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Research paper

Allometric relationships between cutaneous surface area and body mass in anuran amphibians

Wilfried Klein[a,b,*], Lucianne Dabés[c], Vanessa Maria Gomes Bonfim[b], Leandro Magrini[d], Marcelo Felgueiras Napoli[b,c,e]

[a] Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes 3900, 14040-901 Ribeirão Preto, São Paulo, Brazil
[b] Programa de Pós-Graduação em Diversidade Animal, Instituto de Biologia, Universidade Federal da Bahia, Rua Barão de Jeremoabo, Campus de Ondina, 40170-115 Salvador, Bahia, Brazil
[c] Programa de Pós-Graduação em Ecologia e Biomonitoramento, Instituto de Biologia, Universidade Federal da Bahia, Rua Barão de Jeremoabo, Campus de Ondina, 40170-115 Salvador, Bahia, Brazil
[d] Departamento de Psicologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes 3900, 14040-901 Ribeirão Preto, São Paulo, Brazil
[e] Museu de Zoologia, Instituto de Biologia, Universidade Federal da Bahia, Rua Barão de Jeremoabo, Campus de Ondina, 40170-115 Salvador, Bahia, Brazil

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ABSTRACT

Several allometric relationships exist in the literature describing the relationship between surface area (A_S) and body mass (M_B) for different taxa of Anura and these are frequently used in physiological studies. Here, we gathered literature data of A_S (cm^2) and M_B (g), added new original data, and allometric relationships between A_S and M_B were evaluated using linear and phylogenetic generalized least-square (PGLS) regressions. Data from 453 specimens from 44 species were included. Intraspecific allometric relations between A_S and M_B were determined for 18 species, with ten of those showing regressions not significantly different from the respective family regression; four species showing a significantly different y-intercept; and three species showing a significantly different slope. Only the families Bufonidae, Ranidae, and Hylidae were represented by several species (9, 11, and 12, respectively) and with larger specimens' numbers (54, 215, and 127, respectively). These three families exhibited significantly different OLS linear regressions on $\log_{10}$-transformed data, with Hylidae showing the steepest (0.7735 ± 0.0110), Bufonidae an intermediate (0.6772 ± 0.0220), and Ranidae the lowest slope (0.6091 ± 0.0114). The relationship between A_S and M_B for Anura could be described by the linear regression $A_S = 9.8537\ M_B^{0.6745}$ or by the PGLS regression $A_S = 8.7498\ M_B^{0.685}$.

1. Introduction

Body size varies considerably in anuran amphibians, the smallest known species are the Papua New Guinean *Paedophryne amauensis* Rittmeyer et al. 2012 [Microhylidae; adult snout-vent length (SVL) 7.0–8.0 mm; Rittmeyer et al., 2012] and the Brazilian flea-toads *Brachycephalus pulex* Napoli et al. 2011 and *B. sulfuratus* Condez et al. 2016 (Brachycephalidae; combined adult SVL 7.4–10.8 mm; Napoli et al., 2011; Condez et al., 2016), while the largest species is the African *Conraua goliath* Boulenger, 1906 (Conrauidae), generally considered the largest frog in the world, with some individuals having a SVL of 32 cm SVL (Sabater-Pi, 1985). The integument of amphibians interacts with the environment, and is involved in gas, water, and ion exchange (Hillman et al., 2009). All of these processes depend on the surface area (A_S) available for passive diffusion and/or active transport.

Measurements of actual anuran A_S, however, are scarce and difficult to obtain due to the irregularly shape of these animal's bodies. To measure A_S in anurans, most studies removed the integument from euthanized animals, cut the skin into small pieces and determined the area of each piece (Rubner, 1883; Fry, 1913; Voit, 1930; Terroine and Delpech, 1931; Hutchison et al., 1968; McClanahan and Baldwin, 1969; Talbot and Feder, 1992; Dabés et al., 2012). Indirect methods to determine A_S have also been used,

* Corresponding author at: Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo. Av. Bandeirantes 3900, Bairro Monte Alegre, 14040-901 Ribeirão Preto, São Paulo, Brazil.
E-mail address: wklein@usp.br (W. Klein).

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Table 1
Species investigated in the present study. Body mass (M_B) data given first as range followed by mean ± standard deviation. N gives the number of individuals sampled.

Species	N	M_B (g)	M_B (g)
Dendropsophus branneri	10	0.25–0.38	0.32 ± 0.04
Dendropsophus decipiens	10	0.22–0.46	0.27 ± 0.07
Dendropsophus minutus	10	0.66–1.07	0.78 ± 0.12
Hypsiboas albomarginatus	9	5.68–6.83	6.16 ± 0.42
Leptodactylus natalensis	7	6.03–9.22	7.28 ± 1.05
Leptodactylus macrosternum	10	20.88–55.43	37.31 ± 11.45
Phyllodytes melanomystax	10	0.90–1.17	1.03 ± 0.11
Pristimantis paulodutrai	10	0.58–1.34	0.84 ± 0.25
Rhinella jimi	10	123.03–522.94	338.40 ± 133.42
Scinax auratus	10	0.71–0.94	0.80 ± 0.08
Scinax eurydice	8	6.72–8.81	7.48 ± 0.64
Scinax sp. (*S. ruber* clade)	10	0.87–2.59	2.09 ± 0.54

such as measuring the capacitance of a copper-coated wax model (Buttemer, 1990) or, more recently, applying magnetic resonance imaging to estimate A_S in the Palmate Newt using 3D reconstructions (Wardziak et al., 2014).

From these databases allometric relationships between A_S and body mass (M_B) have been derived and are frequently being used to estimate physiological traits such as evaporative water loss (e.g. Ruibal and Hillman, 1981; Withers et al., 1982; Wygoda, 1984; Tattersall et al., 2006; Young et al., 2006; Tracy et al., 2007). Most studies have relied on the relationship between A_S and M_B for Anura established by McClanahan and Baldwin (1969), which was confirmed by Buttemer (1990). However, McClanahan and Baldwin (1969) used only three species of Bufonidae and one species of Scaphiopodidae in their study, weighing between 11.4 and 90.5 g, while Buttemer (1990) used two species of Australian tree frogs (Hylidae) weighing between 10.0 and 62.0 g and Talbot and Feder (1992) studied the bullfrog *Lithobates catesbeianus* (Shaw, 1802) (Ranidae) weighing between 40.0 and 514.8 g to establish the allometric relationship between A_S and M_B. Another attempt to examine this relationship by Hutchison et al. (1968) used regression analysis based on a larger dataset using bufonids (3 species), hylids (4 species), ranids (5 species) and scaphiopodids (1 species), ranging in overall body mass between 1.1 and 94.8 g. Comparing the commonly used regression given by McClanahan and Baldwin (1969) ($A_S = 9.9\,M_B^{0.56}$) with the regressions given by Hutchison et al. (1968), the latter dataset yielded a regression line with greater scaling exponent when analyzing Bufonidae ($A_S = 9.246\,M_B^{0.645}$), but a greater y-intercept when analyzing Anura ($A_S = 13.521\,M_B^{0.579}$).

Rubner (1883) measured surface area for the first time in a frog (species not given), reporting A_S to be 116 cm^2 for an animal with M_B of 40 g. Applying the regression given by Hutchison et al. (1968) for Anura on a M_B of 40 g would result in an A_S of 114.44 cm^2, whereas McClanahan and Baldwin's regression would yield an A_S of 78.12 cm^2. Given these different allometric relationships between A_S and M_B reported in the literature and the resulting differences in estimates of anuran A_S, our aims in the present study were to compile data on anuran surface area and body mass, add new original data, and perform allometric regression analysis (ordinary and phylogenetic) on the largest datasets available to date, thereby establishing the best model at the level of species, family and order.

2. Material and methods

2.1. Data from this study

Surface area was determined in twelve species of anurans (Table 1). These animals were collected for a previous study (Dabés et al., 2012) under permits of the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (License numbers 13001-1 and 2239548) and have been deposited in the amphibian collection of the Museu de Zoologia of the Universidade Federal da Bahia (UFBA). No animal has been killed for the sole purpose of determining its cutaneous surface area.

To determine A_S, animals were euthanized following the Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research (Beaupre et al., 2004) and preserved for up to one month in alcohol (70%). The integument (except hands and feet) was removed, stained in methylene blue, cut into pieces that would lay out on a flat surface, and all pieces were photographed together at 5-megapixel resolution using a digital camera at a constant distance. Total A_S of a specimen was determined from the digital photographs, transforming the pictures into black and white pixels and counting the number of black pixels representing integument using an *ad-hoc* developed program, Area (Camacho and Rocha, 2010). Anuran surface area was calibrated against a square of known area (1 cm^2). Body mass of well-hydrated animals was determined during the experiments in Dabés et al. (2012) using a semi-analytical scale (Shimadzu). Bladder content was not removed before weighing.

2.2. Data from previous studies

To compare our results with previously published results, data of M_B and "ratio of surface of skin (in cm^2) to body mass (in g)" of twenty species of anurans were taken from Table II of Czopek (1965) and used to calculate A_S (cm^2). We also included in our analyses data of A_S and M_B taken from Fry (Table IV, 1913), from Voit (Table VI, 1930), from Terroine and Delpech (Tables I–IV, 1931), and from Chen et al. (2014), as given in their Supplemental S1. For the data from Voit (1930), we used his mean values of A_S, since he determined surface area both by skinning and by calculations using the animals' circumference. Furthermore, Fig. 18 from Hutchison et al. (1968), Fig. 2 from McClanahan and Baldwin (1969), Fig. 8 from Heatwole et al. (1969), and Fig. 2 from Talbot and Feder (1992) were extracted from pdf-files downloaded from the publisher's archives. Coordinates of data points were determined using the free software PlotDigitizer version 2.6.2. In all cases only data for whole animal surface area (except hand and feet) were included into our analysis (see Supporting Information 1).

We verified the quality of data obtained from the literature once the extracted data of A_S and M_B were $\log_{10}$ transformed and analyzed with Prism Graphpad 6.0, performing an ordinary least square (OLS) linear regression analysis to compare the data extracted from the graphs published in Hutchison et al. (1968); Heatwole et al. (1969), McClanahan and Baldwin, 1969, and Talbot and Feder (1992) to the regression equations given in these papers.

2.3. Comparison and analyses of datasets

Data from Fry (1913), Voit (1930), Terroine and Delpech (1931), Czopek (1965) Hutchison et al. (1968), Heatwole et al. (1969), McClanahan and Baldwin (1969), Talbot and Feder (1992) and Chen et al. (2014) were pooled together for each family (Bufonidae, Hylidae, Ranidae, Scaphiopodidae) and again into one complete data set for all Anura. The pooled data of the four families and Anura as a whole were then compared to our data, grouped into Bufonidae, Craugastoridae, Hylidae, Leptodactylidae, and Ranidae, as well as Anura. A combined total of 433 data points from our study and from literature data, comprising 44 different species from 11 families, were used to analyze the relationship between A_S and M_B of Anura.

Data for individual species were analyzed separately when data from at least seven individuals were available to provide intraspecific allometric relations between A_S and M_B and to permit