASTIAANS, E.; VILLAGRÁN-SANTA CRUZ, M.; LARA-RESENDIZ, R.; MARTÍNEZ-MENDEZ, N.; CALDERÓN-ESPINOSA, M.L.; MEZA-LÁZARO R.N.; GADSDEN, H.; AVILA, L.J.; MORANDO, M.; DE LA RIVA, I.J.; SEPULVEDA, P.V.; DUARTE, C.F.; IBARGUENGOYTÍA, N.; AGUILAR, C.; MASSOT, M.; LEPETZ, V.; OKSANEN, T.A.; CHAPPLE, D.G.; BAUER, A.M.; BRANCH, W.R.; CLOBERT, J.; SITES Jr.J.W. Erosion of lizard diversity by climate change and altered thermal niches. *Science* 328: 894–899, 2010.

- SINSCH, U. Behavioural thermoregulation of the Andean Toad (*Bufo spinulosus*) at high altitudes. *Oecologia* 80: 32–38, 1989.
- SKELLY, D.K.; HALVERSON, M.A.; FREIDENBURG, L.K.; URBAN, M.C. Canopy closure and amphibian diversity in forested wetlands. *Wetlands Ecology and Management* 13: 261–268, 2005.
- SPOTILA, J.R. Role of temperature and water in the ecology of lungless salamanders. *Ecological Monographs* 42: 95–125, 1972.
- SOMERO, G.N. The physiology of global change: linking patterns to mechanisms. *Annual Review of Marine Science* 4: 39–61, 2012.
- STADMÜLLER, T. Los Bosques Nublados tropicales: distribución, características ecológicas e importancia hidrológica. pp.47–54. Em: M. Liberman & C. Baied (eds.). *Desarrollo de Ecosistemas de Montaña: Manejo de áreas frágiles en los Andes*. Editorial del Instituto de Ecología, La Paz. 1997.
- THORSON, T. The relationship of water economy to terrestriality in amphibians. *Ecology* 36: 100–116, 1955.
- THORSON, T.B.; SVIHILA, A. Correlation of the habitats of amphibians with their ability to survive the loss of body water. *Ecology* 24: 374–381, 1943.
- TITON, Jr.B.; GOMES, F.R. 2015. Relation between Water Balance and Climatic Variables Associated with the Geographical Distribution of Anurans. 2015. *PLoS ONE* 10(10): e0140761.
- TRACY, C.R. A model of the dynamic exchanges of water and energy between a terrestrial amphibian and its environment. *Ecological Monographs* 46: 293–326, 1976.

- TRACY, C.R.; CHRISTIAN, K.A.; TRACY, R.C. Not just small, wet, and cold: effects of body size and skin resistance on thermoregulation and arboreality of frogs. *Ecology* 91: 1477–1484, 2010.
- TRACY, C.R.; REYNOLDS, S.J.; MCARTHUR, L.; TRACY, C.R.; CHRISTIAN, K.A. Ecology of Aestivation in a Cocoon-forming frog, *Cyclorana australis* (Hylidae). *Copeia* 4: 901–912, 2009.
- TYLIANAKIS, J.M.; DIDHAM, R.K.; BASCOMPTE, J.; WARDLE, D.A. Global change and species interactions in terrestrial ecosystem. *Ecology Letters* 11: 1351–1363, 2008.
- VALDIVIESO, D.; TAMSITT, J.R. Thermal relationships of the neotropical frog *Hyla labialis* (Anura: Hylidae). *Life Sciences Occasional Royal Ontario Museum* 26: 1–10, 1974.
- WALTHER, G.R. Community and ecosystem responses to recent climate change. *Philosophical Transactions of the Royal Society B: Biological Sciences* 365: 2019–2024, 2010.
- WILBY, R.L.; WIGLEY, T.M.L.; CONWAY, D.; JONES, P.D.; HEWITSON, B.C.; MAIN, J.; WILKS, D.S. Statistical downscaling of general circulation model output: a comparison of methods. *Water Resources Research* 34: 2995–3008, 1998.
- WILMER, P.G. Microclimate and the environmental physiology of insects. *Advances in Insect Physiology* 16: 1–57, 1982.
- WITHERS, P.C.; LOUW, G.N.; NICHOLSON, S. Water loss, oxygen consumption and color change in 'waterproof' reed frogs (*Hypernlius*). *South African Journal of Science* 78: 30–32. 1982.

- WYGODA, M.L. Low cutaneous evaporative water loss in arboreal frogs. *Physiological Zoology* 57: 329–337, 1984.
- WYGODA, M.L. Adaptive control of water loss resistance in an arboreal frog. *Herpetological* 44: 251–257, 1988.
- YOUNG, J.E.; CHRISTIAN, K.A.; DONNELLAN, S.; TRACY, C.R.; PARRY, D. Comparative analysis of cutaneous evaporative water loss in frogs demonstrate correlation with ecological habits. *Physiological and Biochemical Zoology* 78: 847–856, 2005.
- ZHENG, D.; CHEN, J.; SONG, B.; XU, M.; SNEED, P.; JENSEN, R. Effects of silvicultura treatments on summer forest microclimate in southeastern Missouri Ozarks. *Climate Research* 15: 45–59, 2000.

Anexos e Apêndices

Comissão Técnico Científica – COTEC

TERMO DE COMPROMISSO JUNTO AO INSTITUTO FLORESTAL¹

Processo SMA Nº	
Título do Projeto:	Efeito do microclima sobre aspectos da fisiologia termal dos anfíbios anuros no Parque Estadual Intervales, estado de São Paulo, Brasil.
Período de Execução:	15 / Janeiro / 2015 a 30 / Dezembro / 2017.
Equipe executora do projeto:	Jesús Eduardo Ortega Chinchilla, Gustavo Adolfo Agudelo Cantero, Laura Camila Cabanzo Olarte

Responsável pelo Projeto : Carlos Arturo Navas Iannini	
RG V172810-S	CIC 213419128-76
Residência: Av. Dr C. Motta Filho 183, apto 83E	CEP 0553-000
Email: carlos.a.navas2@gmail.com	DDD e Telefone: (11) 987686898

Instituição Instituto de Biociências, USP	Unidade: Departamento de Fisiologia
CGC 63.025.530000 4-57	Inscrição Estadual Isenta
Endereço Rua do Matão, Travessa 14 No. 321	CEP 05508-090
Representante legal da Instituição: Gilberto Fernando Xavier	Cargo: Diretor

Projeto financiado por:

O responsável e a(s) instituição(ões) relacionada(s) ao projeto supra, devidamente autorizado pelo Conselho Técnico do Instituto Florestal, compromete-se a:

1. Cumprir a Resolução SMA-25, de 8/11/2000, que dispõe sobre a Medida Provisória n.º 2052-3, de 27/11/2000, sobre o acesso ao patrimônio genético, a proteção e o acesso ao conhecimento tradicional associado, a repartição dos benefícios e o acesso à tecnologia e transferência de tecnologia para sua conservação e utilização.
2. Cumprir a Portaria do Diretor Geral do Instituto Florestal, de 23/01/90 que estabelece normas de uso para pesquisa nas áreas do Instituto Florestal por Técnicos externos, especialmente no que concerne a:
 - Remessa ao Instituto Florestal de qualquer tipo de publicação, gerada pela pesquisa;
 - Divulgação dos resultados da pesquisa, na qual deverá constar o nome da dependência onde ela foi realizada e do Instituto Florestal e,
 - Quando houver coleta de material botânico, exsiccata(s) deverá(ão) ser encaminhada(s) ao Herbário D. Bento Pickel (SPSF) do Instituto Florestal.
3. Cumprir a Portaria do Diretor Geral do Instituto Florestal, de 19/07/93 "Estabelecendo que todas as pesquisas desenvolvidas, em quaisquer das dependências do Instituto Florestal ficam sujeitas à prévia assinatura de termo de compromisso sobre direitos e eventuais patentes delas decorrentes".
4. Cumprir a Instrução Normativa do Ministério do Meio Ambiente, IBAMA nº 154, de 1º de março de 2007, retificada em 14/03/2007, que fixa normas para a coleta de material biológico em unidades de conservação.
5. Cumprir o Decreto n.º 98.830 de 15/01/90, que dispõe sobre a coleta, por estrangeiros, de dados e materiais científicos no Brasil.

Declaro, outrossim, que eximo o Instituto Florestal de toda e qualquer responsabilidade em decorrência de acidentes que possam ocorrer comigo ou demais membros da equipe executora, em dependências do Instituto Florestal, e que me comprometo a retirar, até o final dos trabalhos, todos os materiais utilizados para a coleta de dados, tais como armadilhas, colares, fitas, placas, plaquetas, etc.

Local e Data

Assinatura do Responsável pela Pesquisa

Assinatura e carimbo do Representante Legal da Instituição à qual o pesquisador é filiado

¹ A área sombreada deve ser preenchida pelo responsável pelo projeto.

Prof. Dr. Gilberto F. Xavier
Diretor
Instituto de Biociências - USP

Comissão Técnico Científica - COTEC

TERMO DE RESPONSABILIDADE - RESOLUÇÃO SMA-25 DE 8/11/2000***PROCESSO SMA N.º _____**

Eu Carlos Arturo Navas Iannini, profissão Professor Universitario, pesquisador da (o) Instituto de Biociências, USP, R.G. V172810-S, CIC 213419128-76, e-mail carlos.a.navas2@gmail.com, residente na Av. Dr C. Motta Filho 183, apto 83E, CEP 0553-000, Cidade: São Paulo, Estado: São Paulo, Telefone (11) 987686898, na qualidade de responsável pelo desenvolvimento do Projeto de Pesquisa intitulado Efeitos do microclima sobre aspectos da fisiologia termal dos anfíbios anuros no Parque Estadual Intervales, estado de São Paulo, Brasil., financiado por FAPESP / CNPq / CAPES, com prazo de execução de 36 a 42 meses, e Instituto de Biociências, Inscrição Estadual n.º Isenta e CGC 63.025.530 0004-57, sediada Rua do Matão, Travessa 14, No. 321, representada legalmente por Gilberto Fernando Xavier, e com o ingresso devidamente autorizado pelo Conselho Técnico do Instituto Florestal, comprometo-me a:

1. Respeitar toda a legislação brasileira e tratados internacionais de proteção dos recursos naturais, toda a legislação brasileira relativa à pesquisa, expedições científicas, patentes e segredos de indústria; bem como todos os termos do Decreto n.º 2.519, de 16 de março de 1998, que promulga a Convenção sobre Diversidade Biológica, principalmente aqueles relacionados com acesso aos recursos genéticos, conhecimento tradicional e transferência de tecnologia;
2. Depositar pelo menos um exemplar de cada espécie ou amostra coletada, devidamente identificada, em instituição indicada pela Secretaria do Meio Ambiente do Estado de São Paulo;
3. Elaborar e entregar ao órgão competente, da Secretaria de Meio Ambiente do Estado de São Paulo, relatórios semestrais da pesquisa, com o conteúdo mínimo de: resumo das atividades já executadas; descrição das coletas já realizadas (localização das coletas, período de coleta); discriminação e quantificação do tipo de material coletado, bem como indicação de seu uso e destino; descrição dos resultados já obtidos; cronograma das próximas atividades. A não entrega dos relatórios no prazo estipulado e/ou a conduta inadequada, ocasionará a imediata interrupção da pesquisa e da autorização concedida para ingressar na Unidade de conservação.
- # 1º - o critério do órgão competente, a periodicidade da entrega dos relatórios poderá ser ampliada,
- # 2º - o pesquisador poderá requerer sigilo sobre os dados apresentados nestes relatórios.
4. Requerer permissão, observada a legislação que rege a matéria, junto ao órgão competente, para o acesso aos componentes do patrimônio genético e prosseguimento da pesquisa quando os produtos ou processos decorrentes do desenvolvimento desta, possuírem aproveitamento comercial ou resultarem em pedidos de patentes.
5. Contribuir para a divulgação da Convenção sobre Diversidade Biológica no meio acadêmico, científico, técnico e popular, especialmente na região alvo da pesquisa.

Local e Data: S. Paulo, 27/11/2019

Assinatura do Responsável pelo Projeto: _____

Assinatura e carimbo do Diretor Geral: _____

* A área sombreada deve ser preenchida pelo responsável pelo projeto.

Prof. Dr. Gilberto F. Xavier
Diretor
Instituto de Biociências - USP

Comissão Técnico Científica – COTEC

(ou equivalente da instituição à qual o pesquisador é filiado)

Assinatura do responsável pela Unidade de Conservação: _____
(será providenciada pela COTEC)

TERMO DE TRANSFERÊNCIA DE MATERIAL - TTM*
OBRIGATÓRIO para projetos que incluem coleta de amostras de qualquer natureza

Processo SMA Nº	
Título do Projeto:	Efeitos do microclima sobre aspectos da fisiologia termal dos anfíbios anuros no Parque Estadual Intervales, estado
Período de Execução:	15 / Janeiro / 2015 a 30 / Dezembro / 2017.
Responsável pelo Projeto e Equipe Executora:	Carlo Arturo Navas Iannini, Jesús Eduardo Ortega Chinchilla, Gustavo Adolfo Agudelo Cantero, Laura Camila Cabanzo Olarte

Assinalar, especificar e estimar a quantidade das amostras a serem coletadas no projeto

Amostra	Especificação	Quantidade estimada
Água		
Ar		
Fauna	10 indivíduos e 50 girinos das espécies mais representativas de anuros presentes no Parque Intervales (ver projeto)	
Fauna - subprodutos		
Material Botânico		
Microrganismo		
Minério		
Outros		

Informações da instituição coletora do material:

Instituição: Instituto de Biociências
CEP e Endereço: Rua do Matão, Travessa 14 No. 321
Nome do representante legal da instituição coletora: Gilberto Fernando Xavier
Documento de Identificação (tipo, nº e órgão emissor): 7.447.473-6
Cargo do representante legal da instituição coletora: Diretor
Especificar o ato que delega competência ao representante legal: Diário oficial de Estado de São Paulo. 11/11/2014

Instituição detentora do material: Instituto Florestal, Secretaria do Meio Ambiente do Estado de São Paulo.

Endereço: Rua do Horto, 931 02377-000 São Paulo, SP.

Representante da instituição: Rodrigo Antônio Braga Moraes Victor

Documento de Identificação: RG nº 23.996.970-4

Cargo do representante legal da instituição detentora do material: Diretor Geral

Especificar o ato que delega competência ao representante legal: Resolução do Secretário de Estado do Meio Ambiente de 14/10/2009, publicado no Diário Oficial do Estado de 16/10/2009, S II, p. 42.

As instituições signatárias, acima qualificadas, por meio de seus representantes devidamente constituídos, tendo em vista o disposto na Convenção sobre a Diversidade Biológica, na Medida Provisória nº 2.186-16, de

* A área sombreada deve ser preenchida pelo responsável pelo projeto.