

Field body temperatures in the rainforest frog *Mantidactylus (Brygoomantis) bellyi* from northern Madagascar: Variance and predictors

Safidy M. Rasolonjatovo^{1,2}, Mark D. Scherz^{3,4},
Andolalao Rakotoarison^{1,3}, Julian Glos⁵, Achille
P. Raselimanana^{1,2} & Miguel Vences³

¹ Mention Zoologie et Biodiversité Animale, Faculté des Sciences, BP 906, Université d'Antananarivo, Antananarivo 101, Madagascar
E-mail: safidymalala@yahoo.com, andomailaka@gmail.com, raselimananaachille@gmail.com

² Association Vahatra, BP 3972, Antananarivo 101, Madagascar

³ Division of Evolutionary Biology, Zoologisches Institut, Technische Universität Braunschweig, Mendelssohnstr. 4, 38106 Braunschweig, Germany
E-mail: mark.scherz@gmail.com, m.vences@tu-braunschweig.de

⁴ Department of Herpetology, Zoologische Staatssammlung München, Münchhausenstr. 21, 81247 Munich, Germany

⁵ Institute of Zoology, Animal Ecology and Conservation, Universität Hamburg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany
E-mail: julian.glos@uni-hamburg.de

Abstract

Given that global amphibian population declines are in part associated with environmental thermal preferences of infectious pathogens, assessing variation of field body temperatures can contribute to a preliminary disease risk assessment. We evaluated the field body temperatures of the semi-aquatic frog *Mantidactylus (Brygoomantis) bellyi* from northern Madagascar during the warm-wet season (November-January), identified predictors of body temperature (T_b) and differences between T_b and substrate temperature (T_{subs}), and investigated diel and elevational variance of T_b . Body temperatures of 353 individuals of *M. bellyi* in the field ranged from 17.0 to 25.5°C, matching documented optimal temperature growth of the chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*). Our results also confirm *M. bellyi* to be a thermoconformer, with body temperatures closely matching those of the surrounding substrate. Body temperature of individual frogs slightly increased as a function of size and differences were

found between the sexes, with males being slightly warmer. Statistical models did not maintain elevation as a relevant predictor of T_b , in spite of a noticeable decrease of T_b with elevation. This was less clearly expressed for diurnal than nocturnal T_b which suggests heat gain through exposure to sunlight. The highest variance of T_b was recorded in the population towards the summit of the massif (≥ 1200 m above sea level), and the lowest at mid-elevation (800-1000 m above sea level). However, these data require further extension to seasonal temperature variation, including measurements in the cool-dry season, to fully understand the thermal regime of this rainforest species and its seasonal thermal fluctuations.

Keywords: Amphibia, Mantellidae, elevation, substrate temperature, diel cycle

Résumé détaillé

Lorsque le déclin de la population mondiale d'amphibiens est associé aux préférences thermiques environnementales des agents pathogènes infectieux, l'étude de la variation de la température corporelle au niveau de leur habitat peut contribuer à une évaluation préliminaire des risques d'infection à une maladie. Une évaluation de la température corporelle de la grenouille semi-aquatique *Mantidactylus (Brygoomantis) bellyi* du Nord de Madagascar pendant la saison chaude et humide (de novembre 2017 à janvier 2018) a été effectuée. Les individus ont été repérés grâce à une fouille active des habitats potentiels et à la localisation des chants des mâles lors de recherches diurnes et nocturnes. Les cours d'eau échantillonnés ont couvert une large gamme d'altitude du Parc National de la Montagne d'Ambre (600 à 1470 m) et différentes structures forestières. La température cutanée externe de la grenouille a été assimilée comme celle de la température corporelle (T_b). Cette dernière ainsi que la température du substrat (T_{subs}) directement en contact avec l'animal ont été mesurées à l'aide d'un thermomètre à laser infrarouge (Benetech® GM700-GM900, Shenzhen Jumaoyuan Science and Technology Co., Ltd.). Les facteurs prédictifs de T_b et de la différence entre T_b et

T_{subs} ont été déterminés, et les variances journalières et altitudinales de T_b ont été étudiées. La température corporelle de 353 individus de *M. bellyi* variait entre 17 et 25,5 °C dans leur milieu naturel, correspondant à la température de croissance optimale connue pour le chytride, *Batrachochytrium dendrobatidis* (Bd). Les résultats confirment également que *M. bellyi* est un thermoconformeur, avec une température corporelle proche de celle du substrat. La température corporelle est positivement corrélée avec la taille de la grenouille et a différé selon le sexe, les mâles présentant des températures légèrement plus élevées. Les modèles statistiques n'ont pas corroboré l'élévation comme étant un facteur de prédiction pertinent de T_b , même si une diminution de T_b avec l'élévation a été notée. De plus, cette baisse de T_b était moins explicite le jour que la nuit suggérant un gain de chaleur dû à l'exposition au soleil. La variance la plus élevée de T_b a été retrouvée chez la population vivant au niveau sommital du massif (> 1200 m) et la plus faible à mi-altitude (800 à 1000 m), rendant peut-être cette dernière population plus vulnérable à la maladie fongique chytridiomycose. Cependant, ces données nécessitent des études supplémentaires de la variation saisonnière de la température corporelle, y compris des mesures faites en saison fraîche et sèche, pour bien comprendre le régime thermique de cette espèce des forêts tropicales humides et ses fluctuations thermiques saisonnières.

Mots clés : Amphibia, Mantellidae, altitude, température du substrat, cycle journalier

Introduction

Ectothermic vertebrates use a large array of physiological and behavioral mechanisms to regulate their body temperatures, such as the adjustments of the cardiovascular system, and control over blood flow to the skin and microvascular regulation of cutaneous gas exchange (Duellman & Trueb, 1994; Pough *et al.*, 2001). Amphibians are more limited in terms of physiological and behavioral thermoregulatory mechanisms (Raske *et al.*, 2012). Thermoregulation is restricted by the conflicting processes of hydro regulation and cutaneous respiratory gas exchange (Duellman & Trueb, 1994; Stebbins & Cohen, 1995; Tattersall *et al.*, 2006; Centeno *et al.*, 2015). Thus, amphibians select environmental conditions that minimize evaporative water loss, sometimes at the expense of thermoregulation, and their physiological and behavioral performance (Tattersall *et al.*, 2006;

Köhler *et al.*, 2011; Centeno *et al.*, 2015; Mitchell & Bergmann, 2016).

As their body temperatures are strongly derived from their immediate surroundings – especially air, water, and substrate temperatures – amphibians are considered mostly thermoconformers (Duellman & Trueb, 1994; Stebbins & Cohen, 1995; Wells, 2007; Iturra-Cid *et al.*, 2014), but behavioral thermoregulation has been reported for some species and might help in balancing insolation against the concomitant evaporation of body water (Wells, 2007). The behavioral mechanism most commonly displayed by amphibians in the field is basking, which is achieved by movement between microhabitats, direct exposure to sunlight or indirect heat gain from the atmosphere, substrate, and vegetation nearby (Hutchison & Dupré, 1992; Tattersall *et al.*, 2006; Centeno *et al.*, 2015). Other behavioral mechanisms involve selection of temporal diversity provided by the diel and seasonal cycles of the thermal environment, postural and orientation responses to maximize solar heat gain, and social interactions (Hutchison & Dupré, 1992; Stebbins & Cohen, 1995). Therefore, the body temperature of amphibians in their natural environment results from complex interactions of numerous environmental factors (ambient temperature, salinity, atmospheric pressure, gases, etc.), internal physical changes, and behavioral and physiological adjustments (genetic adaptation, photoperiod, disease, life cycle stage, orientation, posture, etc.) (Hutchison & Dupré, 1992; Leger & Mathieson, 1997; Tattersall *et al.*, 2006; Tracy & Christian, 2010; Raske *et al.*, 2012; Centeno *et al.*, 2015).

Many aspects of amphibian biology and performance are affected by environmental temperatures, such as locomotion, vocalization, timing and duration of breeding, digestive and assimilation efficiencies, growth and development of eggs, larvae and juveniles, and activity patterns (Rome *et al.*, 1992; Stebbins & Cohen, 1995; Ruthsatz *et al.*, 2018). For instance, breeding is expedited and development and growth are accelerated in warm environments; while the reverse is generally true in colder environments (Turriago *et al.*, 2015; Dastansara *et al.*, 2017; Sinsch & Dehling, 2017; Ruthsatz *et al.*, 2018). Though these ectothermic animals are capable of activity over a broad range of temperatures, optimal thermal range changes with the biological function they need to perform. Elevating body temperature from external thermal sources

accelerates, for example, feeding and digestion, but reduces visual sensitivity (Hutchison & Dupré, 1992).

Apart from organismal performance, assessing the relationship between environmental and body temperatures has become crucial in light of disease risk assessment (Duellman & Trueb, 1994; Daskin & Alford, 2012; Stevenson *et al.*, 2013; Sauer *et al.*, 2018; von May *et al.*, 2018). With the global emergence of the chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), including on Madagascar (Bletz *et al.*, 2015; Scheele *et al.*, 2019), investigations of body temperatures of wild amphibians have gained interest in order to assess *Bd* risk infection in wild populations. *Bd* infection is known to increase when host temperatures match their optimal growth range (Catenazzi *et al.*, 2013; Rowley & Alford, 2013; Stevenson *et al.*, 2013; Sonn *et al.*, 2017; Sauer *et al.*, 2018). On the other hand, higher body temperature can not only prevent infection (Rowley & Alford, 2013) but also eliminate *Bd* and perhaps other pathogens (Duellman & Trueb, 1994; Woodhams *et al.*, 2003).

Records of *Batrachochytrium dendrobatidis* exist from numerous wild amphibian communities on Madagascar, especially between mid- to high-elevation sites (Lötters *et al.*, 2011; Bletz *et al.*, 2015). Yet, so far almost no information on body temperatures of wild Malagasy amphibians has been published. Here, we present an assessment of

body temperatures of a frog occurring in Montagne d'Ambre National Park in northern Madagascar. Our analysis is focused on field body temperatures (T_b) in the Malagasy semi-aquatic frog, *Mantidactylus (Brygoomantis) bellyi* (family Mantellidae) and its relationship to different environmental and biological predictors. Our pilot study provides the first extensive thermophysiological information on a Malagasy frog species, which may prove useful for the assessment of the susceptibility of this and other, co-occurring amphibians to chytridiomycosis and environmental fluctuations.

Materials and methods

Sampling

Fieldwork was carried out in the Montagne d'Ambre National Park, in northern Madagascar. From 11 November 2017 to 7 January 2018, corresponding to the rainy season, individuals of *M. bellyi* were located by active searching or localization of calling males along streams, ponds, edges of lakes, and waterfalls during diurnal and nocturnal searches. A total of 18 water bodies covering known elevations (from ca. 600 to 1470 m above sea level) and diversified forest structure were surveyed and georeferenced using a Global Positioning System (GARMIN GPSMAP®64s). Information about the surveyed sites are given in Table 1. Samples were taken from moist semi-deciduous forest at low elevation, passing

Table 1. Geographical coordinates and elevations of the 18 studied water bodies. Longitudes and latitudes represent haphazardly selected points taken along the sampled water bodies. Cascade Antakarana and RE1 both overlap between mid- and high-elevation. Elevation is given as meters above sea level.

Elevational zone	Sampled body waters	Longitude	Latitude	Elevation (m)
Foothills(400-600 m)	Antsakomboiny	-12.46864	49.22070	467-484
Low elevation (600-800 m)	Betsikoboko	-12.49085	49.17858	623-774
	Antomboko	-12.49012	49.17096	652-726
	Zanakatomboko	-12.48682	49.17038	670-715
	Andrano Créole	-12.49494	49.18488	734-759
Mid-elevation (800-1000 m)	RW4	-12.58600	49.13212	923-941
	RW3	-12.59099	49.13644	927-980
	RW5	-12.58559	49.13422	949-993
	RE1*	-12.53328	49.18724	949-976
	Cascade Antakarana*	-12.52057	49.17232	997
High-elevation (1000-1200 m)	Cascade Antakarana*	-12.52057	49.17228	1000-1054
	RE1*	-12.53310	49.18731	1010-1057
	Gîte	-12.52487	49.17282	1005-1055
	RW2	-12.52080	49.16775	1042-1093
	RW1	-12.52662	49.16807	1089-1125
	Cascade sacrée	-12.52796	49.17180	1045-1192
	Chris camp	-12.53696	49.16800	1151-1196
Summit > 1200 m	Lac Maudit	-12.58554	49.15026	1244-1255
	Grand lac	-12.59824	49.15938	1277-1333
	RtransectGL	-12.59880	49.15889	1307-1394

through medium altitude moist evergreen forest covering the majority of the National Park, to the summit covered by montane ericoid thicket. Sampling sites also included the dry deciduous forest from the western slope, and few moist secondary thickets and secondary prairies in areas where anthropogenic activities have modified the natural vegetation (vegetation types according to Gautier *et al.*, 2018). Body Temperatures of the frog (T_b) at skin level and the nearby substrate (T_{subs}) directly in contact with the animal were measured with an infrared laser thermometer (Benetech® GM700-GM900, Shenzhen Jumaoyuan Science and Technology Co., Ltd.) (Sample size $n = 353$). Substrates used ranged from compact surfaces found along riverbank and in the immediate surrounding microhabitats, or simply the water. When the frog was partially submerged, T_{subs} was taken by pointing the laser thermometer at the water. Thermal records were taken at distance less than 1 m from the individual and the substrate, without physical contact with the subject. External skin temperature was used as an estimate of individual's body temperature (T_b) (Berg *et al.*, 2015), which is justified because the body size of *M. bellyi* is rather small based on snout-vent length (SVL) (24.70-53.90 mm in adults). Following temperature readings, animals were captured, sex noted, and SVL of adult specimens measured. Before being released, individuals were web-clipped for molecular analyses (data not shown in this study) allowing recognition of individuals already sampled. Juveniles were smaller in body size compared to adults. Adult males were recognizable due to their smaller body size and larger tympanum diameter compared to adult females, as well as their prominent femoral glands.

Data analysis

Mean, standard deviation, and variance of T_b and T_{subs} were calculated with Microsoft® Excel. Other analyses were performed in R (R Core Team, 2018). The alpha-level for significance was set at $P = 0.05$. Before the analysis, all dependent variables in the models were tested for autocorrelation using Spearman's rank correlation. Subsequently, variables were included in statistical analysis when the correlation was significant but well below the suggested threshold of 0.7 for eliminating variables (Fielding & Haworth, 1995; Chin, 1998) or not significant. Because a correlation between elevation and body size was found in other studies (Liao *et al.*, 2011; Khatiwada *et al.*, 2019), the interaction between elevation and body size of the

specimen was added as a predictor, as well as the interaction between elevation and diel cycle since some amphibians select temporal diversity provided by the diel and seasonal cycles of the thermal environment for regulation of body temperature (Hutchison & Dupré, 1992; Stebbins & Cohen, 1995). Using the "stepAIC" function implemented in the MASS package (Venables & Ripley, 2002), we then conducted a stepwise model selection to test the influence of elevation, sex, diel cycle, T_{subs} , body size (SVL), interaction between elevation and body size, and interaction between elevation and diel cycle on T_b . The process both adds and removes variables (direction = "both") to/from a model using the Akaike information criterion as a selection criterion. Then, the relationships between the retained predictors on T_b (response variable) were determined with multiple linear regression. We followed the same steps to assess the impact of these variables (except T_{subs}) on the difference between T_b and T_{subs} (response variable). A sign test was applied to analyze the systematic difference between T_b and T_{subs} of each individual. Strength of the correlations between T_b and T_{subs} were measured with Spearman's correlation tests for the entire dataset, and separately for diurnal and for nocturnal temperature records.

Because *M. bellyi* dwells along streams across high to low-elevation sites in the Montagne d'Ambre National Park, we also analyzed variance of T_b across elevational zones on this massif. In this last approach, the elevational range of the massif was subdivided into five zones: (1) the foothills (400-600 m), (2) low elevation (600-800 m), (3) mid-elevation (800-1000 m), (4) high-elevation (1000-1200 m), and (5) the summit (≥ 1200 m). Each individual record was assigned to an elevational zone and the thermal variance of T_b was calculated for each zone. Sample size per elevational zone counted 19 individuals at the foothills, 81 at low elevation, 96 at mid-elevation, 112 at high-elevation and 45 individuals at the summit. Strength of the correlations and the coefficient of correlation between T_b and T_{subs} were measured with Spearman's correlation tests for each elevational zone. We herein consider a species to be a thermoconformer when T_b strongly parallels changes in T_{subs} (i.e. indicating little or no behavioral thermoregulation). On the other hand, a species is considered to be actively thermoregulating when T_b is maintained within a narrow range although T_{subs} significantly varies beyond the controlled T_b (Hutchison & Dupré, 1992). When modelled, the intensity of thermoregulation would be given

by the slope of the linear relationship between the operative environmental temperature and the body temperature ($0 \leq k \leq 1$), with $k = 0$ defining a perfect thermoregulator and $k = 1$ defining a perfect thermoconformer (Huey & Slatkin, 1976; Angilletta, 2009).

Results

A total of 353 body (T_b) and substrate temperatures (T_{subs}) were recorded during the rainy season 2017-2018 across a wide elevational gradient of the Montagne d'Ambre Massif. Table 1 presents the geographical coordinates and elevations of the sampled water bodies. Examined specimens consisted of 186 adult males and 167 adult females. Juveniles and adults which escaped before determination of their sex were excluded.

Temperature ranges of wild *Mantidactylus bellyi*, elevation and diel variances

Body temperatures of *M. bellyi* ranged between 17.0-25.5°C, with a mean \pm SD temperature of 20.5°C \pm 1.3 (Figure 1, Table 2). The lowest temperature was recorded at high-elevation (1095 m) and the highest temperature at low elevation (720 m). Variance of T_b was highest along the mountain summit above 1200 m (1.74°C), lowest at mid-elevation, and relatively high at low elevation (Tables 2 and 3). The overall variance of T_b was 1.71°C. Since *M. bellyi* is

a cathemeral species (active in daylight as well as at night), its body temperatures were measured for both times of the day. Minimal and maximal records of T_b were lower at night, with a difference of 1.4°C and 1.8°C, respectively. The variance of T_b during daylight was higher compared to that of the night (Table 2).

Mantidactylus bellyi was commonly found along riverbanks or in immediate surrounding microhabitats. Individuals were spotted on diverse types of substrates, sometimes half immersed in the water, sitting on rocks, resting on the ground, mud, grass, branches, foam in water, dead or fresh leaves, wood, stems, and sometimes hidden under rocks, between roots, grasses or reeds surrounding the water body. Rocks were the most commonly used substrate (Figure 2). Temperature of substrate across the entire elevational montane range was 20.51°C \pm 1.31. The lowest and highest T_{subs} were respectively recorded at high elevation (1095 m) and low elevation (705 m) (Table 2).

Predictors of body temperature in *Mantidactylus bellyi*

As all variable pairs were not highly correlated (i.e., $\rho < 0.4$), the predictive variables were all included in the initial models. Models including the following variables: elevation, diel cycle (day/night), interaction between elevation and body size of the specimen, and finally the interaction between elevation and diel cycle were excluded via backward elimination

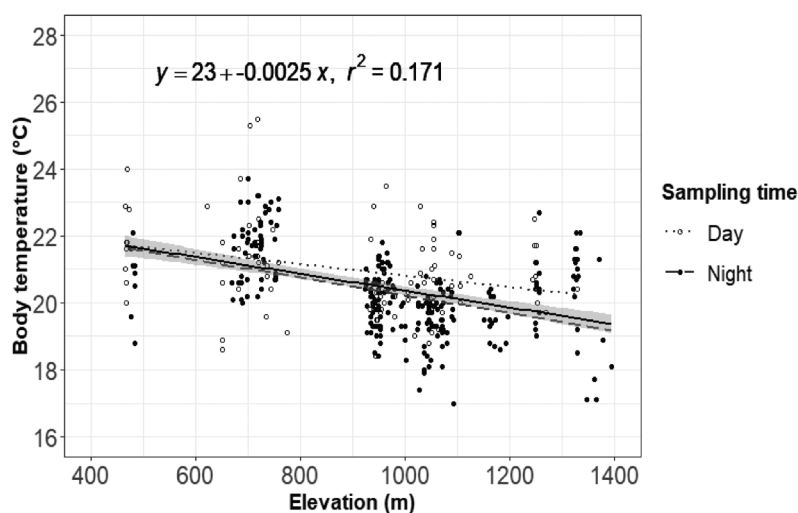


Figure 1. Body temperature T_b of the wild *Mantidactylus bellyi* as a function of elevation on the Montagne d'Ambre Massif in northern Madagascar showing a decrease of T_b with elevation ($R^2 = 0.169$, $F = 72.57$, $P = 4.82e-16$). In multivariate models including elevation, sex, diel cycle, substrate temperature, body size, interaction between elevation and body size, and interaction between elevation and diel cycle, elevation was not retained as predictor of T_b . White and black dots are representative of individual T_b , respectively recorded during diurnal and nocturnal searches. Linear regression is represented in black line, with the gray area representing the 95% confidence level interval for predicting the model, equation of regression is shown at top left corner. Regression lines defining the correlation between T_b and elevation during the day and at night are respectively shown in dotted and dashed gray lines.

Table 2. Mean body temperature T_b , mean substrate temperature T_{subs} , minimum and maximum T_b and T_{subs} values, Spearman's correlation coefficient r between T_b and T_{subs} and P values of Spearman's rank correlation rho tests across the elevational zones and between day and night cycles. $Var T_b$ and $Var T_{subs}$ represent respectively the variance of body temperature and substrate temperature. Diff in Min-Max T_b gives the difference between the maximum and minimum values in body temperatures.

Elevational zone (m)	Mean±SD T_b (°C)	Mean±SD T_{subs} (°C)	Var T_b	Var T_{subs}	Min-Max T_b (°C)	Diff in Min-Max T_b (°C)	Min-Max T_{subs} (°C)	Rho (T_b/T_{subs})	Spearman's correlation test (T_b/T_{subs}) P -value
Foothills (400-600)	21.35 ± 1.20	21.42 ± 1.21	1.44	1.45	18.8-24.0	5.2	18.4-23.7	0.94	< 0.0001
Low elevation (600-800)	21.64 ± 1.22	21.63 ± 1.20	1.48	1.44	18.6-25.5	6.9	18.6-25.5	0.99	< 0.0001
Mid-elevation (800-1000)	20.20 ± 0.89	20.21 ± 0.88	0.79	0.78	18.4-23.5	5.1	18.4-23.5	1.00	< 0.0001
High-elevation (1000-1200)	19.79 ± 1.04	19.80 ± 1.05	1.08	1.10	17.0-22.9	5.9	17.0-22.9	0.99	< 0.0001
Summit (≥1200)	20.42 ± 1.32	20.50 ± 1.39	1.74	1.95	17.1-22.7	5.6	17.1-23.5	0.96	< 0.0001
Sampling time									
Day	20.98 ± 1.36	21.02 ± 1.37	1.84	1.86	18.4-25.5	7.1	18.4-25.5	0.98	< 0.0001
Night	20.29 ± 1.24	20.31 ± 1.24	1.53	1.53	17.0-23.7	6.7	17.0-23.7	0.99	< 0.0001

Table 3. P values of the Tukey Post hoc test results revealing differences of body temperature variances ($Var T_b$) between two elevational zones.

Elevational zone	Foothills (400-600 m)	Low elevation (600-800 m)	Mid-elevation (800-1000 m)	High elevation (1200-1400 m)	Summit (1200-1475 m)
Foothills (400-600 m)	-	-	-	-	-
Low elevation (600-800 m)	0.83	-	-	-	-
Mid-elevation (800-1000 m)	< 0.001	< 0.00001	-	-	-
High-elevation (1000-1200 m)	< 0.0001	< 0.0001	0.05	-	-
Summit (1200-1475 m)	< 0.05	< 0.0001	0.81	< 0.05	-

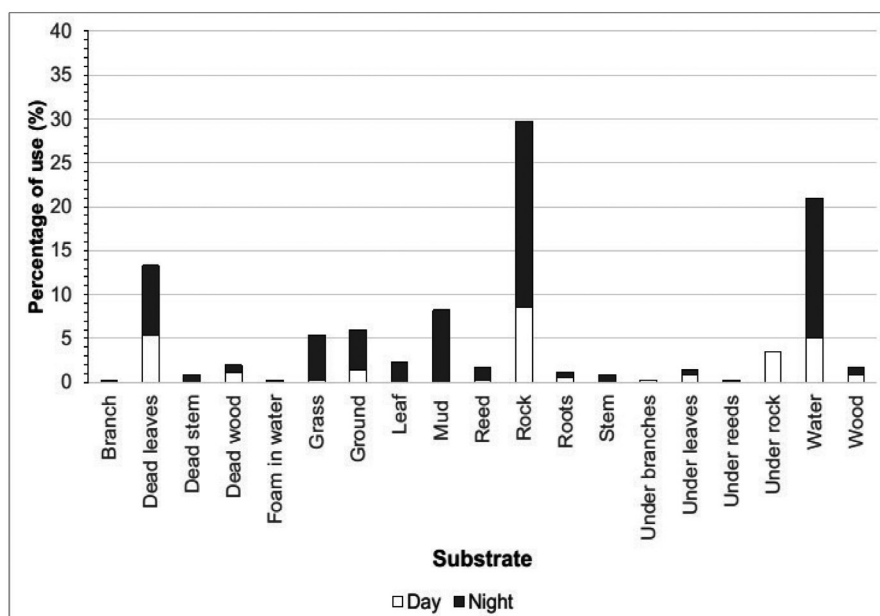


Figure 2. Substrate preferences and percentage of use of the different types of substrates by *Mantidactylus bellyi* related to total observations during day and night cycles between November 2017 and January 2018.

in a stepwise fashion. The final model only retained sex ($P = 0.01$), frog body size ($P = 0.01$), and especially T_{subs} ($P < 0.0001$) as significant predictors of T_b ($R^2 = 0.98$; Table 4). Body temperatures increased slightly with body size and strongly with T_{subs} , and were slightly higher on average in males ($T_b = 20.58^\circ\text{C} \pm 1.34$) compared to females ($T_b = 20.38^\circ\text{C} \pm 1.26$). The difference between T_b and T_{subs} was not, but close to being significant (Sign test, $S = 40$, $n = 353$, $P = 0.06$); T_b was slightly lower ($T_b = 20.49^\circ\text{C} \pm 1.31$) than T_{subs} ($T_{\text{subs}} = 20.51^\circ\text{C} \pm 1.31$). A Spearman correlation confirmed the tight positive correlation between T_b and T_{subs} both during day and night (Figure 3, Table 2).

When modelling the difference between T_b and T_{subs} , the predictive variables elevation, diel cycle, interaction between elevation and body size, and interaction between elevation and diel cycle were discarded throughout the stepwise regression procedure. The final model included the variables sex ($P = 0.02$) and body size ($P = 0.02$) as relevant predictors of the difference between T_b and T_{subs} . However, the coefficient of determination ($R^2 = 0.01$) was very low, indicating a low goodness of fit of this model.

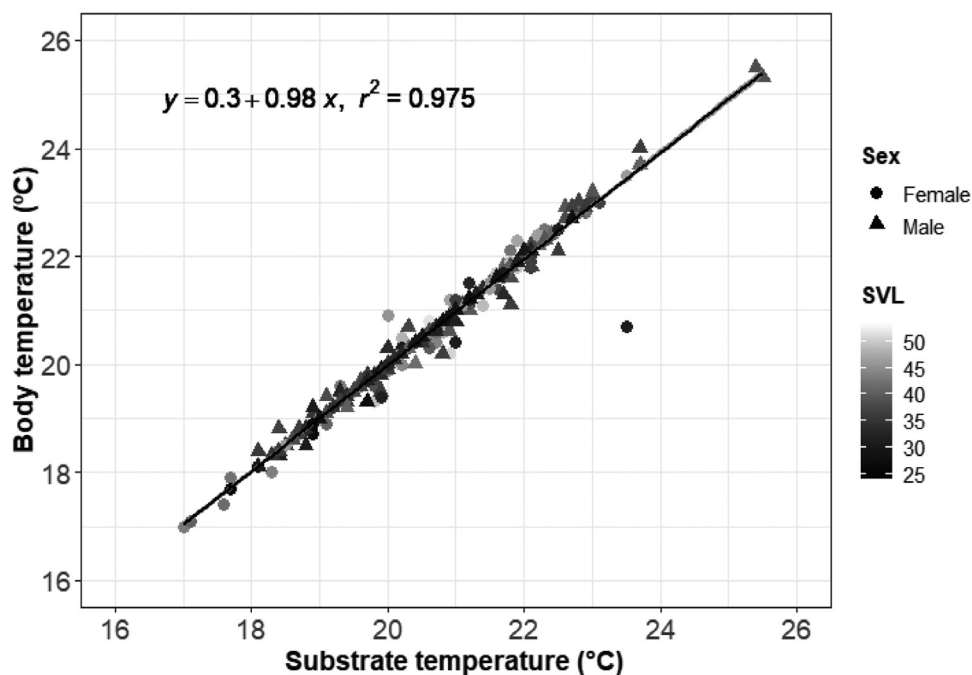


Figure 3. Regression line defining strong positive correlation between body temperatures of the tropical rainforest frog *Mantidactylus bellyi* and substrate temperatures ($k = 0.98$).

Table 4. Retained multiple linear models using the Akaike information criterion as a selection criterion and showing the influence of biological (sex and body size) and environmental (T_{subs}) parameters on T_b of *Mantidactylus bellyi* and on the difference between T_b and T_{subs} (Diff).

Model	Predictors	Regression coefficient	Standard error	t-value	P-value
$T_b \sim T_{\text{subs}} + \text{sex} + \text{SVL}$ Adjusted R-squared = 0.98 $P < 0.0001$	(Intercept)	0.09	0.19	0.47	0.64
	Substrate temperature	0.98	0.01	116.86	< 0.0001
	Sex	0.07	0.03	2.59	< 0.05
	Snout-vent length	0.01	0.00	2.55	< 0.05
Diff \sim sex + SVL Adjusted R-squared = 0.01 $P < 0.05$	(Intercept)	-0.27	0.10	-2.70	< 0.01
	Sex	0.06	0.03	2.36	< 0.05
	Snout-vent length	0.01	0.00	2.36	< 0.05

Discussion

Temperature ranges, elevational trend and diel variance

The Malagasy rainforest frog *Mantidactylus bellyi* showed a mean body temperature of $20.49^{\circ}\text{C} \pm 1.31$ between 470 to 1395 m in Montagne d'Ambre National Park. These values fit within the T_b range (-4.3 - 29°C) of other montane amphibian species previously recorded in tropical regions ranging from 73 to 3550 m above sea level (Navas *et al.*, 2013). In Navas *et al.*'s (2013) overview of thermal regime of tropical amphibians, the investigated species showed a decrease of T_b when the elevation of their collection sites increased, though this trend does not seem to be universal, as reported in some temperate species (Trochet *et al.*, 2018). We herein confirm for *M. bellyi* a general expected decrease of T_b along an increasing elevational gradient, but the influence of elevation on T_b was not significant in a model also taking into account sex, body size (SVL), and substrate temperature. This lack of significance most likely was caused by frogs from the 1300-1400 m elevational zone having higher body temperatures than those found around 1200 m, which is probably associated with the effect of air temperature on T_b . We hypothesize that *M. bellyi* living on this mountain probably warm their body by basking or by shuttling among the available range of environmental temperatures during the day, which could explain how these frogs maintain the relatively constant body temperature trend observed during the day compared to night observations (Figure 1). In fact, we found the correlation between T_b and elevation was slightly stronger at night than during the day.

Predictors of T_b and predictors of the difference between T_b and T_{subs}

Our results identified T_{subs} as the dominant driver of body temperature of *M. bellyi*, though biological traits (sex and SVL) jointly impacted T_b , and the difference between T_b and T_{subs} as well. By moving from one spot to another, this amphibian probably selects temperatures available within its microhabitat while controlling for evaporative water loss. In other amphibians, such behavioral mechanisms include, for example, direct body contact with water or moist surfaces (substrate or soil) to regulate the amount of water being absorbed and evaporated from the skin (Hutchison & Dupré, 1992; Duellman & Trueb, 1994; Stebbins & Cohen, 1995). In support of this hypothesis, this species was frequently seen

immersed in water. In agreement with the pattern expected in a primary thermoconformer, we observed no significant thermal difference between T_b of *M. bellyi* and the substrate it used, despite the diverse forest types of the mountain (Goodman *et al.*, 2018). This is consistent with patterns observed in other amphibian species (Raske *et al.*, 2012; Navas *et al.*, 2013).

Indeed, temperature regulation is crucial for many aspects of amphibian biology and physiological performance. A specific physiological function is controlled by the thermal tolerance limits, itself delimiting a thermal range of physiological performance, and the thermal optimum allows optimal biological function (Rome *et al.*, 1992; Turriago *et al.*, 2015; Ruthsatz *et al.*, 2018). Additionally, the various physiological systems do not share a single thermal optimum and fluctuations of body temperature can have contradictory effects on different physiological processes (Hutchison & Dupré, 1992). For example, high or low thermal ranges can both be beneficial, but for different aspects of an amphibian's life: high temperatures can accelerate larval development and growth of juveniles, enhance digestion and other physiological processes, or help to combat some diseases. Lower temperatures, on the other hand, promote recovery from daily or seasonal high metabolic demands, favor tolerance to prolonged periods of deprivation and unfavorable environmental conditions (Stebbins & Cohen, 1995). Where and when possible, amphibians likely choose preferred temperatures based on their biological needs and constraints; but in some thermally homogeneous environments amphibians are necessarily thermoconformers, as behavioral thermoregulation may not be possible. In this study, T_b regulation by *M. bellyi* seems to be highly dependent on T_{subs} , besides other possible environmental factors that were not surveyed here.

Correlation between T_b and T_{subs} and variance of T_b related to elevational zone

Despite environmental heterogeneity of the study site (Goodman *et al.*, 2018), T_b tightly correlated with T_{subs} across the five elevational zones ($\rho > 0.94$). In such a case, being thermally constrained to available T_{subs} could be a handicap for *M. bellyi*. Increase of T_b by exposure to solar radiation or other external sources of heat has been demonstrated in other amphibians living at relatively high elevations (Navas, 1997; Vences *et al.*, 2002; Tattersall *et al.*, 2006). Although a tight relationship between T_b and

T_{subs} was demonstrated here, it must be remembered that heat can also be obtained from the ambient air/water temperatures or from the sun (Duellman & Trueb, 1994) and merits attention in future studies. Beyond heat generated by T_{subs} , the decline of the correlation between T_b and T_{subs} at the summit could be associated with these sources of heat considering high-elevation sites are subjected to large seasonal and diel weather fluctuations.

Variance of T_b was lowest at mid-elevations and highest at the summit (Table 2). This reflects that animals living at high elevations are subjected to more variable environmental temperatures daily (Duellman & Trueb, 1994; Catenazzi *et al.*, 2013). Our results are similar to those of Navas *et al.* (2013) in that frogs living at higher elevation had higher variance compared to species living at lower elevation. But we conducted our investigation on a single species because amphibians do not share equally broad ranges of thermal tolerance, or similar abilities to undergo thermal extremes from adverse environmental conditions (through acclimation, acclimatization or “temperature hardening” mechanisms) (Duellman & Trueb, 1994). This reduces bias driven by inter-specific morphological, physiological, and phylogenetic variability, such as expected by studying wide-ranging amphibian species (Tracy & Christian, 2010; Trochet *et al.*, 2018) or multi-species datasets without phylogenetic correction.

Implications for conservation

We found that temperature preferences of the semi-aquatic *M. bellyi* ($20.5^{\circ}\text{C} \pm 1.3$) matched suitable temperatures for optimal *Bd* growth and reproduction ($17\text{-}23^{\circ}\text{C}$; Stevenson *et al.*, 2013; Sonnet *et al.*, 2017). In addition, frog populations living at the summit of Montagne d’Ambre had higher variance in T_b , probably useful in fighting *Bd* infection. In that respect, one might think that frog populations living at the summit would present greater chance to cope with the infection, comparatively to amphibians living between 800–1000 m. Indeed, some amphibians raise their body temperature to prevent or cure infection from certain emerging diseases (Woodhams *et al.*, 2003; Sauer *et al.*, 2018) but most current knowledge about thermoregulation and *Bd* growth is derived from laboratory experiments. As pointed by Stevenson *et al.* (2020), thermoregulation may allow tropical frogs to reduce disease impacts by limiting pathogen growth (using available range of environmental temperatures for basking) or, in the reverse case,

the environment may also limit the ability of frogs to thermoregulate in the field. Even though some skin bacteria of Malagasy frogs are able to inhibit *Bd* (Bletz *et al.*, 2017), no further information is available on how Malagasy wild frogs would behave when infected, and populations of *M. bellyi* may or may not be capable of raising their body temperature when necessary. Here, the only confirmed information is that *M. bellyi* is a thermoconformer in its natural habitat, with body temperatures closely matching those of the substrate. In such Least Concern species, serious ecological damage could still affect the population size after disease outbreaks. Specifically, being thermoconformers could be a major handicap for species distributed in locally restricted areas, primarily those that are endemic to higher altitudes in Madagascar, as body temperature is highly dependent on variation of its thermal environment.

With our dataset, we have highlighted that habitat preferences of *M. bellyi* also matched habitat suitable for disease transmission. The detectability of the pathogenic fungus *Batrachochytrium dendrobatidis* is associated with different environmental factors such as ambient temperature, geographic and elevational range distribution, wetness of the region, and connectivity between streams (Rödger *et al.*, 2009; Sapsford *et al.*, 2013; Scheele *et al.*, 2019). The severity of amphibian decline caused by chytridiomycosis is strongly associated with perennial aquatic habitats and wet regions (Scheele *et al.*, 2019), like the streams inhabited by *M. bellyi* on Montagne d’Ambre. During our study period, *M. bellyi* was mostly found dwelling in streams, and was present across the entire elevational range of the Montagne d’Ambre massif. Dead leaves also figured among the three most-used substrates by *M. bellyi*. Leaf litter temperatures were reported to play a significant role in *Bd* growth in other amphibians (von May *et al.*, 2018). However, extending this study to the cool-dry season is required to fully understand the thermal regime of the wild moist evergreen forest frogs, especially because *Bd* prevalence appears to vary across seasons (Sapsford *et al.*, 2013; Bletz *et al.*, 2015; von May *et al.*, 2018). We know that streams and leaf litter temperatures during the cool-dry season in other localities of Madagascar are different from that of the warm-wet season (Wollenberg *et al.*, 2008). In that respect, body temperatures of thermoconforming species could differ across seasons, even though frogs living in streams are expected to be less vulnerable to body

temperature fluctuations due to little change in annual temperature of streams (Wollenberg *et al.*, 2008).

At present, our data allow us only to conclude that *M. bellyi* occurs in a thermal zone and has a body temperature range conducive to *Bd* infection during the warm-wet season. Although investigations on thermophysiological variation across seasons are lacking, it may therefore be susceptible to infection, should *Bd* arrive on the mountain. At present, no mass die-offs attributable to disease have occurred on Madagascar, but species like *M. bellyi* should be among those most closely monitored, if we want to notice it and mitigate it if/when such events take place.

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