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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS (ZOOLOGIA)

Temperature and dehydration effects on respiratory and cutaneous water flux in the terrestrial toad, *Rhinella schneideri* (Anura, Bufonidae)

LUIS MIGUEL SENZANO CASTRO

Dissertação apresentada ao Instituto de Biociências do Campus de Rio Claro, Universidade Estadual Paulista Júlio de Mesquita Filho, como parte dos requisitos para obtenção do título de Mestre em Ciências Biológicas (Zoologia).

Rio Claro, SP - 2018

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As ectotherms, amphibians may experience wide fluctuations in body temperature and, due to their high skin permeability, terrestrial species face a constant risk of desiccation. These two variables (temperature and dehydration) are centrally relevant for water balance regulation because they directly affect water flux through the integument, *i.e*. skin evaporative water loss (EWL_{Skin}) and water uptake (WU). In addition, as nearly all anurans are lung breathers, the respiratory water loss (EWL_{Resp}) will add to EWL_{Skin}. Although the contribution of EWL_{Resp} to total EWL (EWL_{Total}) is generally assumed to be negligible, the partitioning between EWL_{Skin} and EWL_{Resp} varies among species and is affected by temperature and dehydration. Therefore, we investigated the combined effects of temperature and dehydration on the rates of EWL through the skin and the lungs of the terrestrial toad, *Rhinella schneideri*. Subsequently, we evaluated how water uptake (WU) through the pelvis skin was affected by temperature and dehydration. To this aim, we measured rates of EWL_{Total} and EWL_{Skin} in intact and masked adult toads at 15, 25 and 35 ^{o}C under fully hydrated condition and dehydrated until they have lost 10% and 20% of their initial body mass. Masked toads were able to breath normally during the measurement of EWL_{Skin}; EWL_{Resp} was calculated as EWL_{Total} minus EWL_{Skin}. Rates of EWL were also determined using biophysical agar models of *R. schneideri* specimens, which allowed the estimation of skin resistance (R_s) to evaporation. WU rates were determined by measuring body mass gain against rehydration time of toads placed on a thin film of water. EWL_{Skin} and EWL_{Resp} increased with temperature, however, this effect was much more pronounced for EWL_{Resp} than for EWL_{Skin} and, as a result, the partitioning between cutaneous and respiratory water loss was significantly altered with temperature. Indeed, the contribution of EWL_{Resp} to EWL_{Total} increased from 2.44% at 15 ^{o}C to 8.1% at 35 ^{o}C. This result may be attributed to a limited capacity for EWL_{Skin} regulation combined with a temperature induced increment in pulmonary ventilation resulting from the elevation in metabolic rate with temperature. The contribution of EWL_{Skin} to EWL_{Total} decreased with dehydration which may be related to a loss in skin water content and the subsequent compression of cell layers, which limit water efflux to the environment. Therefore, the relative contribution of EWL_{Resp} augmented with temperature and dehydration accompanied by the corresponding decrease in the relative contribution of EWL_{Skin}. Rates of WU increased with dehydration but not with

temperature, which indicate the central role of the osmotic gradient in driving water flow through the anuran skin.

Como animais ectotérmicos, os anfíbios experimentam longas flutuações na temperatura corpórea e, devido à sua alta permeabilidade cutânea, eles também enfrentam o risco constante da dessecação. Essas duas variáveis (temperatura e desidratação) são muito relevantes no contexto do balanço hídrico dado que elas afetam diretamente o fluxo de água através da pele, ou seja, a perda evaporativa de água (PEA_{Pele}) e a absorção de água (RE). Além disso, dado que a maioria dos anuros possuem respiração pulmonar, a perda de água respiratória (PEA_{Resp}) irá se adicionar à cutânea. Embora a contribuição da PEA_{Resp} para a perda de água total (PEA_{Total}) seja geralmente considerada insignificante, a divisão entre PEA_{Pele} e PEA_{Resp} varia de acordo com as espécies e é afetada pela temperatura e desidratação. Portanto, nós investigamos os efeitos combinados da temperatura e desidratação nas taxas de PEA através da pele e pulmões no sapo terrestre, *Rhinella schneideri*. Posteriormente, avaliamos como a absorção de água através da pele ventral (RE) foi afetada pela temperatura e desidratação. Para isso, medimos as taxas de PEA_{Total} e PEA_{Pele} em sapos adultos intactos e mascarados à 15, 25 e 35 ^{o}C e desidratados até perderem 10% e 20% da massa corpórea inicial. Os sapos mascarados foram capazes de respirar normalmente durante a medição da PEA_{Pele}; PEA_{Resp} foi calculado como PEA_{Total} menos PEA_{Pele}. As taxas de perda de agua também foram determinadas usando modelos de ágar de espécimes de *R. schneideri*, o que permitiu a estimativa da resistência da pele (RP) à evaporação. As taxas de RE foram determinadas medindo o ganho de massa corpórea durante o tempo de reidratação dos sapos colocados sobre uma fina camada de água. PEA_{Pele} e PEA_{Resp} aumentaram com a temperatura, no entanto, este efeito foi muito mais marcado para a PEA_{Resp} do que para a PEA_{Pele} e, como resultado, a divisão entre a perda de água cutânea e respiratória foi significativamente alterada com a temperatura. De fato, a contribuição da PEA_{Resp} para a PEA_{Total} aumentou de 2,44% a 15 ^{o}C para 8,1% aos 35 ^{o}C. Este resultado pode ser atribuído a uma capacidade limitada para a regulação da PEA_{Pele}, combinada com um aumento na ventilação pulmonar induzido pela temperatura devido à elevação na taxa metabólica. A contribuição de PEA_{Pele} para a PEA_{Total} diminuiu com a desidratação, o que pode estar relacionado à perda de conteúdo de água da pele e a subsequente compressão das camadas celulares, o que limita o fluxo de água para o ambiente. Portanto, a contribuição relativa da PEA_{Resp} aumentou com a temperatura e a desidratação, acompanhada da diminuição correspondente da contribuição relativa de PEA_{Pele}. As taxas de RE aumentaram com a

desidratação, mas não com a temperatura, o que indica o papel central do gradiente osmótico na condução do fluxo de água através da pele.

Terrestrial anuran amphibians are capable of performing their routine activities away from standing water, yet they still depend on moisture to rehydrate (Wells 2007). Nonetheless, terrestriality may entails hydric constraints since most amphibians are susceptible to high rates of evaporative water loss (EWL) because of their highly permeable skin (Hillman *et al*. 2009). As a consequence, terrestrial anurans are prone to experience variable degrees of hydration states while active (see Tracy *et al*. 2014). As ectothermic organisms, amphibians may also experience wide fluctuations in body temperature, which mirroring the variation of their thermal environment (Seebacher and Alford 2002, Noronha-de-Souza *et al.* 2015). Due to the fact that EWL rates are influenced by temperature (Tracy 1976), and because evaporation from the skin has a cooling effect on body temperature (Andrade *et al*. 2016), thermoregulation and water balance are highly intertwined in terrestrial anurans (Feder and Burggren 1992, Navas *et al*. 2008). Therefore, the interaction between these two important physiological functions may involve important trade-offs that will vary from species to species, among different organismal conditions, and as a function of the thermal and hydric environment (Rogowitz *et al*. 1999, Seebacher and Alford 2002, Anderson and Andrade 2017).

In general, terrestrial species may withstand large losses of body water (Wells 2007) however, diverse detrimental effects are expected (see Lillywhite 1975, Hillman 1987). Dehydration is also accompanied by a decrease in EWL and an increase in water uptake (WU) through the ventral skin area, the "pelvic patch" (see Anderson *et al*. 2017). This dehydration-induced dynamic on cutaneous water flux is, as expected, influenced by the temperature (Preest *et al*. 1992, Rogowitz *et al*. 1999). Indeed, if relative humidity is fixed, rises in temperature increases the rates of EWL by affecting air capacitance for water vapor. Thus, dehydrated anurans can minimize water loss by behaviourally selecting cooler sites (Tracy *et al*. 1993, Dohm *et al*. 2001, Anderson and Andrade 2017). Finally, although behavioral performance is also negatively impacted by dehydration, the optimal temperature to attain optimal performance is also shifted to low temperatures under more dehydrated states (Anderson and Andrade 2017). These responses illustrate the complex trade-offs involving thermoregulation and water balance in anurans.

Evaporative water loss is not a process that occurs solely through skin surface (EWL_{Skin}) but, for lung breathers, also involves evaporation through the lung surface (EWL_{Resp}) (Mautz 1982, Hillman *et al*. 2009). Nonetheless, since most anurans exchange a substantial fraction of their respiratory gases through the skin and since they have a relatively low pulmonary surface area (Czopek 1965) associated to low rates of ventilation while resting (Burggren and Doyle 1986), EWL_{Resp} is quite commonly assumed to be negligible in these animals (Spotila and Berman 1976, Bentley and Yorio 1979, Wygoda 1981, 1984). However, lung ventilation in anurans can vary considerably among species (Hutchison *et al*. 1968), under different circumstances (Whitford 1973) and in response to environmental factors (Boutilier 1992). For example, lung ventilation is known to be affected by temperature (Kruhøffer *et al.* 1987, Branco *et al*. 1993, Bícego-Nahas *et al*. 2001, Zena *et al*. 2016) and dehydration (Boutilier *et al*. 1979). However, the interactive effects of both of these factors, temperature and dehydration, on the relative contribution of EWL_{Resp} and EWL_{Skin} to total evaporation (EWL_{Total}) is not available. This evaluation is ecologically and functionally meaningful since these two routes of water loss involve different sets of constraints that may be differently affected by changes in temperature and hydration state (Hutchison *et al*. 1968, Geise and Linsenmair 1986, Rogowitz *et al*. 1999, Burggren and Vitalis 2005). Accordingly, we examined the combined effects of temperature and dehydration on the partitioning of water balance between the skin and the lung in the terrestrial toad, *Rhinella schneideri*, under different temperatures and hydration levels. More specifically, we measured EWL rates of intact and masked toads, for the estimation of EWL_{Total} and EWL_{Skin}, respectively. Thereafter, we estimated EWL_{Resp} as the difference between EWL_{Total} and EWL_{Skin}. Finally, we determined the influence of temperature and dehydration on the rate of water uptake (WU) through the pelvic patch of the toads. We chose *R. schneideri* to perform the present study because this species has a terrestrial lifestyle and is broadly distributed in tropical biomes of South America (Haddad *et al*. 2013) and, therefore, it is likely to experience natural fluctuations in body temperature and hydration state.

Our specific hypothesis for the present study includes: 1) the rise in temperature will increase the rates of EWL_{Skin} and EWL_{Resp}; 2) this effect will be more pronounced for EWL_{Resp}, as metabolism and lung ventilation will likely be elevated at higher temperatures; 3) dehydration will cause a temperature-independent decrease in EWL_{Skin} but not on EWL_{Resp}; 4) however, the partitioning between EWL_{Skin} and EWL_{Resp} is not expected to be affected by hydration state; and 5) the WU rates will be leaded by dehydration and not by temperature.

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