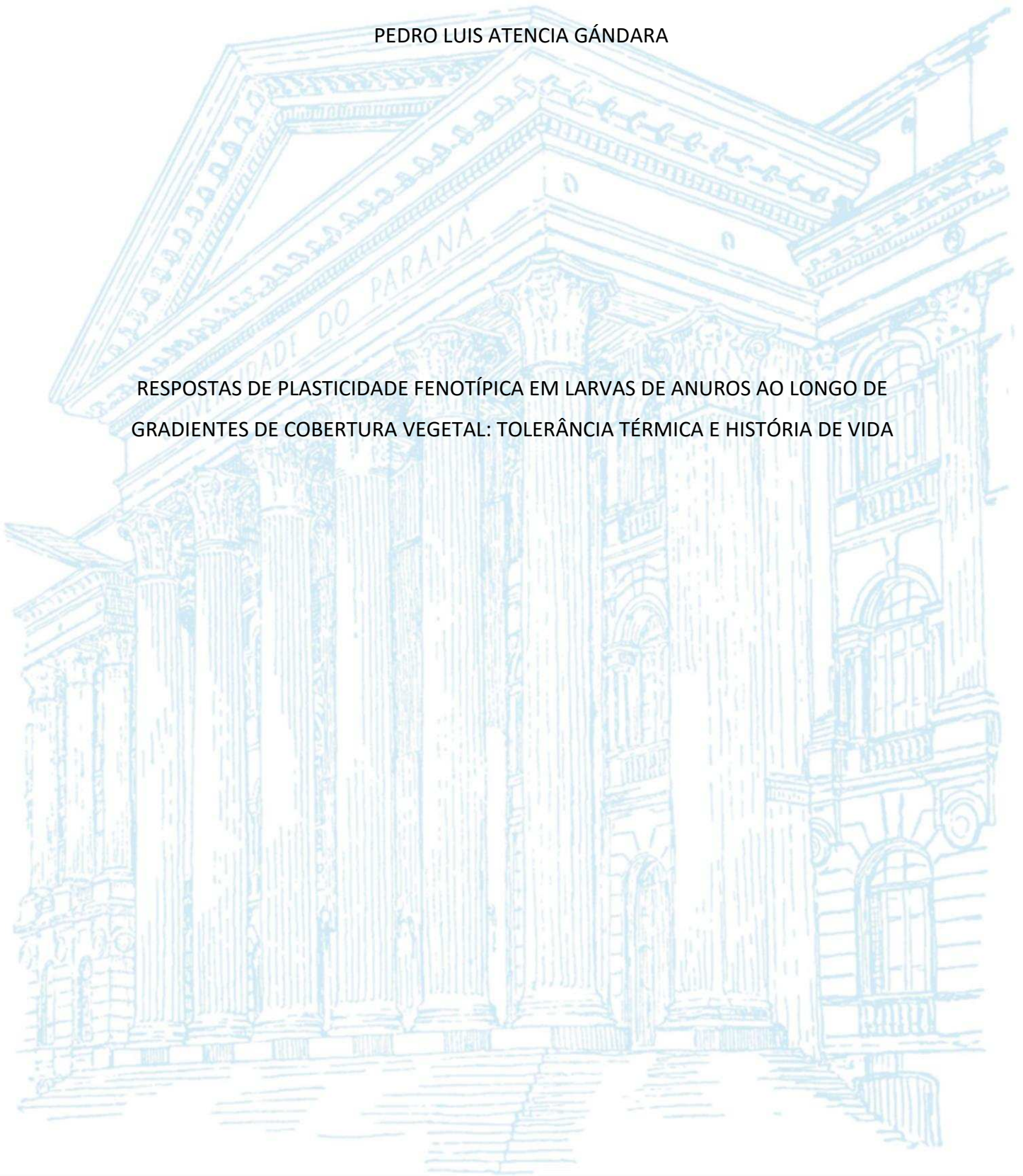UNIVERSIDADE FEDERAL DO PARANÁ

PEDRO LUIS ATENCIA GÁNDARA

RESPOSTAS DE PLASTICIDADE FENOTÍPICA EM LARVAS DE ANUROS AO LONGO DE GRADIENTES DE COBERTURA VEGETAL: TOLERÂNCIA TÉRMICA E HISTÓRIA DE VIDA

CURITIBA

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Dissertação apresentada ao Programa de Pós-Graduação em Zoologia, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Zoologia.

Orientador: Prof. Dr. Rodrigo Lingnau
Coorientador: Prof. Dr. Carlos Arturo Navas

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Presidente da Banca Examinadora (UNIVERSIDADE TECNOLÓGICA FEDERAL DO PARANÁ)

Assinatura Eletrônica
15/03/2021 15:26:47.0
MARCO JACINTO KATZENBERGER BAPTISTA NOVO
Avaliador Externo (UNIVERSIDADE FEDERAL DE PERNAMBUCO)

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JOSÉ EDUARDO DE CARVALHO
Avaliador Externo (UNIVERSIDADE FEDERAL DE SÃO PAULO)

DEDICATÓRIA

À minha família,

especialmente a meus pais: Juan e Doris;

meu irmão: Cristian

pelo amor, exemplo e suporte.

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Aos meus pais por sempre incentivar-me para conseguir minhas metas.

EPÍGRAFE

It has been a beautiful fight. Still is.

Charles Bukowski

RESUMO

O grau de variação térmica ambiental experimentado por animais ectotérmicos influencia profundamente as características de desempenho e as demandas energéticas. Portanto, é importante saber se animais que experimentam maior variabilidade térmica melhoraram sua capacidade de montar uma resposta fisiológica que amortece o desempenho da variação ambiental. No presente estudo, investigamos como o desenvolvimento em diferentes regimes de variação térmica pode influenciar a norma de reação de desenvolvimento de CTmax e de traços de história de vida em girinos de duas espécies de anuros que diferem em seu microambiente térmico. Girinos obtidos a partir de desovas foram criados em condições de temperatura constante (23ºC), flutuação pequena (19-24 ° C) e grande (19-30 ° C). Estimamos a temperatura crítica máxima (CTmax) usando o método dinâmico de Hutchison e taxas de aquecimento ecológicas. Medimos parâmetros de desenvolvimento, como taxa de crescimento, massa na metamorfose e período larval (idade). Os girinos de *P. cuvieri* aumentaram seu CTmax em resposta a grandes flutuações de temperatura, enquanto os girinos de *P. moreirae* não apresentaram nenhuma resposta de aclimatação. Uma massa reduzida na metamorfose foi observada em ambas as espécies sob condições térmicas variáveis. *P. cuvieri* também apresentou um tempo de desenvolvimento reduzido no tratamento de alta variação térmica e seu crescimento foi insensível à temperatura, enquanto *P. moreirae* exibiu um desenvolvimento mais longo e taxa de crescimento lenta sob tratamentos flutuantes. Nossos resultados sugerem que *P. cuvieri* tem capacidade de amortecer o desempenho e a aptidão das variações térmicas diárias atuais. Por fim, nossos resultados também contribuem para identificar uma vulnerabilidade de *P. moreirae* às mudanças microclimáticas atuais (desmatamento) e futuras (aquecimento global), uma vez que sua incapacidade de lidar de forma eficiente com a variabilidade térmica está relacionada a desequilíbrios energéticos e um desempenho prejudicado.

Palavras-chave: aclimatação; variação térmica diária; desempenho; girinos; desenvolvimento

ABSTRACT

The degree of environmental thermal variation that ectothermic animals experience profoundly influences performance traits and energetic demands. Hence, it is important to know if animals that experience greater thermal variability improved their ability to mount a physiological response that buffer performance from environmental variation. In the present study, we investigated how development in different regimes of thermal variation can influence the developmental reaction norm of CTmax and of life-history traits in tadpoles of two species that differ in their thermal microenvironment. Tadpoles of clutches were raised in small (19-24 ° C) and large fluctuate conditions (19-30 ° C), as well in a constant condition (23 ° C). We estimated maximum critical temperature (CTmax) using Hutchison's dynamic method and ecological heating rates. We measured developmental parameters such as growth rate, mass at metamorphosis and larval period (age). Tadpoles of *P. cuvieri* increased their CTmax in response to large temperature fluctuations, while *P. moreirae* tadpoles did not show any acclimation response. A reduced mass at metamorphosis was observed in both species under variable thermal conditions. *P. cuvieri* also showed a reduced development time under the high thermal variation treatment and their growth was insensitive to temperature, while *P. moreirae* exhibited a longer development and slow growth rate under fluctuant treatments. Our findings suggest that *P. cuvieri* has some capacity to buffer performance and fitness from current daily thermal variations. Finally, our results also contribute to identify a vulnerability of *P. moreirae* to both, current (deforestation) and future microclimatic changes (global warming), since its inability to efficiently cope with thermal variability is related to energetic imbalances and an impaired performance.

Key-words: acclimation; daily thermal variation; performance; tadpoles; development

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INTRODUÇÃO GERAL

HETEROGENEIDADE AMBIENTAL

Variação térmica em escalas organísmicas

A variação ambiental é um importante determinante da distribuição, abundância e desempenho ecológico das espécies (Parmesan, 2006; Bellard et al., 2012). O ambiente pode variar em parâmetros climáticos como temperatura, umidade relativa, radiação solar, entre outros, e também em fatores bióticos como intensidade de competição, densidade de predadores ou predominância de parasitas (Oke, 2002; Wilmers et al., 2007; Traill et al., 2010). Embora os parâmetros do clima variem de maneira onipresente nas diferentes escalas espaciais e temporais (Geiger et al., 2003), ao nível dos micro-habitats estes são vivenciados de maneira muito particular pelos indivíduos durante seu tempo de vida (Tracy, 1977; Sears et al., 2011). Por esse motivo, as respostas das espécies às variações climáticas podem ser compreensivelmente entendidas através do conceito de microclima usado em ecofisiologia, que resulta da interação clima-indivíduo em uma escala equivalente a seu tamanho corpóreo (Willmer, 1982; Potter et al., 2013). Muitos autores têm mostrado como as variações macroclimáticas são fracamente correlacionadas com a temperatura corpórea de pequenos animais, quando comparados aos preditores microclimáticos que realmente experimentam em seus microambientes (Kaspari et al., 2015; Pincebourde e Suppo, 2016; Gutiérrez-Pesquera et al., 2016b; Katzenberger et al., 2018; Pintanel et al., 2019). Por exemplo, para insetos e vertebrados de pequeno porte que habitam florestas, a variação térmica ecofisiologicamente relevante ocorre em escalas espaciais e temporais muito pequenas (como centímetros e horas), devido às condições climáticas locais estocásticas e características da estrutura física (por exemplo, cobertura vegetal) (Kaspari et al., 2015; Pincebourde e Suppo, 2016; Du et al., 2019). Por esta razão, informações sobre a flutuação de parâmetros biofísicos nas escalas individuais de uma espécie interessam para compreender adequadamente o potencial das respostas fenotípicas à variação climática (Scheffers et al., 2013, 2014).

Dentre os parâmetros do clima, a temperatura influência fortemente a fisiologia dos organismos (Angilletta, 2009). Os impactos termodinâmicos das flutuações térmicas atingem diferentes níveis de organização, desde as reações bioquímicas até o desempenho ecológico

das espécies nos ecossistemas (Angilletta et al., 2002; Hochachka e Somero, 2002). A temperatura também pode ter consequências importantes para a sobrevivência, influenciando funções chave como crescimento, locomoção e até mesmo a respiração aeróbia, processo responsável pelo balanço energético dos vertebrados (Hochachka e Somero, 2002; Niehaus et al., 2006; Seebacher et al., 2014). A temperatura pode variar espacialmente no ambiente, entre habitat e entre micro-habitat de uma paisagem, por exemplo; e também pode variar durante a vida de um indivíduo, em escalas de tempo específicas de uma espécie alvo, desde minutos em organismos que vivem durante pouco tempo até estações do ano para organismos com um tempo de vida mais longo (Kern et al., 2015a; Woods et al., 2015). Indivíduos ou espécies de tamanho comparável em diferentes ambientes podem experimentar diferentes níveis de variação térmica, diferentes valores máximos e diferentes taxas de mudança da temperatura ambiente (Williams et al., 2012; Kern et al., 2015a). Por exemplo, as regiões temperadas apresentam maior variação sazonal de temperatura do que as regiões tropicais (Conover, 1992; Ghalambor et al., 2006), poças temporárias expostas à radiação solar, apresentam maiores taxas de mudança de temperaturas do que poças sombreadas (Martin, 1972; Oke, 2002). Além disso, drásticas variações térmicas diárias têm sido registradas em muitos ambientes (Navas, 1997; Kingsolver et al., 2004b; Niehaus et al., 2012; Kern et al., 2015a), e em alguns são tão grandes quanto a variação sazonal (Morris e Taylor, 1983) ou anual (Sarmiento, 1986), apresentando faixas de flutuação diária acima de 20°C (Podrabsky e Somero, 2004; Kern et al., 2014). Alguns exemplos comuns disso são, os ecossistemas rochosos entre marés (Helmuth e Hofmann, 2001), os habitats dos páramos andinos (Sarmiento, 1986) e as poças temporárias (Scholnick, 1994), ambientes caracterizados por grandes variações de temperatura de curto prazo. As condições acima são extremamente desafiadoras e requerem várias formas de adaptação fisiológica por parte dos organismos que vivem ali (Sinclair et al., 2006; Williams et al., 2012).

Gradientes de cobertura vegetal e variação térmica

Como é bem conhecido, as florestas desempenham um papel importante na regulação da variação térmica em escala local. Principalmente através da interceptação da radiação solar; assim quando a radiação solar, em particular a radiação de onda curta, incide no dossel esta é refletida e absorvida, e nas florestas primárias apenas uma pequena porcentagem é transmitida ao solo (Oke, 2002; Geiger et al., 2003). Os processos fisiológicos das plantas

através da evapotranspiração também contribuem para reduzir a variação da temperatura nas áreas subjacentes (Woods et al., 2015). Além disso, aspectos da estrutura física das florestas, como sua arquitetura e morfologia, pode dar conta da heterogeneidade espacial e temporal da paisagem térmica disponível para animais de pequeno porte (Pincebourde et al., 2016). Isto é particularmente evidente nos gradientes locais, como os gradientes de cobertura vegetal, os quais podem filtrar o clima produzindo diferentes regímenes de variação térmica dentro de uma paisagem (Figura 1). Nestes gradientes o grau de variação térmica e a taxa de mudança de temperatura nos micro-habitats de pequenos animais dependem de seu nível de cobertura vegetal (Chen e Robinson, 2014). Micro-habitats expostos à radiação solar passam por uma maior variação térmica, além das mudanças térmicas abruptas e o oposto ocorre em micro-habitats sombreados (menor variação e mudanças térmicas lentas) (Burton e Likens, 1973; Skelly et al., 2002). Os diferentes regimes de variação térmica que ocorrem nos gradientes de cobertura vegetal podem influenciar o desempenho fisiológico de diversos animais, principalmente de animais ectotérmicos, os quais refletem em sua temperatura corpórea as flutuações da temperatura ambiental (Skelly et al., 2002; Pike et al., 2011; Chen e Robinson, 2014; Ilha et al., 2018).
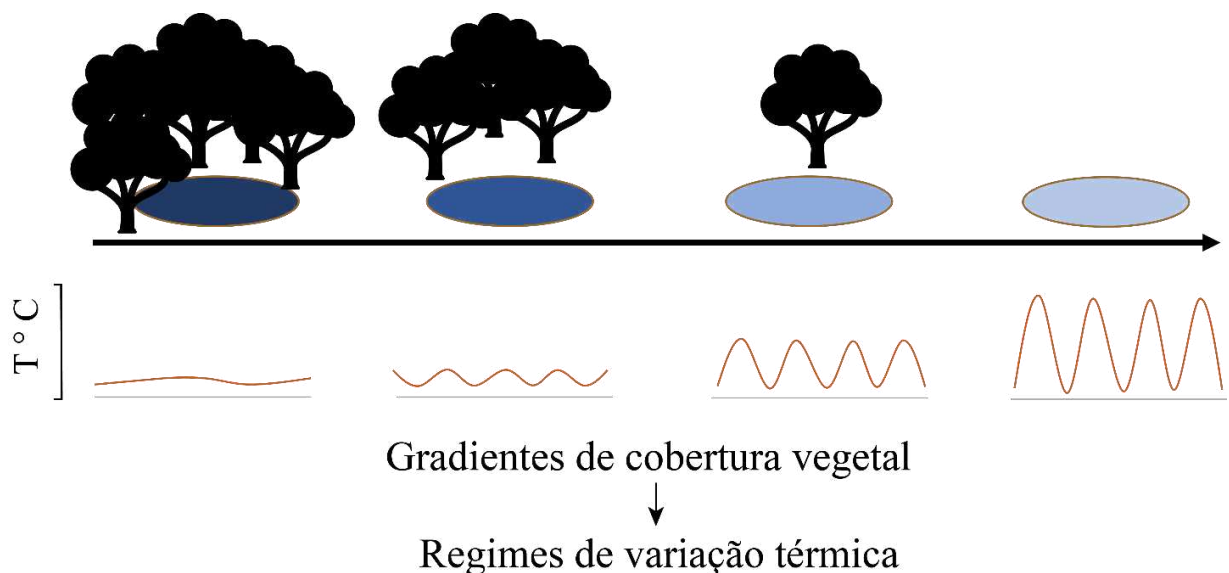
Figura 1. Ilustração esquemática de um gradiente de cobertura vegetal produzindo diferentes regimes de variação da temperatura da agua em poças temporárias onde larvas de anfíbios se desenvolvem.

Os gradientes de cobertura vegetal influenciam os regimes de variação térmica não apenas nos ambientes terrestres, mas também em muitos ambientes aquáticos (Skelly e

Freidenburg, 2000; Batzer et al., 2004; Schiesari, 2006; Van Buskirk, 2010; Richter-Boix et al., 2015). Entre estes destacam-se as poças temporárias, nas quais ocorrem as diferenças mais drásticas na variação térmica já registradas em ambientes aquáticos (Podrabsky et al., 1997; Kern et al., 2015a). Grandes áreas de superfície, em relação aos volumes de água, fazem com que o ambiente térmico disponível para animais ectotérmicos seja extremamente variável (Scholnick, 1994). Por causa disso, as poças temporárias podem refletir rapidamente as variações térmicas diárias que resultam dos gradientes de cobertura vegetal, bem como suas consequências no desempenho fisiológico dos organismos aquáticos (Skelly et al., 2002; Richter-Boix et al., 2015). Animais ectotérmicos em poças temporárias são expostos a grandes flutuações diárias de temperatura, com possibilidades limitadas de manter temperaturas corpóreas dentro de faixas estreitas devido à ausência de gradientes térmicos para seleção comportamental (Navas et al., 2008; Gutiérrez-Pesquera et al., 2016a).

CONSEQUÊNCIAS DA TEMPERATURA NA FISIOLOGIA DE ECTOTERMOS

As flutuações de temperatura afetam profundamente o desempenho, comportamento e sobrevivência dos animais ectotérmicos (Angilletta, 2009). Temperaturas corpóreas em vertebrados ectotérmicos em grande parte refletem a paisagem térmica que ocupam, imprimindo particular sensibilidade térmica a muitas de suas funções (Navas et al., 2008). A sensibilidade térmica do desempenho orgânismal é comumente representada por meio de uma função não linear denominada "curva de desempenho" (Figura 2). Alguns exemplos de variáveis biológicas comumente analisadas na curva de desempenho incluem: locomoção, crescimento, desenvolvimento ou sobrevivência de organismos. O pico dessa curva representa a temperatura ótima na qual o desempenho da variável biológica estudada é maximizado. Os limites de tolerância térmica na curva de desempenho delimitam a amplitude de temperaturas corpóreas na qual o funcionamento orgânismal é garantido (Huey e Stevenson, 1979). Os dois limites térmicos, temperatura crítica mínima (CTmin) e temperatura crítica máxima (CTmax), na curva de desempenho representam pontos nos quais o desempenho é reduzido a níveis nos quais o organismo é incapaz de escapar de situações, que em condições naturais, levariam à morte (Cowles e Bogert, 1944; Lutterschmidt e Hutchison, 1997), por exemplo, escapar de seus predadores.
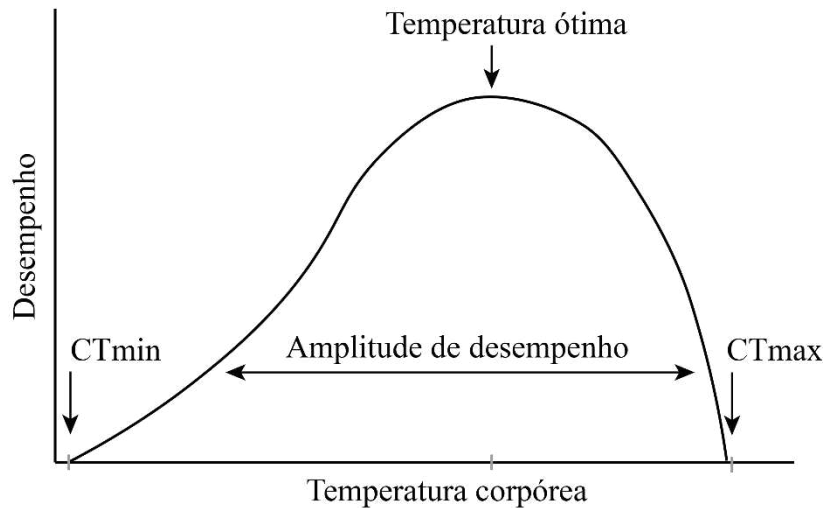
Figura 2. Exemplo de uma curva de desempenho em ectotermos indicando seus principais parâmetros (baseado em Huey e Stevenson, 1979): temperatura ótima, temperatura crítica mínima (CTmin), temperatura crítica máxima (CTmax) e amplitude de desempenho.

Teoricamente, características que congregam menor amplitude de desempenho seriam mais sensíveis às mudanças de temperatura (Huey e Kingsolver, 1993); em determinadas linhagens de vertebrados ectotérmicos a amplitude da faixa em que a taxa de crescimento se mantém em níveis apropriados pode ser estreita (John-Alder et al., 1988; Bennett, 1990; Angilletta et al., 2010; Herrel e Bonneaud, 2012). Inclusive em um mesmo organismo, a sensibilidade térmica de diferentes funções pode variar (ex. pode variar Topt e amplitude de desempenho); a taxa de crescimento pode ser termicamente mais sensível do que o desempenho locomotor (Angilletta et al., 2002), por exemplo. Além disso, as funções biológicas geralmente exibem curvas de desempenho diferentes, que podem variar previsivelmente entre os níveis de organização (Bozinovic et al., 2020). De forma geral, as características na curva de desempenho (ex. forma da curva, ótimo térmico, amplitude de desempenho) e a tolerância térmica podem variar entre e dentro das espécies em relação aos regimes de temperatura que os organismos experimentam (Angilletta et al., 2003). Neste sentido, as curvas de desempenho podem ser também entendidas como normas de reação térmica para um tipo particular de características (funções biológicas). Ajustes fisiológicos que envolvem mudanças na norma de reação térmica permitem que os organismos lidem com as consequências negativas da variação térmica, reduzindo a sensibilidade térmica de suas funções (Niehaus et al., 2006; Kern et al., 2015a).
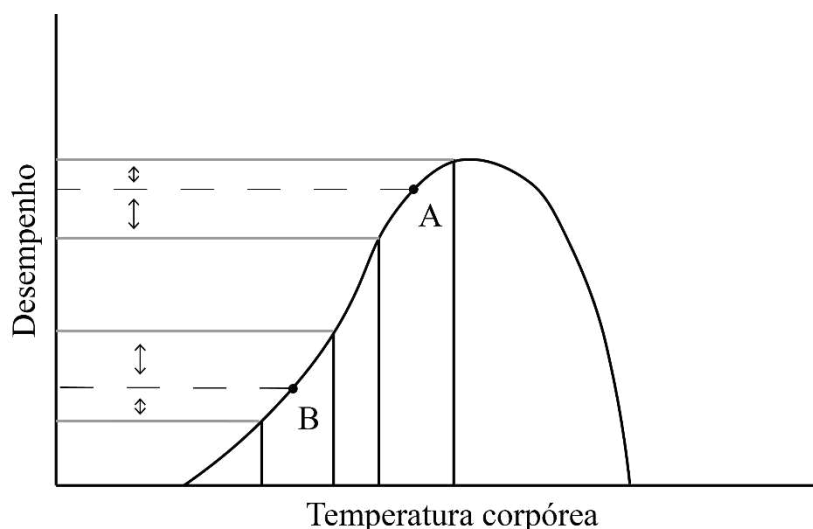
Figura 3. Efeito de temperaturas flutuantes no desempenho de funções biológicas explicado pela desigualdade de Jensen (baseado em Ruel e Ayres, 1999). A. indica a região côncava da curva onde o desempenho é reduzido sob condições variáveis, B. indica a região convexa da curva onde o desempenho é incrementado sob condições variáveis. O efeito de reduzir e aumentar o desempenho ocorre devido ao tempo diferencial que o indivíduo passa experimentando temperaturas acima ou abaixo da média (pontos pretos na curva) em cada ciclo de variação diária.

O efeito da temperatura no desempenho das funções biológicas sob condições constantes nem sempre é igual ao desempenho em condições térmicas flutuantes (Niehaus et al., 2012). Isso ocorre porque as temperaturas flutuantes têm um efeito complexo sobre o valor médio dos traços, que resulta da relação não-linear entre a temperatura e o desempenho da função estudada (Desigualdade de Jensen, Ruel e Ayres, 1999). Portanto, para cada função biológica testada sob temperaturas flutuantes, dois resultados possíveis são esperados, dada a desigualdade de Jensen. Quando a faixa de temperaturas corpóreas experimentada está em uma região côncava da curva de desempenho (Figura 3A), o organismo terá um melhor desempenho sob temperaturas constantes do que flutuantes com a mesma média. Pelo contrário, quando a faixa de temperaturas corpóreas experimentada está em uma região convexa da curva (Figura 3B), o organismo terá um melhor desempenho em condições flutuantes do que temperaturas constantes (Ruel e Ayres, 1999; Bozinovic et al., 2011). Devido ao acima mencionado, ao analisar o desempenho das funções biológicas sob regimes de variação térmica diária, a desigualdade de Jensen dificulta nossa capacidade de prever corretamente os efeitos da temperatura sobre o desempenho dos organismos.

Principalmente porque as respostas à variação térmica dependem da forma da curva de desempenho (grau de curvatura e assimetria) e da faixa de temperaturas corpóreas experimentada pelo organismo (Ruel e Ayres, 1999).

PLASTICIDADE FENOTÍPICA E NORMA DE REAÇÃO

Plasticidade fenotípica e ambientes heterogêneos

Todo os organismos têm a capacidade de expressar algum grau de plasticidade em suas características morfológicas, fisiológicas e comportamentais em resposta à variação ambiental (Schlichting e Pigliucci, 1998). A plasticidade fenotípica foi definida como a capacidade dos genótipos individuais de produzir diferentes fenótipos em resposta a diferentes condições ambientais (Pigliucci, 2005). Em ambientes heterogêneos, genótipos flexíveis podem ser favorecidos por suas respostas fenotípicas plásticas; enquanto os genótipos especializados que possuem um fenótipo fixo podem sofrer as consequências negativas da variação ambiental (Lewontin, 1957; Levins, 1962; Bradshaw e Hardwick, 1989; Schlichting e Pigliucci, 1998). Porém, a plasticidade fenotípica pode ser restringida em algumas espécies/populações devido aos custos associados, mesmo em ambientes variáveis. Isto ocorre porque as respostas plásticas podem evoluir apenas se o benefício da plasticidade supera o custo de manutenção dos mecanismos que a produzem (Via e Lande 1985; DeWitt et al. 1998; West-Eberhard, 2003). Levins (1962) propôs que a resposta plástica "ideal" em ambientes heterogêneos depende de três fatores: magnitude, frequência e previsibilidade da variação ambiental. De acordo com isto, a plasticidade fenotípica deveria ter um benefício adaptativo quando a variabilidade ambiental é previsível através da associação com dicas confiáveis (Levins, 1962).

A variação térmica é um sinal ambiental importante que induz inúmeras respostas plásticas em características fisiológicas (Angilletta, 2009). Essa plasticidade fenotípica permite aos indivíduos reduzir as demandas energéticas e manter seu desempenho em uma ampla faixa de temperaturas ambientais (Leroi et al., 1994; Angilletta, 2009; Seebacher et al., 2015). A plasticidade fisiológica pode ter efeitos persistentes em estágios sucessivos da ontogenia (irreversível = plasticidade do desenvolvimento), quando os indivíduos são expostos a variação térmica durante o desenvolvimento (Angilletta, 2009; Beaman et al., 2016), ou pode ocorrer repetidamente em respostas à desafios ambientais recorrentes (plasticidade

reversível = aclimatação), quando os indivíduos experimentam variação térmica durante o resto da vida (juvenis e adultos) (Gabriel et al., 2005; Angilletta, 2009). Teoricamente, a plasticidade do desenvolvimento deveria ter um alto valor adaptativo em ambientes que mudam entre gerações, mas que são relativamente estáveis dentro das gerações; enquanto a plasticidade reversível deveria ser benéfica do ponto de vista adaptativo em ambientes que mudam recorrentemente dentro de uma geração, já que permite aos indivíduos compensar os efeitos negativos potenciais das mudanças frequentes de temperatura (Levins, 1968; Angilletta, 2009). Como em outros tipos de plasticidade fenotípica, a capacidade para expressar respostas fisiológicas plásticas depende da magnitude, frequência e previsibilidade da variação térmica que os indivíduos vivenciam durante sua vida (Gabriel et al., 2005; Botero et al., 2015). Nesse sentido, regimes de variação térmica diária conseguem induzir plasticidade fisiológica em muitos animais, especialmente quando os indivíduos podem antecipar as mudanças de temperatura por sua previsibilidade (Niehaus et al., 2006; Kingsolver et al., 2016).

Abordagem de norma de reação

O conjunto de fenótipos que podem ser produzidos por um único genótipo quando exposto a diferentes condições ambientais, é chamado de norma de reação (Woltereck, 1909; Schlichting e Pigliucci, 1998). Embora o conceito de norma de reação seja comumente utilizado para referir-se à plasticidade fenotípica, a presença da dita plasticidade é apenas uma propriedade da norma de reação, bem como sua ausência, que gera fenótipos insensíveis à variação ambiental (Delasalle e Blum, 1994; Pigliucci, 1997). Neste último caso, indica-se que o traço fenotípico é canalizado segundo Waddington (1957), pois existem mecanismos genéticos para manter a estabilidade do desenvolvimento de dito traço, garantindo o fenótipo ótimo que favorece uma maior aptidão. As normas de reação são geralmente representadas como funções lineares ou não-lineares, que relacionam diferentes valores de uma variável ambiental (temperatura, salinidade, pH etc.) com os valores de uma ou mais características fenotípicas (Figura 4). Em uma função linear, a inclinação da norma de reação indica o grau de plasticidade da característica, quando é analisada em dois ambientes (Via et al., 1995). Então, quando a inclinação da norma de reação é acentuada, diz-se que a característica varia muito à medida que o ambiente muda (ou seja, tem alta plasticidade, Figura 4A), e quando a

inclinação da norma de reação é horizontal, diz-se que a característica mantém o valor em toda a faixa ambiental avaliada (não é plástica, Figura 4B).
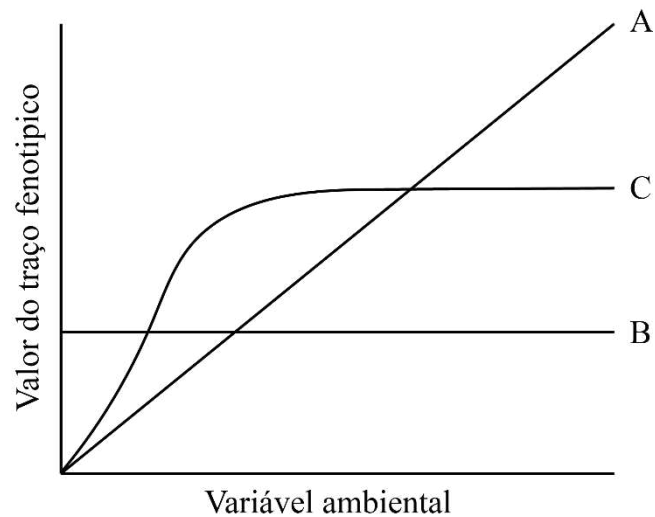
Figura 4. Normas de reação para um caráter hipotético em três genótipos distintos (baseado em Schlichting e Pigliucci, 1998): A. é um genótipo plástico em uma função linear, B. é um genótipo não plástico em uma função linear, enquanto C. é um genótipo plástico em uma função não-linear.

As normas de reação linear são comumente utilizadas para estudar diferentes tipos de caracteres morfológicos, fisiológicos ou de história de vida, os quais são conhecidos por variar mais frequentemente em resposta a alterações ambientais previsíveis (Via, 1993). As normas de reação de desenvolvimento são normas de reação linear, que permitem avaliar o efeito irreversível da temperatura em diferentes características fenotípicas de organismos em desenvolvimento (e.g. tamanho corpóreo, tempo de desenvolvimento) (Pigliucci et al., 1996). A abordagem de norma de reação de desenvolvimento fornece uma descrição clara dos atributos das respostas plásticas (magnitude e padrão), bem como da interação genótipo-ambiente (De Jong, 1990). A interação genótipo-ambiente é possível de ser analisada através de experimentos de ninhada dividida, nos quais membros de uma mesma família ou clones são expostos a duas ou mais condições ambientais para que expressem plasticidade (Kingsolver et al., 2004a). Nestes experimentos costuma-se distinguir o valor médio dos traços em todos os ambientes, e a plasticidade dos traços fenotípicos em diferentes ambientes (inclinação). Então, quando as normas de reação dos diferentes genótipos analisados têm a mesma inclinação (paralelas), a mudança fenotípica resultante pode ser associada

unicamente à variação ambiental, já que uma variação genética para plasticidade não está envolvida na expressão fenotípica (Via, 1993). Pelo contrário, quando os genótipos analisados exibem respostas plásticas diferentes e as normas de reação têm inclinação diferente (não paralelas), estabelece-se uma interação genótipo-ambiente, na qual há um componente de variação genética para a plasticidade (Via, 1993; Scheiner, 1993). Essa variação genética é de fato o requisito mínimo que permite a evolução da plasticidade fenotípica e a norma de reação nas populações (Via e Lande, 1985).

As curvas de desempenho térmico são normas de reação térmica de tipo não-linear (Figura 4C), utilizadas comumente para estudar a sensibilidade térmica do desempenho em funções biológicas, como locomoção, crescimento e desenvolvimento, por exemplo. Nas curvas de desempenho os valores das características estudadas variam de maneira contínua em função da temperatura ambiental (Huey e Stevenson 1979). Conforme detalhado acima, as curvas de desempenho têm uma forma de "U" invertida típica e diferentes parâmetros, como: Topt (temperatura ótima), amplitude de desempenho e limites térmicos críticos (CTmin e CTmax). A alteração desses parâmetros causa mudanças na forma e na posição da curva ao longo do eixo de temperatura. Três modos alternativos de variação da curva de desempenho têm sido propostos, o que nos permite entender muitas respostas fisiológicas plásticas e a evolução das normas de reação não-lineares (Huey e Kingsolver, 1989): (1) deslocamento vertical (mais rápido-mais lento) que produz mudanças no desempenho médio, (2) deslocamento horizontal (mais quente-mais frio) que produz variação na temperatura Topt, e (3) mudanças na amplitude de desempenho (especialista-generalista) associadas com um trade-off deste parâmetro com o desempenho máximo (Huey e Hertz 1984; Huey e Kingsolver, 1989). Os modos de variação acima, fazem que o entendimento da interação genótipo-ambiente, especialmente a variação genética para plasticidade, em normas de reação não-linear apresenta maiores dificuldades do que uma norma linear de duas ou três condições térmicas. Porém, diferentes tipos de interação genótipo-ambiente foram reconhecidos para cada um dos modos de variação da curva de desempenho (Kingsolver et al., 2004a).

Base genética da plasticidade

Uma rica evidência tem suportado a existência de variação genética para plasticidade fenotípica, indicando que esta propriedade dos genótipos claramente pode evoluir pela

seleção natural (Schlichting, 1986; Schlichting e Levin, 1986; Stearns, 1989; Scheiner, 1993; David et al., 1994; Gilchrist, 1996; Schlichting e Pigliucci, 1998). A evolução da plasticidade fenotípica e da norma de reação, pode ocorrer pelos dois mecanismos que têm sido propostos para explicar a base genética da plasticidade (Via, 1993; Schlichting e Pigliucci, 1993). O primeiro mecanismo é a sensibilidade alélica, no qual a plasticidade fenotípica pode evoluir como um subproduto da seleção de diferentes valores fenotípicos ótimos em diferentes ambientes (não como um caráter em si mesmo), sem necessitar de uma base genética exclusiva para plasticidade (Via, 1993). Nesse mecanismo, os diferentes valores fenotípicos são assumidos como estados de caracteres expressos para cada ambiente quando a seleção direcional atua sobre os valores médios das características (Via, 1995). O segundo mecanismo é a regulação genética, em que a plasticidade pode evoluir independentemente do valor médio dos traços fenotípicos, visto que existe uma variação genética exclusiva para respostas plásticas (Schlichting e Pigliucci, 1993). Nesse mecanismo, há uma regulação genética ambientalmente controlada, segundo a qual loci (reguladores) específicos regulam a expressão de diferentes genes estruturais (Schlichting e Pigliucci, 1998). Essa ação reguladora controla as respostas plásticas, uma vez que nem todos os loci dos genes são expressos em todos os ambientes.

Ambos os mecanismos genéticos mencionados acima podem produzir respostas fenotípicas plásticas em populações naturais. Inclusive um locus com sensibilidade alélica e um locus regulador com uma ação específica do ambiente podem atuar de forma independente ou interativa em alguns casos para gerar diferentes valores de uma característica fenotípica em cada ambiente (Via et al., 1995). Respostas plásticas com base genética específica (regulação genica) tem sido observadas, alguns exemplos disso são: a resposta de aclimatação facilitada por proteínas de choque térmico (Pigliucci, 1996), resposta à luz nas plantas com flores que é facilitada por genes de fitocromos (Pigliucci, 1996), ou os polifenismos sazonais em padrões de asas de borboletas (Brakefield et al., 1996). Esses exemplos são importantes para demonstrar que a plasticidade é um caráter em si, que evolui por meio da seleção natural (Bradshaw, 1965).

PLASTICIDADE FENOTÍPICA EM LARVAS DE ANUROS

As larvas de anuros são modelos ideais para estudar respostas de plasticidade fenotípica induzidas por variações de temperatura. Por serem aquáticas, a temperatura corpórea das larvas reflete a temperatura de seu ambiente devido à alta condutividade térmica da água (Spotila et al. 1992). Ao contrário da fase adulta, as larvas têm pouca chance de manter comportamentalmente suas temperaturas corpóreas dentro de faixas estreitas devido à ausência de gradientes térmicos pronunciados nas poças de reprodução (Gutiérrez-Pesquera et al., 2016a; Simon et al., 2015). As larvas que se desenvolvem em corpos de àgua temporários (e.g. gêneros *Physalaemus, Engystomops, Leptodactylus*) podem experimentar aquecimento intenso, especialmente as que usam poças rasas que recebem radiação solar direta (Duarte et al. 2012; Simon et al., 2015). Por esta razão, as larvas de anfíbios de poças temporárias dependem de diversos ajustes fisiológicos plásticos para lidar com as variações térmicas diárias, como a aclimatação da CTmax por exemplo (Tejedo et al., 2012). As larvas também dependem de sua capacidade para reduzir a sensibilidade térmica de funções como desenvolvimento, crescimento e respiração aeróbica, que devem ser mantidas em faixas térmicas apropriadas para atingir o estágio adulto (Niehaus et al., 2012; Kern et al., 2015a).

Os efeitos da temperatura no desenvolvimento das larvas de anuros têm sido extensamente estudados (Newman, 1989, 1992; Richter-Boix et al., 2011), e diferentes modelos foram propostos para explicar a plasticidade induzida pela temperatura no tempo de desenvolvimento e na massa na metamorfose (Wilbur e Collins, 1973; Smith-Gill e Berven, 1979; Werner, 1986). O modelo de Wilbur e Collins (1973) prediz que o tempo de desenvolvimento é regulado por fontes de estresse ambiental que influenciam a taxa de crescimento. Assim as larvas com taxas de crescimento reduzida (pelo efeito de temperatura) estenderão seu período de desenvolvimento até atingirem um limite de tamanho mínimo para metamorfose (Wilbur e Collins, 1973; Werner, 1986). Por outro lado, Smith-Gill e Berven (1979) propuseram um modelo que supõe a existência de um trade-off entre a taxa de desenvolvimento e a taxa de crescimento, segundo o qual girinos expostos a altas temperaturas sofrem metamorfose mais rapidamente, resultando em recém-metamorfoseados com tamanho corpóreo reduzido, devido a um desenvolvimento mais acelerado que o crescimento. Este modelo é amplamente utilizado para explicar a redução no tempo de metamorfose em resposta à dessecação de poças temporárias. Bem como outros dois modelos alternativos que foram discutidos por Richter-Boix et al. (2011): o primeiro

modelo prevê trajetórias ontogenéticas onde as taxas de crescimento e desenvolvimento são desacopladas, portanto, a redução no tempo de desenvolvimento deve ser acompanhada por uma redução semelhante na massa na metamorfose, sem alterações na taxa de crescimento. O segundo modelo prevê que os incrementos na taxa desenvolvimento larval devem ser acompanhados por incrementos compensatórios na taxa de crescimento, resultando em um tamanho maior na metamorfose (Richter-Boix et al., 2011).

No contexto particular da variação térmica em gradientes de cobertura vegetal, que é o tema central desta dissertação, as larvas de anfíbios têm mostrado uma série de mudanças fenotípicas induzidas pela temperatura, tais como, plasticidade na tolerância ao calor (CTmax), que permite aos indivíduos resistir a temperaturas altas extremas em poças sem cobertura vegetal (Skelly e Freidenburg, 2000; Richter-Boix et al., 2015), e ajustes em traços de história de vida como taxas crescimento e desenvolvimento, correlacionados com o nivel de cobertura vegetal nesses gradientes locais (Skelly, 2004; Richter-Boix et al., 2010; 2015). Todos esses trabalhos utilizaram uma abordagem de normas de reação de desenvolvimento, testando as respostas fenotípicas das larvas em condições térmicas constantes, e também em experimentos semi-naturais (mesocosmos) que não permitem o isolamento da variação térmica natural como variável de interesse nos gradientes de cobertura vegetal (Hawley, 2010). Portanto, as respostas fisiológicas das larvas de anuros à variação térmica diária dos gradientes de cobertura vegetal são desconhecidas. O acima é correto, uma vez que as respostas fisiológicas à variação térmica diária podem ser subestimadas ou superestimadas quando as normas de reação são caracterizadas sob temperaturas constantes, como foi demonstrado para traços de desenvolvimento e tolerância térmica em larvas de anuros (Niehaus et al., 2006; Niehaus et al., 2012; Kern et al., 2015a; Gutiérrez-Pesquera et al., 2016a). Nesse sentido, os estudos das normas de reação sob regimes de variação térmica natural fornecem informações ecologicamente relevantes sobre a capacidade de resposta fenotípica das espécies/populações às flutuações de temperatura atuais e futuras (Bozinovic et al., 2011; IPCC, 2014; Kern et al., 2015a).

Diante do cenário exposto, o objetivo principal desta dissertação foi avaliar as respostas fisiológicas à variação térmica diária registrada em gradientes de cobertura vegetal em larvas de anuros que utilizam poças temporárias. Esta dissertação pretende contribuir para preencher lacunas de conhecimento relacionadas com os efeitos da variação térmica em

traços de desenvolvimento e tolerância térmica (CTmax) de larvas de anfíbios em gradientes de cobertura vegetal.

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OBJETIVOS

OBJETIVO GERAL

Avaliar como as flutuações térmicas diárias num gradiente de cobertura vegetal influem nas respostas de traços de tolerância térmica (CTmax) e desenvolvimento de larvas de *Physalaemus cuvieri* e *Physalaemus moreirae*.

OBJETIVOS ESPECÍFICOS

- Determinar se os padrões de variação térmica (descritores microclimáticos) das poças temporárias utilizadas pelas espécies estão associados com seus níveis de cobertura vegetal.
- Comparar as normas de reação térmica de desenvolvimento da tolerância ao calor (CTmax) e características da história de vida em *Physalaemus cuvieri* e *Physalaemus moreirae* sob regimes térmicos variáveis*.*
- Analisar se a sensibilidade térmica (desenvolvimento) e capacidade de aclimatação das larvas das duas espécies se correlacionam com os regimes de variação térmica que experimentam em seus microambientes.

HIPÓTESE

Devido a que os gradientes de cobertura vegetal podem gerar diferentes padrões de variação térmica diária dentro de uma paisagem, nós hipotetizamos que a capacidade para ajustar as características fisiológicas e trajetórias ontogenéticas sob regimes variáveis depende do grau de variação térmica diária que as larvas experimentam em seus ambientes (Niehaus et al., 2006; Kern et al., 2015a; Gutiérrez-Pesquera et al., 2016a). Especificamente, nós esperamos que as larvas que experimentam maior amplitude térmica diárias e altas taxas de mudança de temperatura em seus ambientes mostrem uma maior capacidade para realizar ajustes fisiológicos do que as larvas de ambientes termicamente mais estáveis.

CAPÍTULO ÚNICO

Phenotypic plasticity responses in anuran larvae along vegetation cover gradients: thermal tolerance and life history[1]

1. INTRODUCTION

Temperature fluctuations profoundly influence on physiological function, performance (e.g. metabolic rate, growth rate, development, locomotion) and fitness of ectothermic animals (Huey and Kingsolver, 1989; Angilletta, 2009). Thermal performance is usually described by a non-linear function in these organisms (Huey and Stevenson, 1979), where the performance of a given trait increases gradually with temperature from a critical thermal minimum (CTmin) to an intermediate maximum point (its optimal temperature), and then decreases rapidly to a critical thermal maximum (CTmax) in an asymmetric gaussian trend (Lynch and Gabriel, 1987; Angilletta et al., 2002). Thermal limits of organisms and performance on different traits have thermal reaction norms that may be shaped by both, genetic local adaptation and phenotypic plasticity, the ability of a single genotype to express variable phenotypes under different environments (Angilletta et al., 2002; Beaman et al., 2016). Phenotypic plasticity can be reversible when is induced by periods shorter than life span (thermal acclimation or heat hardening) or nonreversible when takes place during development (developmental plasticity), changing the phenotypic trait values throughout life and modifying developmental trajectories (Gilbert and Huey, 2001; Gabriel, 2005; Bodensteiner et al., 2020). Both types of phenotypic plasticity aid resist stressful thermal regimes, since that improve individual's capacity to buffer performance and fitness from daily thermal variation they experience (Schaefer and Ryan, 2006; Seebacher et al., 2015; Kern et al., 2015a).

Phenotypic plasticity in ectothermic animals can be induced in diverse environmental thermal gradients, even at scales relevant to the organism (Sinclair et al., 2006; Williams et al., 2012; Jimenez et al., 2015). The particular spatial dynamics of the daily thermal variation (and seasonal) in this gradients is an important environmental cues to induce plasticity (Du et al., 2019). For instance, in local gradients of vegetation cover (GVC) the degree of thermal

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variation and the rate of temperature changes in the microhabitats of small animals depend on the level of vegetation cover (Schiesari, 2006; Pike et al., 2011). Uncovered microhabitats experiences extreme warm temperatures and are more variable, while the opposite occurs in covered microhabitats (moderate warm extremes and slow thermal changes) (Burton and Likens, 1973; Skelly et al., 2002). Daily thermal variation in GVC can affect the phenotypic expression of small ectothermic animals at microgeographic scales (Pincebourde et al., 2016), particularly of those that are in development, since daily thermal variation can lead to critical energetic imbalances due to increased metabolic demands (Arrighi et al., 2013; Kern et al., 2015a).

Several studies on vegetation cover gradients have showed differentiation in development characteristics (Skelly, 2004; Richter-Boix et al., 2010; Hawley, 2010), and thermal tolerance of developing animals as anuran larvae (Skelly and Freidenburg, 2000; Richter-Boix et al., 2015). The aforementioned studies evaluated thermal reaction norm using constant temperatures (Skelly, 2004; Richter-Boix et al., 2010; 2015) or semi-natural approaches that do not allow isolating the multifactorial components in the GVC, i.e. predators, dissolved oxygen, pH (Hawley, 2010). It is known that constant temperatures can underestimate or overestimate the developmental reaction norm of life-history traits and thermal tolerance of tadpoles in relation to natural fluctuating thermal regimes (Niehaus et al., 2006; Niehaus et al., 2012; Kern et al., 2015a; Gutiérrez-Pesquera et al., 2016a). Therefore, experimental fluctuating thermal regimes are ecologically relevant, and reproduce the physiological challenges of tadpoles in heterogeneous environments (Niehaus et al., 2006; Kern et al., 2015a). In this context, it is important to know if tadpoles that experience greater thermal variability can increased thermal tolerance and reduce the sensitivity of performance traits through phenotypic plasticity (Beaman et al., 2016). Since species with the ability to flexibly modify their physiological functions in response to changes in temperature will be less affected by global and local climatic disturbances, at least in the short term (Schulte, 2013; Seebacher et al., 2014; Gunderson and Stillman, 2015; Bodensteiner et al., 2020).

Tadpoles are suitable models for the study of phenotypic plasticity induced by daily thermal variation in GVC, since their body temperatures reflects the temperature of their environment (Spotila et al., 1992). Furthermore, because tadpoles of phylogenetically related species can be found in sympatry, using ponds that differ widely in the degree of thermal variation and in

the rate of temperature changes (Gutiérrez-Pesquera et al., 2016b). In the present study, we investigated tadpoles of *Physalaemus cuvieri* (Fitzinger, 1826) and *Physalaemus moreirae* (Miranda-Ribeiro, 1937) to understand their physiological responses to diurnal thermal fluctuations from GVC. We hypothesized that tadpoles that experience greater daily thermal variation and high rates of temperature change in their environment would exhibit high values of CTmax and greater capacity for acclimation than tadpoles from more stable environments (Angilletta, 2009; Duarte et al., 2012; Gutiérrez-Pesquera et al., 2016a). We also expected that tadpoles that experience greater thermal variability in their environment would have ability to reduce the thermal sensitivity of biological functions and adjust the ontogenetic trajectories under varying thermal conditions than tadpoles that experience low thermal variation (Niehaus et al., 2006; Kern et al., 2015a).

2. METHODS

2.1. *Animals*

We investigated thermal variation effects on tadpoles of two sympatric species that develop in different thermal microenvironments from Brazilian Atlantic forest, *Physalaemus cuvieri* and *Physalaemus moreirae*. These species represent to two different species-group within genus *Physalaemus* such as *P. cuvieri* group and *P. signifer* group respectively (Lourenço et al., 2015). Species within genus *Physalaemus* are characterized by building foam nests in ephemeral pond, which experiment quick temperature changes compared to permanent ponds (Pombal and Haddad, 2007).

Physalaemus cuvieri is widely distributed, occurring from north to south and the central-western region of Brazil, with populations in the Argentinean Provinces of Misiones and Corrientes, Eastern Uruguay, Eastern Paraguay and Bolivia (Lavilla et al., 2000; Maneyro and Beheregaray, 2007; Frost, 2020). These frogs are found breeding in subtropical and tropical forest, open areas and humid savannas always at low altitudes (Heyer et al., 1990). During the breeding season (October to March) individuals of *P. cuvieri* build foam nests in temporary ponds and floodplain where they spawn about 600 eggs (Andrade, 1995). This species has small exotrophic tadpoles (total length ~ 26 mm), which can develop under natural conditions in less than two months (Andrade, 1995; Pombal and Haddad, 2007). The tadpoles

of *P. cuvieri* from temporary ponds are subject to a large spatio-temporal thermal variation in Atlantic forest (Simon, 2010).

Physalaemus moreirae is an endemic species of the Brazilian Atlantic forest, distributed in several localities in the Serra do Mar range in the state of São Paulo, including Santos, Guarujá, Salesópolis, Bertioga, and Santo André (Provete et al., 2011). Individuals of *P. moreirae* can be found in temporary ponds or flooded litter (swamp) inside forests, where these builds foam nest during the breeding season between August and March (Heyer et al., 1990; Provete et al., 2011). *P. moreirae* has small (total length ~ 23 mm) and exotrophic tadpoles, which develop in ephemeral ponds protected from extreme solar radiation and high temperatures (Provete et al., 2011).

2.2. Collection and Maintenance

We collected five egg masses of *P. moreirae* and three of *P. cuvieri* from ephemeral and semi-permanent water bodies (in 06 to 21 February/2020) in the Boracéia Biological Station, near the city of Salesópolis, SP (EBB 28 ° 38' S, 45 ° 52' W). We recorded the day of capture of each egg mass to calculate the ages of the tadpoles in development experiments, so we assume that spawning occurred around 02:00h on the day of collection. We transported field-collected clutches to the laboratory of Ecophysology and Evolutionary Physiology (LEFE) at the University of São Paulo. Once in the laboratory, we placed egg masses in separate 20 L containers with chlorine-free water, at a constant temperature of 20 ° C and a 12L:12D photoperiod until hatching. Animal collection was approved by the Chico Mendes Institute for Biodiversity Conservation (ICMBio/SISBIO – license number: 72843-2), and all experiments were conducted with the authority of the Ethics Committee for Animal Use of the Federal University of Paraná (CEUA/BIO – approval number: 1338).

2.3. Thermal regimes and Vegetation cover gradient

We recorded water temperature at a depth of 10 cm in different ponds (collection sites) every ten minutes from a minimum of four days up to 12 days according to the duration of the water bodies and the period of tadpole presence (ibutton, Maxim Integrated Products, California, USA). Data on forest canopy cover for each pond were estimated using hemispheric

photographs and gap analysis with the Gap Light Analyzer 2.0 (GLA) software (Frazer et al., 2001).

2.4. *Experimental Design and Sampling*

2.4.1. Thermal treatments

Three experimental temperature regimes were simulate: a highly variable treatment (HighT: 19 – 30 °C, mean: 25 °C), a low variable treatment (LowT: 19 – 24 °C, mean: 22 °C), and a constant temperature equivalent to the average observed at our field sites (Constant: 23 °C). Fluctuating regimes simulated daily thermal fluctuation ranges recorded from temporary ponds where *Physalaemus* species develop (Table 1, Fig. S1 and S2). For the HighT, the maximum temperature was selected according to the mean Tmax recorded in uncovered ponds (around 31°C) and thermal environment data previous informed for *P. cuvieri* at the same study site (mean Tmax = 29.6±5.0 °C, Simon et al., 2015). The maximum temperature in the LowT was selected according to the mean Tmax values informed for *P. moreirae* in this study. Finally, the minimum temperature in both fluctuating treatments corresponded to the mean value of Tmin that we recorded in several temporary ponds in the study area (19 °C). Fluctuating thermal regimes were recreated using 100W aquarium heaters connected to electronic timers, inside 100L large plastic containers. The timers were turned on at 06:00 h to reach the maximum temperatures for each fluctuating regime (around 14:30 h), and then turned off at 15:30 h, simulating realistic daily thermal cycles. Fluctuating thermal treatments had ecological ramping rates, LowT = 0.64 °C / h and HighT = 1.27 °C / h. Once the maximum temperature was reached, this condition was kept constant for 1 h. We located the plastic containers in a climatic room at a temperature of 18 °C and a photoperiod of 12 L: 12 D. Aaerators were installed to maintain adequate levels of oxygen dissolved in the water during the experiments and water was partially changed every week (~ 60 % renewed volume). We occupied 12 larger containers, six destined for CTmax experiments and six for tadpole development. For the CTmax experiments we maintained tadpoles of the same clutch and the same experimental group in shared space inside the large plastic container using four medium-sized aquarium nets on a polystyrene support (approximate nets volume: 6 L). In turn, for the development experiment we kept the tadpoles in individual spaces within the

large plastic container using 42 small-sized aquarium nets on a polystyrene support (approximate nets volume: 0.5 L).

2.4.2. Acclimation response of CTmax

After 15 days in the thermal treatments (to acclimation), the CTmax of tadpoles from each experimental group were tested. Tadpoles were tested individually between the stage 25-32 for *P. cuvieri* and 25-36 for *P. moreirae* (Gosner, 1960), because CTmax tends to decrease once tadpoles are close to metamorphic climax (Sherman, 1980; Floyd, 1983). To determine the critical thermal maximum (CTmax) of each tadpole from each species, we used Hutchison's dynamic method (Lutterschmidt and Hutchison, 1997). Tadpoles individually were placed in 300 mL plastic container with 100 mL water, inside a manually regulated thermal bath. They were maintained for 10 minutes at the initial experimental temperature (23 ° C), then we started CTmax test using a constant ecological heating rate: Mean = 0.05 ± 0.01 °C/min (N = 21, range = 0.046 – 0.054 °C/min); all test started between 8:00 am and 10:00 am (UTC-2). The experimental end-point was total immobilization, which we identified when tadpoles not reacted after 5 consecutive touches applied every 2 s with the probe of a fast-reading thermometer. Then we recorded the water temperature as a proxy for tadpole CTmax (to the nearest 0.1°C), assuming isothermia (Navas et al., 2010). After that, tested tadpoles were immediately transferred to individual plastic containers with water at 23 °C to allow for recovery. We only analyzed the CTmax data of tadpoles that survived 24 hours after the tests, for these individuals we measured the body mass using an analytical balance to the nearest 0.1 mg and determined the developmental stage according to Gosner (1960).

2.4.3. Developmental reaction norm of life-history traits

Tadpoles between the stage 25 and 26 were randomly distributed in the three thermal treatments to assess their life-history traits: 8 tadpoles/clutch for *P. moreirae* and from 10 and 16 tadpoles/clutch for *P. cuvieri*. During tadpoles develop the photoperiod was set at 12 L: 12 D, the water in big container was changed every week and the same food ration was provided to all individuals every two days. All tadpoles were weighed on the initial day (to the nearest 0. 1 mg) and then were individually kept in small-sized aquarium nets in respective thermal treatments. We monitor tadpoles develop every two days, and when we

observed individuals in an advanced stage, the nets were checked every day. So when tadpoles reached the stage 42 (Gosner, 1960: showing emergence of some anterior limb) we recorded the larval period (days) and the wet mass at metamorphosis using an analytical balance (to the nearest 0.1 mg). The growth rate is a simple abstraction of complex and nonlinear growth trajectories in anuran larvae. Estimation of such growth trajectories usually require repeated periodic measurements and continuous manipulation, which produces additional stress in the studied animals. In order to avoiding the effects of handling stress on tadpoles growth and survival, we used a linear metric to calculates growth rate (Richter-Boix et al., 2011), defined as the mass gain as a function of time (mg / day). In addition, we verified that the initial body mass did not have differences among experimental groups (*P. moreirae*: ANOVA: $F_{2,117}$ = 0.39, P = 0.673; *P. cuvieri*: ANOVA: $F_{2,105}$ = 0.71, P = 0.492). Tadpole mortality was recorded daily throughout the experiment to estimate survival rates.

2.5. Statistical analysis

The thermal environment of both species was analyzed using graphical and descriptive statistics (mean, maximum and amplitude), from the 24-hour records obtained during several days in the temporary ponds. The rate of temperature changes in the temporary pond was calculated as the thermal hating recorded in ten-minute intervals during the diurnal hours (06:00 and 18:00).

To assess normality and homogeneity of variance of the data we used the Shapiro-Wilk and Bartlett tests. The CTmax data of *P. moreirae* did have a normal distribution (Shapiro-Wilk normality test W = 0.98, P = 0.183). Despite CTmax data of *P. cuvieri* was slightly skewed (Shapiro-Wilk normality test W = 0.90, P < 0.05), the raw data distribution approached normal more than other distributions tested. So we used an ANCOVA analysis, including species as a categorical variable and body mass as a covariate, to determine if species differed in their CTmax. Then, we constructed linear mixed-effects models (LMMs) in order to explain CTmax variation according to our acclimation experiments. Fixed factors were the categorical predictors 'treatments' and 'Gosner stages' (including their interactions), and the continuous covariate 'body mass (mg)'. The random factor was 'Clutch (random intercept)', since the possible variation generated by individuals belonging to different clutches is not predictable.

For the ordinal categorical variable developmental stage, two intervals were defined: (1) Gosner stage 25, and (2) Gosner stage above 26 for both species.

To measure the level of thermal CTmax plasticity in response to temperature treatments we used acclimation response ratio. The ARR corresponds to the change in the CTmax per degree change in acclimation temperature (Claussen, 1977). We calculated this index as the difference in the CTmax among thermal treatments (only fluctuating regimes, Heerwaarden et al., 2016), which is then divides by the difference between the means temperature of both treatments (i.e. 3 °C in this case):

$$ARR = \frac{CTmax\ at\ temp.\,1 - CTmax\ at\ temp.\,2}{\text{mean HighT} - \text{mean LowT}}$$

An acclimation response ratio of 1 indicates a positive 1 °C shift in CTmax for each 1 °C increase in developmental temperature. In contrast, an ARR of 0 indicates that the CTmax is not acclimated in developmental temperature treatments (Gunderson and Stillman, 2015). The ARR can also take negative values, indicating a detrimental effect during acclimation. Warming Tolerance (WT) were calculated as the difference between CTmax measured in tadpoles and maximum water temperatures (Tmax) that these regularly experiences in temporary pond during the breeding season (summer) (Deutsch et al., 2008). In order to avoiding bias from extreme temperatures, which rarely are experiences for tadpoles we used as maximum water temperature a calculated mean value from 25% of the highest temperatures recorded in each pond.

To evaluate the effect of the treatments, initial body mass and their interactions in the survival of both species, we fitted generalized linear models (GLMs) with binomial error and the "logit" link function. Linear mixed models (GLMMs) were used to evaluate the effects of thermal treatments and initial body mass (including their interactions) on age at metamorphosis (days), considering the random effects of 'Clutch' (or not: in case of singularity fit). We assumed a Poisson error distribution ("log" link function) since it was a data set of counts. Additionally, we fitted linear mixed models to explain the variation in body mass at metamorphosis (mg) and growth rate (mg / day) in response to thermal treatments, initial body mass and their interactions, including 'Clutch (random intercept)' as a random factor. We assumed a Normal on error distribution. In both species, the mass at metamorphosis and

the growth rate had a normal distribution (Shapiro-Wilk normality test P > 0.05). Several mixed models built (more parameterized models) to explain the variation in mass at metamorphosis of both species presented singularity fit. So we removed all singularity fits from the list of candidate models to ensure robust estimations. There were also singularity fits in the more parameterized models constructed to explain the variation in age at metamorphosis in *P. moreiae* tadpoles. Only the simplest random effects structure was fitted correctly, but some variance components associated to random intercept were estimated as close to zero or exactly zero in this model. Therefore, we opted not to consider the random factor, constructing generalized linear models (Poisson error distributions, log link function) to evaluate the effect of the treatments and initial body mass (including their interactions) on age at metamorphosis of *P. moreirae* tadpoles.

We selected the best random-effects structures and generalized linear models based on their second-order Akaike information criterion value (AICc), selecting the one with the lowest AICc (Burnham and Anderson, 2002; Burnham et al., 2011). All mixed-effects models (LMMs and GLMMs) and generalized linear models (GLMs) were fitted using the 'lme4' package and the function lmer (Bates et al., 2015) in R programming environment (version 4.0.4) (R Core Team, 2021). Alpha was set as p = 0.05. Data are shown as mean ± standard deviation in the text, and the errors around the means correspond to 95% confidence intervals in the figures.

3. RESULTS

3.1. Thermal regimes in organismic scales

The mean water temperature varied across the vegetation cover gradient, from 25.5 ± 0.1 °C in a 0% cover to 20.7 ± 1.0 °C in a 63.36% cover (Table 1). Mean daily temperature range, as a measure of variability in the species-specific thermal environment, was greater in the uncovered ponds that are used by *P. cuvieri* than in the covered ponds commonly occupied by *P. moreirae* (Table 1, Fig. S2).

The maximum water temperatures experienced for the studied species were very different, for the *P. cuvieri* ponds the mean Tmax was above 31.5 °C, with absolute values

around 40.0 °C. While for the *P. moreirae* ponds the maximum mean was between 24.1 °C and 27.1 °C, with absolute values around 29.0 °C (Fig. 1A). The minimum water temperatures experienced for the studied species were similar (Fig. 1B).

Table 1. Temperature descriptors of species-specific thermal environment and forest canopy cover for five frog breeding ponds in the Boraceia Biological Station. PcC = clutches of *P. cuvieri* and PmC = clutches of *P. moreirae*.

	P1	P2	P3	P4	P5
Forest canopy (%)	0%	19%	51.43%	63.36%	22.45%
Mean temperature	25.6±3.6 °C	25.6±0.1 °C	20.3±2.0 °C	20.7±1.0 °C	24.1±0.7 °C
Mean range temperature	12.5±4.9 °C	13.7±0.5 °C	3.4±6.2 °C	2.4±0.1 °C	7.5±1.5 °C
Maximum thermal change	2.4 °C	2.8 °C	0.1 °C	0.9 °C	0.7 °C
Measurement time	8 days	4 days	12 days	7 days	4 days
Field-collected Clutches	PcC1, 2	PcC3	PmC1, 2, 3	PmC5	PmC4
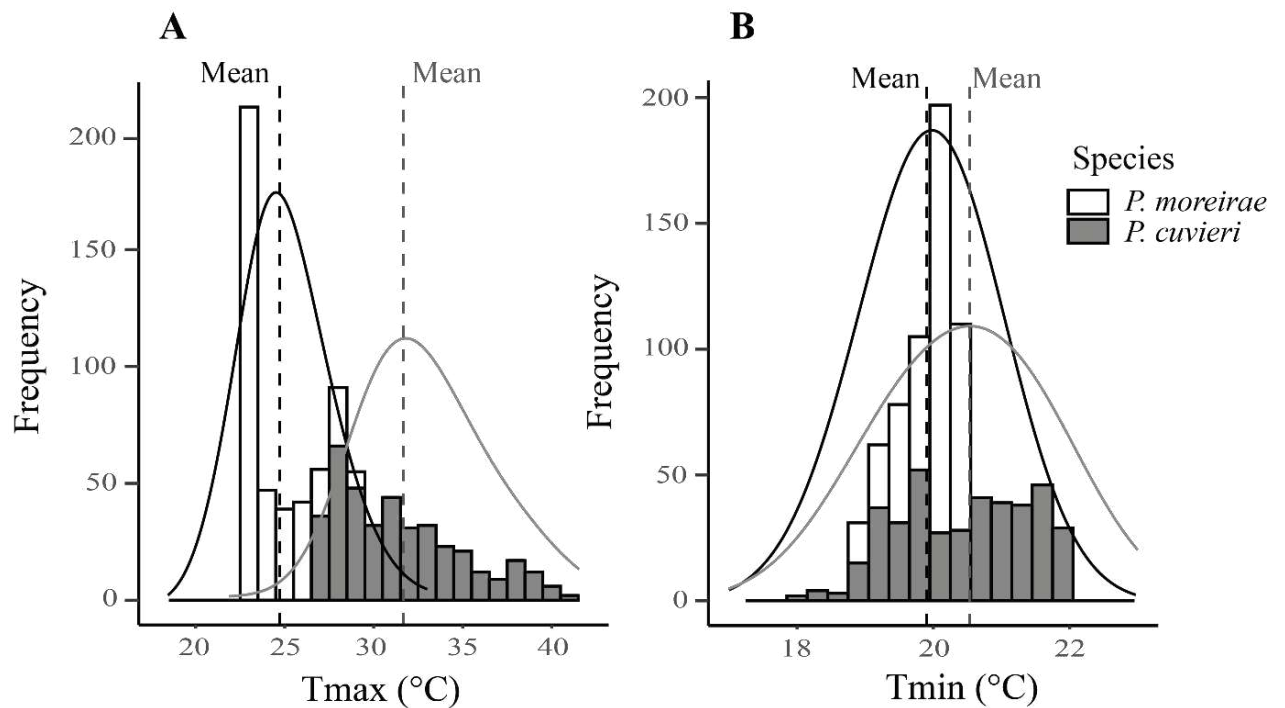
Figure 1. Frequency distribution of (A) maximum and (B) minimum water temperatures recorded from microhabitats commonly occupied by *P. moreirae* and *P. cuvieri* for breeding. Tmin = 25% of the lowest temperatures recorded in temporary ponds, Tmax = 25% of the highest temperatures recorded from temporary ponds every ten minutes during several 24-h cycles.
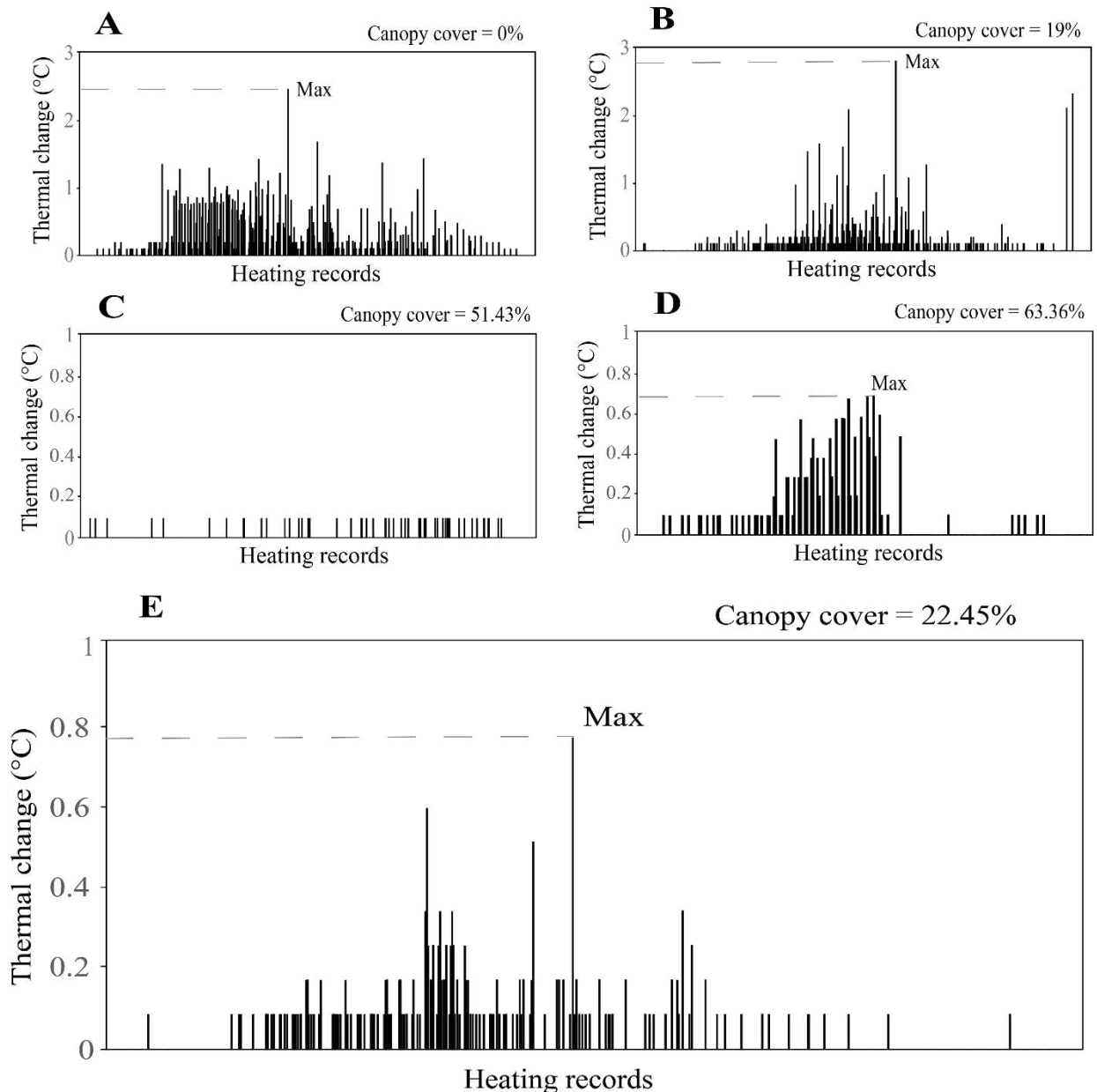
Figure 2. Temperature changes recorded from closed and open canopy ponds every ten minutes during daytime hours (06:00 - 18:00 h). The bars correspond to water heating records that occurred at ten-minute intervals. (A) Temporary pond with 0% canopy cover, (B) Temporary pond with 19% coverage, (C) Temporary pond with 51.43% coverage, (D) Temporary pond with 63.36% coverage, (E) Temporary pond with 22.45% coverage.

Water heating in open canopy ponds occurs faster than in closed canopy ponds (Fig. 2), involving frequent thermal changes above 1 °C in ten minutes with maximum between 2 °C and 2.8 °C (Fig. 2 A and B). Whereas, in ponds with canopy cover level greater than 22.45%

the heating not exceed 1 °C in the same period of time, and large thermal changes were less frequent with maximum between 0.6 °C and 0.8 °C (Fig. 2 C, D, and E).

3.2. Upper thermal tolerance: species comparison

Physalaeumus cuvieri had a higher larval thermal tolerance than *P. moreirae* (ANCOVA: $F_{1,208}$ = 538, P < 0.001; Fig. 3). The CTmax of *P. cuvieri* tadpoles was in mean = 40.8 ± 0.1 °C (39.0 – 42.0 °C) and that of *P. moreirae* was in mean = 38.7 ± 0.1 °C (37.1 – 41.3 °C).
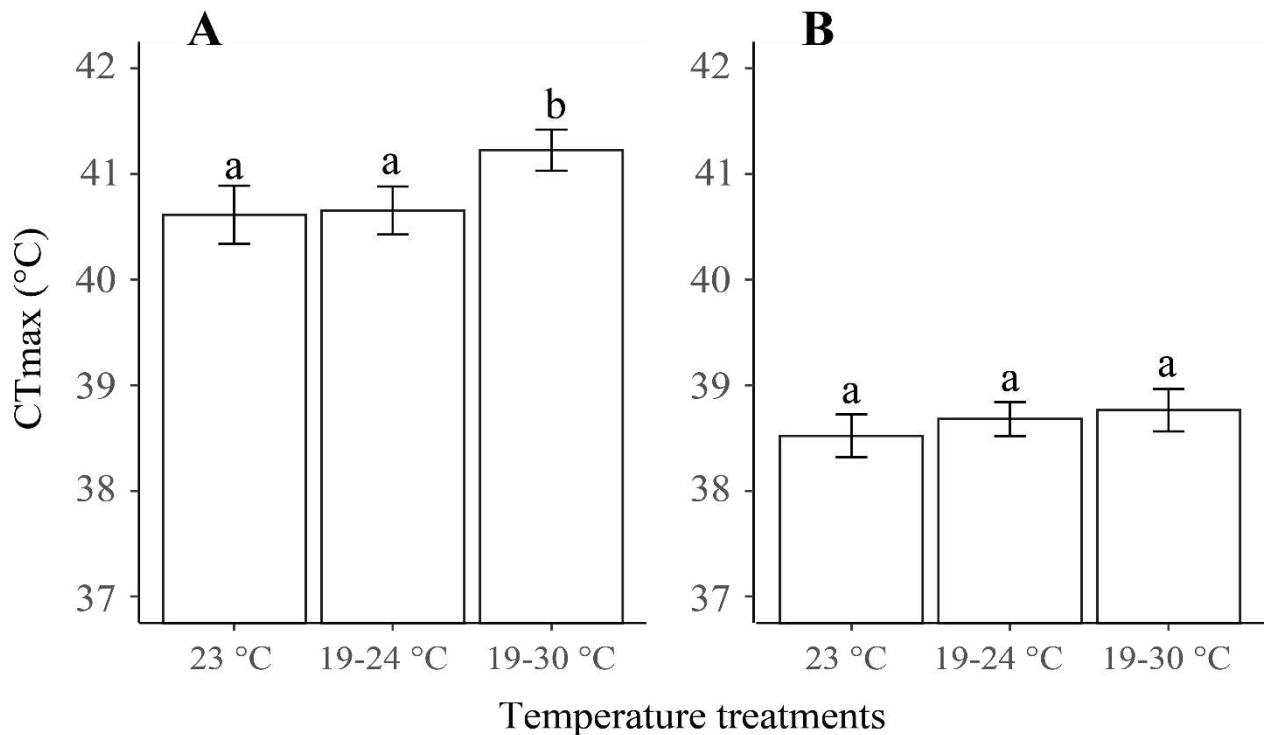
Figure 3. Critical thermal maximum (CTmax) of tadpoles of (A) *P. cuvieri* and (B) *P. moreirae* across the developmental acclimation treatments. Error bars represent 95% confidence intervals. Letters denote significant differences.

3.3. Acclimation response of CTmax

The random effects structure that best explained the variation in the CTmax of *P. cuvieri* (model 4), had as the only predictor variable the thermal treatments (Table 2, Table S4). The tadpoles raised in the HighT had CTmax higher than those of other thermal treatments (t = 4.344, P < 0.001, Fig. 3A, Table S1). The effect of treatments on the CTmax of this species was significant in all the models where this predictor was included, but not in its interaction with

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developmental stage (Table 2). The mass of the tadpoles explained a residual variation in the CTmax of *P. cuvieri* only in model 5, while developmental stage does not affect the CTmax in this species.

Table 2. Competing models fitted to explain the variation in CTmax in our acclimation experiments. Significant predictors effects on CTmax are shown in the boldface and the selected random structure is indicated by an asterisk (*). k=the number of estimated parameters for each model; AICc=second-order Akaike information criterion. Gosner stage = developmental stages (25 and 26-36).

Experiment	Model	Structure	k	AICc
1) Effects of treatment	1	CTmax ~ **treatment** + body mass + (1 | clutch)	6	246.328
on CTmax	2	CTmax ~ **treatment** + Gosner stage + (1 | clutch)	6	239.051
P. cuvieri	3	CTmax ~ treatment x Gosner stage + (1 | clutch)	8	239.289
	4*	CTmax ~ **treatment** + (1 | clutch)	5	234.948
	5	CTmax ~ **body mass** + (1 | clutch)	4	256.514
	6	CTmax ~ Gosner stage + (1 | clutch)	4	252.243
2) Effects of treatment	1	CTmax ~ treatment + body mass + (1 | clutch)	6	183.916
on CTmax	2	CTmax ~ treatment + **Gosner stage** + (1 | clutch)	6	169.088
P. moreirae	3	CTmax ~ treatment x Gosner stage + (1 | clutch)	8	175.850
	4	CTmax ~ treatment + (1 | clutch)	5	175.299
	5	CTmax ~ **body mass** + (1 | clutch)	4	177.075
	6*	CTmax ~ **Gosner stage** + (1 | clutch)	4	162.298

The developmental stage effect in model 6 better explained the variation in the CTmax of *P. moreirae* tadpoles (Table 3, Table S4). Tadpoles in intermediate stage (26-36 Gosner stages) had slightly higher CTmax values than those of stage 25: mean = 38.8 ± 0.5 °C and mean = 38.5 ± 0.5 °C respectively (t = - 3.596, P < 0.001). We did not observe any difference in CTmax between treatments for *P. moreirae* (Fig. 3B, Table S1), while body mass had an effect on the response variable only in a model with little explanatory power (model 5).

Overall, acclimation response of CTmax were low in both species (Table S3). However, the acclimation capacity was greater in *P. cuvieri* tadpoles. We observed clutch differences in ARR within species, these differences being more marked among clutches of *P. moreirae* (Fig. S3). Furthermore, mean ARR values in two clutches of this species resulted in small negative values. Regarding Warming Tolerances (WTs), *P. moreirae* tadpoles had WTs above 11 °C, whereas *P. cuvieri* had comparatively low WTs values ranged between 7 °C and 10 °C (Table S1).

3.4. Developmental reaction norm of life-history traits

The survival of *P. moreirae* was better explained by model 1, which had the treatments as a predictor variable (Table 3, Table S5). Survival of tadpoles of *P. moreirae* declined by approximately 32.5% in the highT relative to the constant treatment (z = - 2.853, P < 0.01), and by 7.5% relative to constant (z = - 0.697, P = 0.485, Fig. 4A, Table S2). The initial mass of *P. moreirae* tadpoles had no effect on survival in any of the models where this variable was included. Survival of *P. cuvieri* tadpoles did not differ between thermal treatments. Survival in this species declined by approximately 16.6% in the constant treatment relative to the highT (z = 1.488, P = 0.137), and by 5.5% relative to the lowT treatment (z = 0.978, P = 0.328, Fig. 4A). The initial mass of *P. cuvieri* tadpoles did not affect their survival at metamorphosis.

Age at metamorphosis variation in *P. cuvieri* was better explained by model 1, which included treatments as the only predictive variable (Table 3, Table S5). The tadpoles of this species metamorphosed faster in the HighT relative to the constant treatment (z = - 3.479, P < 0.001); in turn, the age of the larvae of the LowT did not differ from those of the constant treatment (z = - 1.215, P = 0.224, Fig. 4B, Table S2). The effect of body mass was significant only in interaction with the treatments in model 3. The thermal treatments had effect on the age at metamorphosis in *P. moreirae*, and model 1 was the one that best explained the variation in that response variable. The tadpoles of this species in the HighT had an older age at metamorphosis compared to constant treatment (t = 5.133, P < 0.001), in the same way, the average age in the LowT treatment was older relative to the constant treatment (t = 2.470, P = 0.013, Fig. 4B). The interaction between body mass and treatments showed a significant effect only in model 3, which had the worst adjustment.
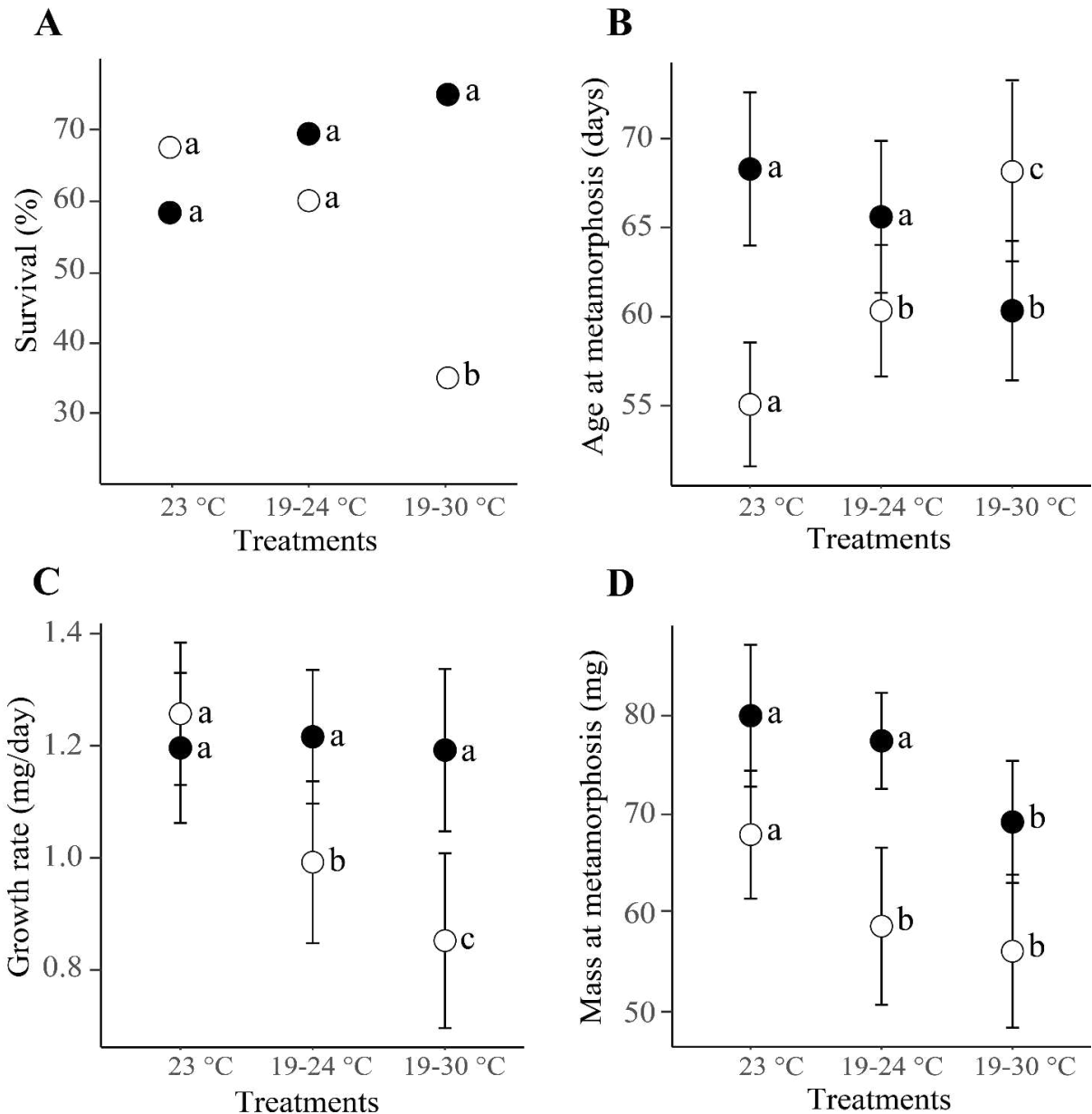
Figure 4. Mean mass at metamorphosis, age at metamorphosis, growth rate and survival of tadpoles of *P. cuvieri* (black) and *P. moreirae* (white) across the developmental acclimation treatments. Error bars represent 95% confidence intervals. Letters denote significant differences.

The growth rate of *P. cuvieri* tadpoles did not differ between thermal treatments (Fig. 4C, Table S5). The random effects structure that best explained this response variable was the model 2, which had significant effects from the initial mass (t = 4.488, P < 0.001, Table 3, Table S2) and had no effect from the treatments. In turn, the interaction between initial mass and treatments had no effect on the growth rate. Model 4 was also the one that best explained

the growth rate in *P. moreirae,* but in this species, it had effects of both treatments and initial mass. The treatment variable alone was a good predictor of growth rate, with effects on the less parameterized model (model 1). *P. moreirae* tadpoles in the HighT had a lower growth rate relative to the constant treatment (t = - 4.168, P < 0.001), similarly the growth rate in the LowT treatment was lower compared to the constant (t = - 2.795, P = 0.007, Fig. 4C).

Table 3. Competing models fitted to explain the variation in life-history traits: age at metamorphosis, mass at metamorphosis and growth rate according to our developmental experiments. Significant predictors effects on life-history traits are shown in the boldface and the selected models are indicated by an asterisk (*). k=the number of estimated parameters for each model; AICc=second-order Akaike information criterion. Age = age at metamorphosis; Body mass = mass at metamorphosis; mass25 = initial body mass.

Experiment	Models	Structure	k	AICc
1) Effects of treatment on survival *P. cuvieri*	1*	Survival ~ treatment	3	139.937
	2	Survival ~ treatment + mass25	4	141.623
	3	Survival ~ treatment * mass25	6	145.999
2) Effects of treatment on survival *P. moreirae*	1*	Survival ~ **treatment**	3	162.290
	2	Survival ~ **treatment** + mass0	4	162.877
	3	Survival ~ treatment * mass0	6	166.291
3) Effects of treatment on age *P. cuvieri*	1*	Age ~ **treatment** + (1 | clutch)	4	550.639
	2	Age ~ treatment + mass25 + (1 | clutch)	5	551.605
	3	Age ~ **treatment x mass25** + (1 | clutch)	7	551.219
4) Effects of treatment on age *P. moreirae*	1*	Age ~ **treatment**	3	471.949
	2	Age ~ treatment + mass25	4	474.148
	3	Age ~ **treatment x mass25**	6	474.744
5) Effects of treatment on Growth rate *P. cuvieri*	1	Growth rate ~ treatment + (1 | clutch)	5	56.773
	2*	Growth rate ~ treatment + **mass25** + (1 | clutch)	6	50.081
	3	Growth rate ~ treatment x mass25 + (1 | clutch)	8	68.727

6) Effects of treatment on Growth rate *P. moreirae*	1	Growth rate ~ **treatment** + (1 | clutch)	5	47.105
	2*	Growth rate ~ **treatment** + **mass25** + (1 | clutch)	6	44.231
	3	Growth rate ~ treatment x mass25 + (1 | clutch)	8	65.088
7) Effects of treatment on body mass *P. cuvieri*	1	Body mass ~ **treatment** + (1 | clutch)	5	591.829
8) Effects of treatment on body mass *P. moreirae*	1	Body mass ~ **treatment** + (1 | clutch)	5	537.919

The mass at metamorphosis of *P. cuvieri* was lower in the HighT relative to the constant treatment (t = - 2.55, P = 0.013), while the tadpoles in LowT metamorphosed with a mass similar to that of the constant (t = -0.562, P = 0.576, Fig. 4D, Table S2). Tadpoles of *P. moreirae* had effects of thermal treatments on the mass at metamorphosis (Table 3). These effects corresponded to with a reduction of body mass in the HighT relative to the constant treatment (t = - 2.501, P = 0.015), and in the same way in the LowT treatment relative to the constant (t = - 2.205, P = 0.031, Fig. 4D, Table S2).

4. DISCUSSION

4.1. Upper thermal tolerance: species comparison

Given that *P. cuvieri* and *P. moreirae* contrast in the thermal descriptors of their microenvironments, the CTmax differences observed among species may be explained from the perspective of thermal adaptation (Arnold, 1987; Angilletta et al., 2003; Angilletta, 2009). Microclimatic descriptors as Tmax and daily temperature ranges represents the main driver of the variation in thermal limits in tadpoles from tropical and temperate communities (Duarte et al., 2012; Simon et al., 2015; Richter-Boix et al., 2015; Gutierrez-Pesquera et al., 2016b; Katzenberger et al., 2018; Bonino et al., 2020), and also in frogs (Pintanel et al., 2019). The CTmax of *P. cuvieri* coincide with the values (range = 40 ° C - 44 ° C) reported for tadpoles of tropical and subtropical open-forest warm communities (Duarte et al., 2012; Simon et al., 2015; Kern et al., 2015a). While the upper thermal tolerance of *P. moreirae* are comparable to that reported for subtropical canopy-protected species that has CTmax below 40 ° C (Duarte

et al., 2012). This is because temporary ponds are thermally homogeneous environments, where tadpoles cannot escape daily temperature fluctuations (Tejedo et al., 2012; Simon et al., 2015). So the selective pressures adjust the CTmax of tadpoles to the thermal extremes in each microenvironment (Navas et al., 2010; Gutiérrez-Pesquera et al., 2016a). In this way, it is likely that a more intense selection for CTmax may occur in the populations of anurans from uncovered and more variable microenvironments (Gutierrez-Pesquera et al., 2016b).

4.2. Acclimation response of CTmax

The acclimation responses observed are in line with our expectations, since the species that experiences high natural thermal variation and warmer temperatures showed plasticity in upper thermal tolerance. Our overall observations agree with previous studies that found increase or no difference in CTmax of tadpoles (Kern et al., 2014, 2015a, 2015b; Gutiérrez-Pesquera et al., 2016a), and other ectothermic animals in fluctuating acclimation to high temperatures (Terblanche et al., 2010; Bozinovic et al., 2011; Hoskins et al., 2020). However, our results disagree with other studies that showed detrimental effects and reduced CTmax under fluctuating heat treatments (Paaijmans et al., 2013). Acclimation response of CTmax under extreme temperatures can be explained for adaptive processes at other levels of organization such as cellular level where the expression of heat shock stabilizing proteins occurs (Feder and Krebs, 1998; Folguera et al., 2011).

Our naturalistic design did not allow us to separate the fluctuation and the mean of temperatures, both the mean of the LowT (mean = 22 ° C) and the constant treatment (23 ° C) was lower than the mean of the HighT (25 ° C). Kern et al. (2015a) indicated that the effect of daily thermal fluctuations on individual performance and fitness depend on both, the magnitude and the average temperature around which such fluctuation occurs. Fluctuating treatments seem not to represent natural environmental cues that induce acclimation responses in *P. moreirae* (Levins, 1968; DeWitt et al., 1998). While tadpoles of *P. cuvieri* did not increase their CTmax under a cold mean and a low thermal fluctuation. Given that *P. cuvieri* is a thermal generalist species that experiences a wide range of warm temperatures, it is probable that the peaks of temperature fluctuation in LowT have not exceeded its Topt (Martin and Huey, 2008; Knies et al., 2009), so acclimation of the CTmax is not expected to occur (Kern et al., 2014).

Physalaemus moreirae did not show ability to acclimate under fluctuating regimes, so its average ARR CTmax was low (0.01 ° C). On the other hand, the tadpoles of *P. cuvieri* showed a compensation of 19%, indicative of low plasticity. Small negative ARR values that we obtained for two clutches of *P. moreirae*, are commonly associated with physiologically detrimental acclimation conditions (Gunderson and Stillman, 2015), so reinforce the idea of lower thermal sensitivity in this species under simulated natural temperature regimes. Despite the fact that aquatic ectotherms, such as tadpoles, exhibit greater CTmax plasticity than their terrestrial counterparts, the estimated ARR values (CTmax ARR < 0.5) suggests a limited ability to cope with increases in environmental temperature (Gunderson and Stillman, 2015). However, it is possible that a certain level of phenotypic plasticity can still evolve in different genotypes, if the benefit of maintaining a plastic phenotype outweighs its relative costs (Dewitt et al., 1998; Relyea, 2002).

Our microclimatic data set allowed us to capture the differences in warming tolerances (WTs) between species. Studies in tropical and temperate tadpole's communities highlighted the importance of using microclimatic data to estimate species vulnerability and responses to climate change, instead of macroclimatic data that can produce a biased perspective of species WTs, as apparent WTs homogeneity in regional/local scale (Gutiérrez-Pesquera et al., 2016b; Katzenberger et al., 2018). The WT values obtained for the species studied fall in the range previously reported for Physalaemus species (8-12 °C), including *P. cuvieri* (Simon et al., 2015), and for other tadpoles from subtropical communities (Duarte et al., 2012; Gutiérrez-Pesquera et al., 2016b). It is important to mention that estimations of warming tolerance depend on both the CTmax and Tmax values, so comparisons with other studies can be difficult due to methodological issues (see Katzenberger et al., 2018). On the one hand, CTmax may be influenced by various external, intrinsic and methodological factors (Agudelo-Cantero and Navas, 2019). While Tmax has generated extensive discussion regarding the use of thermal predictors that appropriately represents the spatio-temporal variation at organismic scales (Garcia et al., 2019).

The canopy-protected species *P. moreirae* is less likely to suffer the direct impact of acute thermal stress, since their CTmax values are further from the maximum environmental temperatures they experience (Duarte et al., 2012; Katzenberger et al., 2018). However, threats other than increases in global temperature are not ruled out. For example, population

declines and extinction of amphibian species from Brazilian Atlantic forest have reported, particularly those species with aquatic larvae (Becker et al., 2009; Ewers and Banks-Leite, 2013). The abrupt microclimatic changes in the breeding ponds produced by deforestation have been proposed as one of the probable causes (Tejedo et al., 2012). In this study, the CTmax of *P. moreirae* tadpoles below 40 °C, would be exceeded in ponds with the lowest level of vegetation cover (canopy cover: 0-19%). Therefore, this species is highly vulnerable to the rapid microclimatic changes associated with deforestation (Becker et al., 2007).

4.3. Developmental reaction norm of life-history traits

Thermal fluctuation seriously compromised the survival of *P. moreirae* tadpoles, but not those of *P. cuvieri*. Previous studies have showed that fluctuating temperatures can impair the survival of tadpoles (Niehaus et al., 2012; Kern et al., 2015a) and other ectotherms (Folguera et al., 2011; Paaijmans et al., 2013). However, some studies have also reported species with a great capacity to reduce the thermosensitivity of physiological processes under thermally variable conditions (Měráková and Gvoždík, 2009; Niehaus et al., 2011). Increased metabolic demands in response to highly fluctuating thermal environments (18-38 ° C) accounted for high tadpole mortality (Kern et al., 2015a). Our results suggest, on the one hand that energetic imbalances may be associated with costly adjustments in *P. moreirae* under thermal fluctuation, and on the other hand that *P. cuvieri* seems to have adaptive mechanisms to reduce the thermosensitivity of physiological processes and ensure survival (Folguera et al., 2011).

The reduced age at metamorphosis in tadpoles of *P. cuvieri* under the HighT agree with a pattern widely reported in the literature, mainly for larvae of generalist species that tolerate wide thermal variation (Niehaus et al., 2006; Kern et al., 2015a). On the other hand, the delay in the development of *P. moreirae* in response to thermal fluctuation is in line with that reported for temperature-sensitive species, which present high energetic demand under fluctuating conditions (Niehaus et al., 2012; Kern et al., 2015a). Our overall results agree with previous studies showing that fluctuating thermal environments can increase energetic demands and consequently affect body mass at metamorphosis (Niehaus et al., 2006; Niehaus et al., 2012; Kern et al., 2015a). Interestingly, we observed that growth rate in *P. moreirae* tadpoles was largely affected in both fluctuating treatments; while in the constant cold

treatment this species grew faster, even more than those of *P. cuvieri* that in turn presented a reduced thermal sensitivity in its growth rate.

The developmental strategy exhibited for *P. cuvieri* can be explained by a life-history model that predicts uncoupled trajectories of growth and development, then a reduction in development time accompanied by a similar reduction in mass at metamorphosis, without changes in growth rate (Richter-Boix et al., 2011). On the other hand, our results disagree with the classical model that foresees a trade-off between growth and development, with a resulting small size at metamorphosis (Smith- Gill and Berven, 1979). It is likely that the development strategy of *P. cuvieri* allows it to shorten the metamorphosis time due to a high risk of desiccation of its microhabitats (Newman, 1989; Denver, 1997; Morey and Reznick, 2004). The ability to shorten the metamorphosis time will provide an adaptive advantage to tadpoles if they can anticipate environmental changes (Newman, 1992). In nature, daily temperature fluctuations in larval microhabitats serve as reliable environmental cues that precedes high rates of water evaporation (Denver, 1997). Thus, genotypes that are capable of increasing their development rate (greater than evaporation rate) in response to thermal variations, will be able to metamorphose before temporary ponds dry. Although the ability to shorten the metamorphosis time ensures survival in temporary ponds, some fitness disadvantages are associated with a small size at metamorphosis (Kulkarni et al., 2011).

The developmental strategy of tadpoles of *P. moreirae* is consistent with a life-history model that predicts that larvae with slow growth rates will extend their development period until they can reach a minimum size threshold for metamorphosis (Wilbur and Collins, 1973; Werner, 1986). A similar result was obtained by Niehaus et al. (2012), who used a high variation regime (18–34 ° C) where the larvae metamorphosed later and with smaller sizes compared to a constant temperature treatment with same mean of 26.0 °C. Further, their results showed an impaired performance under warm thermal fluctuation (Niehaus et al., 2012). Our observations is in line with above results mainly the low growth rate and high mortality that *P. moreirae* exhibited under ecologically relevant thermal fluctuations.

CONCLUDING REMARKS

We conclude that different conditions of thermal fluctuation induce, on the one hand, phenotypic plasticity in CTmax and developmental traits of *P. cuvieri* tadpoles, and on the other hand, important consequences on the physiological performance and survival of *P. moreirae* tadpoles. Adjustment of thermal tolerance and development strategy in *P. cuvieri* can be correlates with cellular level mechanisms (i.e. Hsp70) and with alterations in hormonal levels (thyroid activity) respectively (Denver, 1998). Small increases in daily thermal fluctuation can cause impaired performance and reduced survival in *P. moreirae*, but current this species is relatively safe of direct impacts of extreme temperatures. The inability to efficiently compensate for daily thermal variations identifies a vulnerability of *P. moreirae* to future climatic changes, as well as to rapid microclimatic changes product from the deforestation of its habitat. Finally, we recreated simultaneous variation in mean and variance of temperatures recorded from temporary ponds commonly occupied by *P. cuvieri* and *P. moreirae* at the study area. A traditional approach used to analyze the separate effect of these two components of the daily thermal variation, validates the lack of ecological realism from constant mean equivalent to fluctuating regime (Bozinovic et al., 2011; Niehaus et al., 2012; Kern et al., 2015).

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SUPPLEMENTARY DATA

Figure S1. Variation pattern of air temperature near a pond with an open canopy (0% canopy) and a pond with a closed canopy (70.12% canopy) at the Boracéia Biological Station. Temperature records were made using a Data Logger HOBO U12 located in the shade.
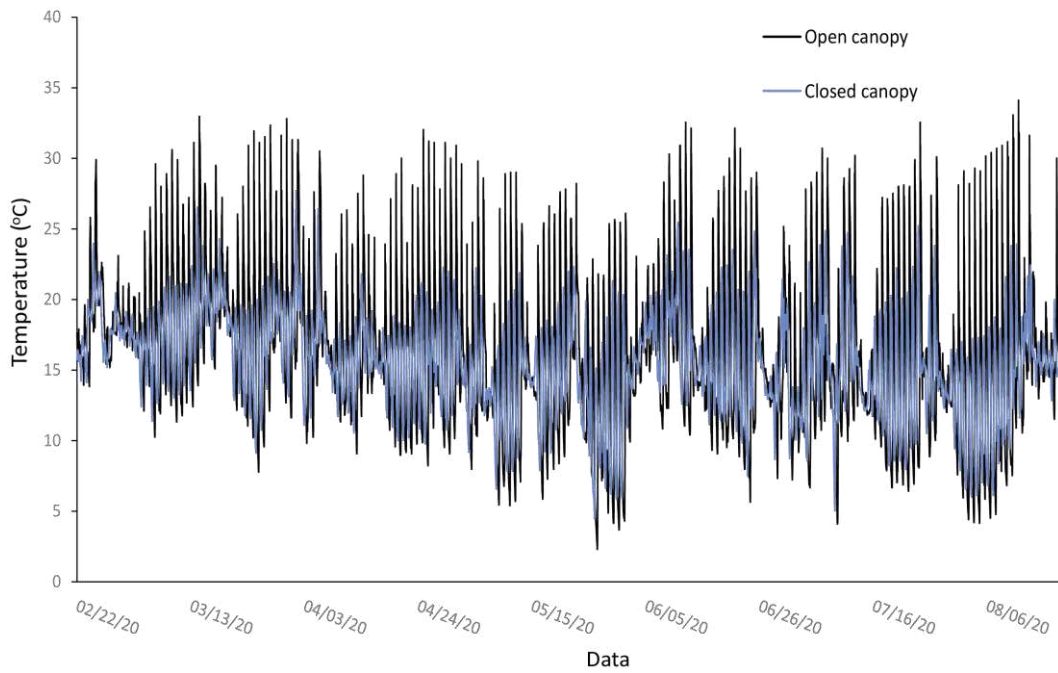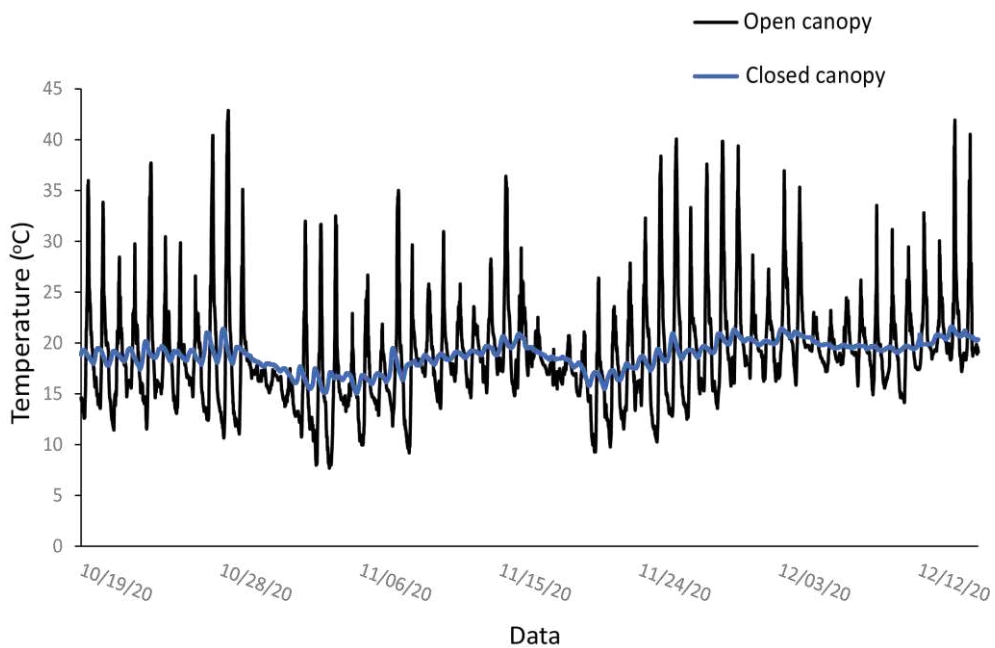

Figure S2. Variation pattern of water temperature in a pond with an open canopy (0% canopy) and one with a closed canopy (70.12% canopy) at the Boracéia Biological Station.
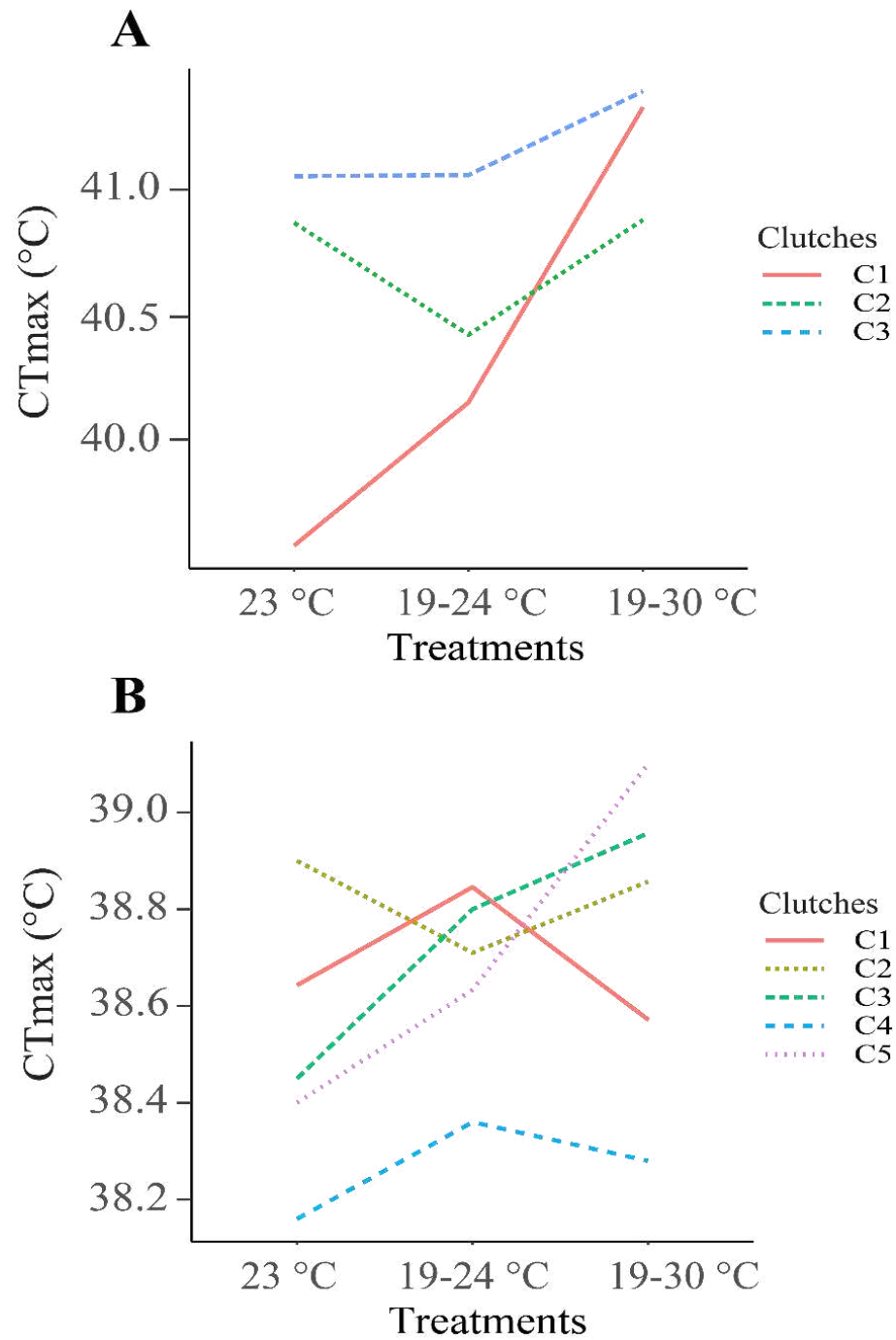
Figure S3. Clutch differences in CTmax of tadpoles (A) *P. cuvieri* and (B) *P. moreirae* across the acclimation treatments.

Table S1. Mean (± SD) of CTmax, mass of tadpoles and warming tolerance (WT) of *P. cuvieri* and *P. moreirae* for each temperature treatments. N = number of tadpoles that recovered after 24 hours of CTMax test. DT = number of tadpoles killed after 24 hours of CTMax test. Min = minimum values for CTmax, Max = maximum values for CTmax, CV = coefficient of variation.

Species	Gosner stage	Treatments	N Final	DT	CTmax (°C)	Min	Max	CV (%)	Mass (mg)	WT (°C)
P. cuvieri	25-30	Constant	34	4	40.6 ± 0.8	39.1	41.5	1.9	47.2 ± 31.5	9.0 ± 0.8
P. cuvieri	25-30	LowT	40	1	40.7 ± 0.7	39.0	41.6	1.7	43.6 ± 31.1	9.0 ± 0.7
P. cuvieri	25-32	HighT	39	1	41.2 ± 0.6	39.7	42.0	1.4	58.7 ± 34.7	9.6 ± 0.6
P. moreirae	25-36	Constant	33	6	38.5 ± 0.6	37.1	39.3	1.5	29.8 ± 15.6	11.5 ± 0.5
P. moreirae	25-34	LowT	42	7	38.7 ± 0.5	37.5	39.6	1.3	31.0 ± 11.7	11.7 ± 0.5
P. moreirae	25-36	HighT	27	2	38.7 ± 0.5	38.0	39.7	1.3	32.8 ± 15.3	11.7 ± 0.5

Table S2. Mean (± SD) of larval period (age), mass at metamorphosis, growth rate and larval survival for each temperature treatment and species.

Species	Treatments	N	N Final	Survival (%)	Age (day)	Mass st 42 (mg)	Growth rate (mg/dia)
P. cuvieri	Constant	36	21	58.3	68.3 ± 9.4	80.1 ± 15.8	1.2 ± 0.3
P. cuvieri	LowT	36	25	69.4	65.6 ± 10.3	77.6 ± 11.8	1.2 ± 0.3
P. cuvieri	HighT	36	27	75.0	60.3 ± 9.9	69.4 ± 15.5	1.2 ± 0.4
P. moreirae	Constant	40	27	67.5	55.1 ± 8.8	68.2 ± 16.2	1.2 ± 0.3
P. moreirae	LowT	40	24	60.0	60.3 ± 8.7	58.9 ± 18.7	1.0 ± 0.3
P. moreirae	HighT	40	14	35.0	68.1 ± 8.7	56.4 ± 13.3	0.8 ± 0.3

Table S3. Acclimation response ratio (ARR) for CTmax across the different clutches of *P. moreirae* and *P. cuvieri*.

Species	Clutches	ARR
P. moreirae	1	-0.06 °C
	2	0.05 °C
	3	0.05 °C
	4	-0.03 °C
	5	0.16 °C
P. moreirae	**all**	**0.01 °C**
P. cuvieri	1	0.39 °C
	2	0.15 °C
	3	0.11 °C
P. cuvieri	**all**	**0.19 °C**

Table S4. Summary of best-fit random-effects structures analyzing the effect of treatments, Gosner stage and body mass on the CTmax of tadpoles of both species.

Experiment	Fixed effects	Estimate	SE	t-value	*P*-value
1) CTmax variation *Physalaemus cuvieri*	Intercept	40.550	0.239	170.047	**< 0.001**
	Treat (HighT)	0.636	0.146	4.344	**< 0.001**
	Treat (LowT)	0.029	0.146	0.198	0.843
	Random effects	Variance	SD		
	Clutch (Intercept)	0.136	0.369	NA	NA
	Residual	0.389	0.624	NA	NA
2) CTmax variation *Physalaemus moreirae*	Intercept	38.778	0.119	324.648	**< 0.001**
	Gosner stage (25st)	-0.388	0.108	-3.596	**< 0.001**
	Random effects	Variance	SD		
	Clutch (Intercept)	0.051	0.226	NA	NA
	Residual	0.236	0.486	NA	NA

Table S5. Summary of best-fit models analyzing the effect of thermal treatments and initial body mass on life-history traits of tadpoles of both species. Mass25 = mass at metamorphosis.

Experiment	Fixed effects	Estimate	SE	z-value	P-value
1) Survival *Physalaemus cuvieri*	(Intercept)	0.336	0.338	0.995	0.320
	Treat (HighT)	0.762	0.512	1.488	0.137
	Treat (LowT)	0.484	0.495	0.978	0.328
2) Survival *Physalaemus moreirae*	(Intercept)	0.731	0.337	2.165	**0.030**
	Treat (HighT)	-1.350	0.473	-2.853	**0.004**
	Treat (LowT)	-0.325	0.467	-0.697	0.485
3) Age variation *Physalaemus cuvieri*	(Intercept)	4.219	0.033	128.375	**< 0.001**
	Treat (HighT)	-0.126	0.036	-3.479	**< 0.001**
	Treat (LowT)	-0.044	0.036	-1.215	0.224
	Random effects	Variance	SD		
	Clutch (Intercept)	0.001	0.033	NA	NA
4) Age variation *Physalaemus moreirae*	(Intercept)	4.009	0.026	154.581	**< 0.001**
	Treat (HighT)	0.213	0.041	5.133	**< 0.001**
	Treat (LowT)	0.091	0.037	2.470	**0.013**

	Fixed effects	Estimate	Std. Error	t-value	P-value
5) Growth rate variation *Physalaemus cuvieri*	(Intercept)	0.517	0.169	3.065	**0.003**
	Treat (HighT)	0.036	0.081	0.452	0.652
	Treat (LowT)	0.022	0.082	0.279	0.781
	Mass25	0.020	0.004	4.488	**< 0.001**
	Random effects	Variance	Std.Dev.		
	Clutch (Intercept)	0.004	0.063	NA	NA
	Residual	0.076	0.276	NA	NA
6) Growth rate variation *Physalaemus moreirae*	(Intercept)	0.725	0.119	6.095	**< 0.001**
	Treat (HighT)	-0.369	0.089	-4.168	**< 0.001**
	Treat (LowT)	-0.212	0.076	-2.795	**0.007**
	Mass25	0.016	0.003	4.996	**< 0.001**
	Random effects	Variance	Std.Dev.		
	Clutch (Intercept)	0.001	0.024	NA	NA
	Residual	0.072	0.268	NA	NA
7) Body mass variation *Physalaemus cuvieri*	(Intercept)	80.285	3.398	23.628	**< 0.001**
	Treat (HighT)	-10.586	4.142	-2.556	**0.013**
	Treat (LowT)	-2.368	4.217	-0.562	0.576
	Random effects	Variance	Std.Dev.		
	Clutch (Intercept)	5.580	2.362	NA	NA
	Residual	202.470	14.229	NA	NA
8) Body mass variation *Physalaemus moreirae*	(Intercept)	68.506	4.570	14.990	**< 0.001**
	Treat (HighT)	-12.440	4.973	-2.501	**0.0152**
	Treat (LowT)	-9.375	4.251	-2.205	**0.0314**
	Random effects	Variance	Std.Dev.		
	Clutch (Intercept)	62.270	7.891	NA	NA
	Residual	224.920	14.997	NA	NA