

Field body temperatures in Malagasy rainforest frogs

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Abstract. Temperature is one of the most prominent environmental factors influencing amphibian physiology and also an important life history trait for amphibian pathogens, such as the chytrid fungus *Batrachochytrium dendrobatidis* (Bd). This pathogen has been reported from wild amphibian communities on Madagascar, but it remains poorly known if field body temperatures (T_b) of wild Malagasy amphibians are in a range suitable for infection by this fungus. Here, we evaluated field T_b of 845 rainforest frogs from 38 species in two hyperdiverse amphibian communities in Madagascar during the warm-wet and cool-dry seasons, the first such comprehensive data set from the island. T_b of Malagasy rainforest frogs differed between habitat types and thus appears to correlate with ecological niche. In particular, frogs measured in pond habitats had a higher T_b across seasons than those sampled from streams, riparian zones, and terrestrial (including arboreal) habitats. Overall, T_b of the 38 frog species ranged between 9.3 and 33.5 across habitats and diel cycle. This temperature range matches the optimal temperature range for Bd growth (17–23°C). We hypothesize that susceptibility to Bd might differ between frogs occupying different habitats. Pond-dwelling frogs may have more opportunities to clear Bd infection by choosing warm operative temperatures in sun-heated pond water. Our results highlight the need for more studies evaluating the seasonal fluctuations of field body temperatures in the studied amphibian species to contribute to a more reliable disease risk assessment.

Keywords. Madagascar, amphibians, thermoregulation, seasonal fluctuations, diel cycle.

Introduction

Temperature shapes the performance of organisms (Pörtner, 2001; Little and Seebacher, 2016) and is therefore often considered as the most influential environmental factor in ectotherms (Angilletta et al., 2002; Angilletta and Angilletta, 2009; Beltrán et al., 2019). In amphibians, temperature is known to control growth and development (Ruthsatz et al., 2018), phenology (Ficetola and Maiorano, 2016), locomotion (Rome et al., 1992), behaviour (Navas and Bevier, 2001; Hauselberger and Alford, 2005), and overall survival. All these physiological functions have different temperature sensitivities and differ in their optimal temperature (Huey and Stevenson, 1979; Martin and Huey, 2008). Consequently, maintaining optimal body temperature (T_b) for task performance is important

for fitness (Kingsolver and Huey, 2008; Kingsolver et al., 2011; Mitchell and Bergmann, 2016).

To maintain optimal T_b and ensure good physiological performance, amphibians mainly rely on physiological (e.g., acclimation/acclimatization) and behavioural thermoregulation (e.g., basking, microhabitat selection) (Angilletta et al., 2002; Trochet et al., 2018). Whereas temperature preference is determined by genetic adaptation, life cycle stage, and body condition in ectothermic animals such as amphibians (reviewed by Rasolonjatovo et al., 2020), body temperature in these animals is strongly derived from their immediate environment. Most amphibians are considered as thermoconformers but there are examples of active thermoregulation, such as basking (Duellmann and Trueb, 1994; Stebbins and Cohen, 1995; Navas et al., 2008).

In contrast to other ectothermic vertebrates, amphibians are more limited in terms of thermoregulatory mechanisms since their thermal biology is characterized by a complex interrelationship between temperature regulation and skin morphology and colour, and concomitant gas exchange and evaporative water loss (Duellmann and Trueb, 1994; Navas et al. 2008; Centeno et al., 2015). Amphibians thermoregulate behaviourally by selecting favourable microhabitats (Vences et al., 2002; Huey et al., 2003; reviewed by Bodensteiner et al., 2020), which provide various thermal environments for basking, indirect heat gain, or cooling due to diel and

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seasonal cycles (Hutchison and Dupré, 1992; Centeno et al., 2015). Whereas aquatic habitats are known to be more stable during the seasonal and diel cycles, terrestrial habitats tend to be more variable in temperature (Woods et al., 2015; Sunday et al., 2014; Gunderson and Stillman, 2015). However, behavioural thermoregulation is limited in the majority of amphibians, since most species are nocturnal and their heat exchange is dominated by convection (Brattstrom, 1979; Feder and Lynch, 1982).

Temperature is not only one of the most influential environmental factors on amphibian physiology but also an important life history trait for pathogens (reviewed in Sonn et al., 2017), such as the chytrid fungus, *Batrachochytrium dendrobatidis* (Bd). Infection with Bd causes the disease chytridiomycosis and is in part responsible for dramatic global amphibian declines (Rohr et al., 2011; Scheele et al., 2014, 2019; Sauer et al., 2018). The relationship between ambient temperature and amphibian T_B as well as the ability to thermoregulate behaviourally have become a focus of attention since infections with Bd are known to accelerate when the T_B of an amphibian host matches the optimal growth temperature of Bd (Stevenson et al., 2013, 2020). Behavioural thermoregulation might especially help tropical amphibians to prevent infections, as it allows for frequenting microhabitats beyond the pathogen's optimal growth temperature (Woodhams et al., 2003; Rowley and Alford, 2013; Stevenson et al., 2013). However, an individual's ability to thermoregulate behaviourally, and thus the possibility of deliberately inducing behavioural fever when infected, depends on habitat temperature variation and the ability of individuals to select favourable microclimates (Beukema et al., 2021). A match between the optimal temperatures of the amphibian host and the pathogen, as is expected with increases in environmental temperature due to ongoing global climate change, may produce better conditions for the pathogen with little or no recourse for the amphibian and is consequently worrying. Evaluating the variation of field body temperatures can thus contribute to a preliminary disease risk assessment (Rasolonjatovo et al., 2020).

Bd has been recorded from wild amphibian communities on Madagascar (Kolby et al., 2014; Bletz et al., 2015a; reviewed by Monzon et al., 2020) and macroclimatic patterns on the island are suitable for the pathogen (Lötters et al., 2011). However, studies investigating T_B of wild Malagasy amphibians are still rare. A recent pilot study by Rasolonjatovo et al. (2020) assessed the T_B of *Mantidactylus bellyi* Mocquard, 1895, a frog species occurring in northern Madagascar, and found the T_B of these frogs closely correlated to environmental (substrate)

temperatures, and matching the optimal temperature growth of Bd.

Here, we evaluated the field T_B of 845 adult rainforest frogs from 38 species at two sites in Madagascar during the warm-wet and cool-dry season. The sampling comprised individuals from species with different habits (predominantly terrestrial, arboreal, or semi-aquatic). Independently of general preferences, we classified sampled individuals based on whether they had been captured in or near flowing water, non-flowing water, or in a non-aquatic habitat far from any water body. We aimed to identify predictors of T_B and differences in T_B and substrate temperature (T_{Subs}). Since environmental temperature varies seasonally, daily, and geographically along latitudinal and altitudinal clines (Feder and Lynch, 1982; Gvozdkik, 2002; Sunday et al., 2014; Trochet et al., 2018) and most amphibians are considered thermoconformers, we also expected the T_B of Malagasy rainforest frogs to vary with diel cycle and season. We further expected lotic aquatic habitats to be more stable in temperature than terrestrial and lentic aquatic habitats, which would suggest more variable T_B across those individuals collected in non-aquatic habitat or in pond water.

Material and Methods

Sampling. Fieldwork was carried out in two protected area of central Madagascar, the Analamazaotra Forest Station managed by the Mitsinjo Association (ca. 18.93°S, 48.44°E; elevation 964 m) and Ranomafana National Park (21.26°S, 47.41°E; elevation 1214 m) in November 2016 (both sites) and February 2017 (Analamazaotra). Whereas February is the peak of the warm rainy season, November represents the late cool dry season (Wollenberg et al., 2008; Bletz et al., 2015b). For logistical reasons, sampling effort per site and season differed, and therefore sample sizes cannot be standardized by sampling effort. However, general sampling methods were similar across sites and seasons. At both sites, we sampled amphibians for about 2–3 h during the day and at night by walking along trails encompassing habitat far from water bodies (non-aquatic; usually within rainforest), ponds, and streams. Whereas night sampling started always after sunset and not before 18:00 h, day sampling took place before the first signs of sunset.

At both study sites, diverse amphibian communities occur. These mostly comprise stream-breeding *Boophis* and *Mantidactylus* species but also various leaf-litter dwelling cophyline microhylids (*Plethodontohyla*, *Stumpffia*) and mantellids (*Gephyromantis*; Glaw and

Vences, 2007), in addition to a community of pond breeders. Frogs were located opportunistically either by locating calling males or spotting frogs with the aid of headlamps, on the ground, in lotic and lentic water bodies, and on perches in the vegetation up to a height of about 3 m above ground. As no frogs were collected from hiding places, and no arboreal frogs of nocturnal habits (e.g., genus *Boophis*) were collected while resting during the day, we consider all measurements to refer to active frogs (i.e., sampled while active). Body temperatures (T_B) of 845 frogs from 38 species were measured to the nearest 1°C at the dorsal body surface with an infrared laser thermometer (Benetech GM700 or GM900, Shenzhen Jumayuan Science and Technology Co. Shenzhen, China). Temperatures of the nearby substrate (T_{Subs}) directly in contact with the animal and air temperature (T_{Air}) were also measured to the nearest 0.1°C with a digital thermometer (Volkraft K101, Conrad Electronics, Hirschau, Germany; Rowley and Alford, 2007).

External skin temperature was used as an estimator for individual T_B (Berg et al., 2015), which is justified because the body size of the sampled species is rather small based on snout–vent length. A table of raw data in Excel format, including all original measurements, was deposited in Figshare and is available at <https://doi.org/10.6084/m9.figshare.14459988.v1>. In our analyses, we focus on substrate temperature as this is more ecologically relevant than air temperature.

Depending on the site and circumstance of observation, the encountered frogs were assigned to different habitat and substrate categories, as follows:

(1) Frogs were assigned to three habitat types based on the particular conditions where they were found, namely (i) flowing water (streams), (ii) non-flowing water (ponds), and (iii) non-aquatic habitat. Frogs were assigned to one of the two aquatic categories if they were either sitting directly in the water or within 100 cm of the water's edge. Frogs were assigned to the non-aquatic habitat category if they were not encountered near any water body, usually at estimated distances > 100 m, either in forest leaf litter or on perches in the vegetation. The rationale for assigning frogs in the vicinity of water bodies to aquatic categories is founded on their activity patterns, with frogs at the water's edge often having been inside the water shortly before being recorded. Sample size per habitat type was 406 individuals in or near flowing water, 250 in or near non-flowing waters, and 189 in non-aquatic habitat.

(2) Frogs were assigned to substrate categories depending on the specific substrate on which they were

sitting. In this categorization, we only distinguished two types of substrate, (i) aquatic (frogs sitting directly in the water) and (ii) non-aquatic (frogs sitting out of the water, irrespective of the distance from the next water body). Obviously, all frogs in the non-aquatic habitat category were also counted in the non-aquatic substrate category, whereas frogs from flowing or non-flowing habitat categories could be in either the aquatic or the non-aquatic substrate category. Sample size per substrate type counted 656 individuals in aquatic substrates and 189 in non-aquatic substrates. The substrate height (in metres) was measured for non-aquatic substrates.

Data analysis. All statistical tests were performed in R for Windows (version 4.0.3; R Core Team, 2020). All plots were constructed using *ggplot2* (Wickham, 2009) and Adobe Illustrator 2021. We calculated means, standard deviations, and variances of T_B and T_{Subs} . Before analysis, all dependent variables in the models were tested for autocorrelation using Spearman's rank correlation coefficient. 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 and Haworth, 1995; Chin, 1998). A sign test was applied to analyse the systematic difference between T_B and T_{Subs} of each individual.

Using the *stepAIC* function implemented in the MASS package (Venables and Ripley, 2002), we then conducted a stepwise model selection to test the influence of habitat type, diel cycle (night/day), T_{Subs} , season, substrate height, interaction between habitat type and diel cycle, and interaction between substrate and substrate height 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). Site and species were added to the models as nested random effects to control for phylogenetic effects due to niche partitioning. The analysis was carried out for the entire dataset ($n = 845$) to assess general differences between habitat types. A separate analysis was carried out for the Andasibe temperature records ($n = 497$) to investigate seasonal differences in T_B in these rainforest frogs. For the Andasibe data set, species were added as random effect to the models. The thermal variance of T_B was calculated for each habitat type and analysed with an ANOVA and post-hoc Tukey HSD test.

In accordance with Rasolonjatovo et al. (2020), we considered frogs from a habitat type to be thermoconformers when T_B strongly paralleled changes in T_{Subs} (i.e., indicating little or no behavioural thermoregulation). In contrast, a species was generally considered to be actively thermoregulating when T_B was maintained within a narrow range even though T_{Subs} significantly varied beyond the controlled T_B (Hutchison and Dupré, 1992). When modelled, the intensity of thermoregulation would be given by the slope (k) 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 and Slatkin, 1976; Angilletta and Angilletta, 2009).

We calculated linear regressions between body temperatures (°C) of Malagasy rainforest frogs and

substrate temperatures (°C; Fig. 1A, B). Differences in T_B between relevant categorical predictors from the models (i.e., habitat type, diel cycle, season, and substrate type) were analyzed by Mann–Whitney U test with Bonferroni correction (Fig. 1C, D).

Results

Temperature ranges of rainforest frogs, habitat type, and diel variances. Body temperatures of sampled frogs ranged from 9.3–33.5°C, with a mean \pm SD temperature of $18.2 \pm 3.2^\circ\text{C}$ across both sites and seasons (Fig. 1A). The lowest temperature was recorded in a frog in or near a flowing water body, whereas the highest temperature was measured in a frog in or near a non-flowing water body, both at Andasibe in November (Fig. 1A, C). T_B minima and maxima were lower at night, with a difference of

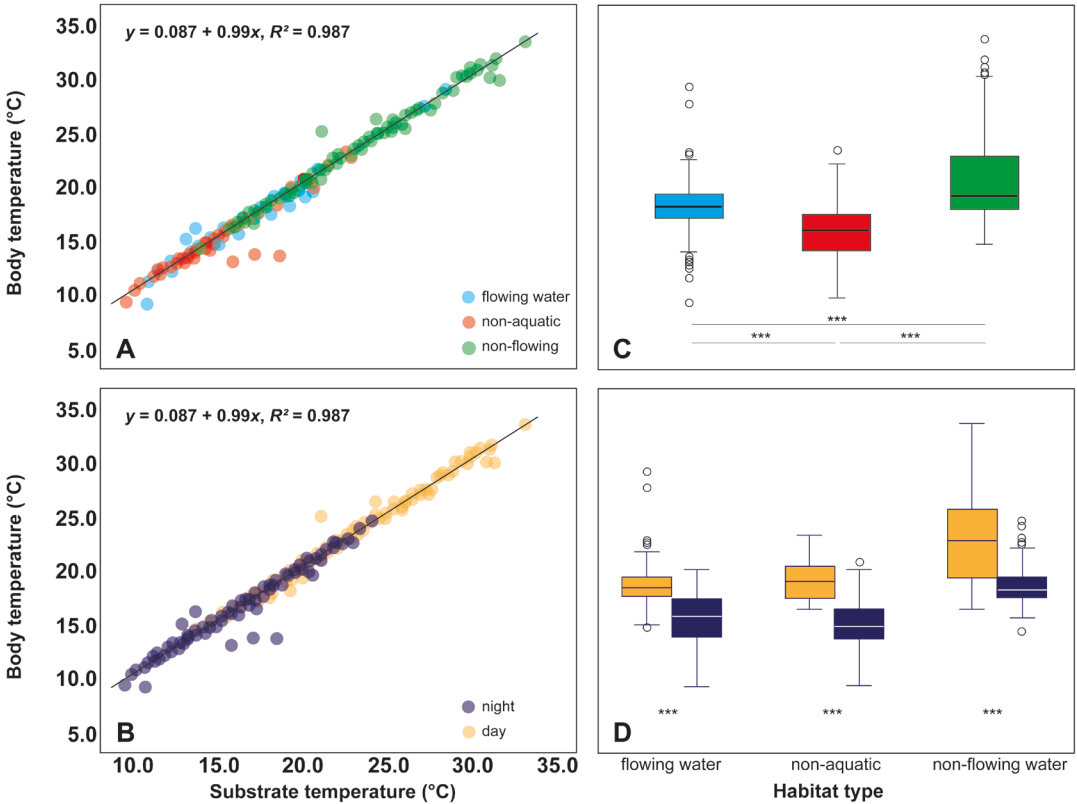


Figure 1. Graphic representations of the interaction between Malagasy rainforest frog body temperatures and substrate temperatures (°C). (A) Regression line indicating a strong, positive correlation between body and substrate temperatures in three different habitats, flowing water (blue circles), non-flowing water (green circles), and non-aquatic environments (red circles). (B) Regression line showing that the correlation persists throughout the 24-hour day, during daytime (orange circles) and at night (dark blue circles). (C) Body temperatures of frogs in three different habitat categories with the same colour-coding as in (A). (D) Body temperatures separated by diel cycle (i.e., day, night), using the same colour scheme as (B). Asterisks (*) indicate significant differences at $p < 0.001$ using a Mann–Whitney U Test with Bonferroni Correction.

5.5°C and 8.9°C, respectively (Fig. 1B, D). The overall variance in T_B was 10.6°C, and it was highest in non-flowing water habitats (14.1°C) and lowest in flowing water habitats (4.5°C) (Table 1; $p < 0.001$ between all groups). The variance of T_B at night was higher compared to that during the day in all flowing water and forest habitats but not in non-flowing water habitats (Table 1). Substrate temperature across all habitats was $18.2 \pm 3.2^\circ\text{C}$ and equal to that of sampled frogs. All measured frogs were thermoconformers (flowing water: $k = 0.98$; non-aquatic habitat: $k = 0.97$; non-flowing water: $k = 1.01$). The lowest and highest T_{Subs} were recorded in non-aquatic habitats (10.1°C) and in non-flowing water (31.7°C), respectively (Table 1). Variance of T_{Subs} was highest in non-flowing water (14.1°C) and lowest in flowing water (4.6°C). T_{Subs} minima and maxima were lower at night with a difference of 4.2°C and 9.1°C, respectively.

For the comparison of two seasons, we evaluated temperature records of a reduced dataset from Andasibe taken in February ($n = 471$) and November ($n = 26$) (Table 2). There were no temperature records at night in non-aquatic and non-flowing water habitats for November (Fig. 2C, D). Body temperatures of sampled frogs ranged between 9.5–31.7°C in February and 9.3–33.7°C in November. Variance of T_B was highest in November (36.7°C) and lowest in February (13.2°C). Frogs had the highest T_B in non-flowing water (16.1°) in

February, whereas T_B of frogs sampled in flowing water was highest in November (Fig. 2B, D). Temperature of substrate across all habitats was equal to the T_B of sampled frogs in both seasons.

Predictors of field body temperatures in Malagasy rainforest frogs. As all variable pairs were not highly correlated (i.e., $\rho < 0.6$), the predictive variables were all included in the initial models. The difference between T_B and T_{Subs} was significant (Sign test, $z = -3.65$, $n = 845$, $p < 0.001$; $T_B = 18.3 \pm 3.3^\circ\text{C}$; $T_{\text{Subs}} = 18.3 \pm 3.3^\circ\text{C}$). T_B was higher than T_{Subs} in 341 samples, whereas both temperature variables were equal in 253 samples. A Spearman correlation coefficient calculation confirmed highly significant positive correlations between T_B and T_{Subs} in general ($\rho = 0.992$) and both during day ($\rho = 0.994$) and night ($\rho = 0.982$; Fig. 1A, B).

All models that included substrate type, season, substrate height, and the interaction of substrate type and substrate height as well as the interaction between habitat type and diel cycle were excluded via backward elimination in a stepwise fashion. The final model only retained substrate temperature ($p < 0.001$), habitat type ($p < 0.001$) and the interactive effect of habitat type and diel cycle ($p = 0.029$) as significant predictors of T_B ($r^2 = 0.98$; Table 3). Diel cycle ($p = 0.382$) was also in the final model but had no significant effect on T_B . Body temperatures increased strongly with T_{Subs} (Table 3) and

Table 1. Body temperature (T_B) and substrate temperature (T_{Subs}) parameters (in °C) obtained in three habitat types from a diverse group of frogs in Madagascar. Included are ranges with means \pm standard deviations, variances (Var T_B , Var T_{Subs}), and Spearman’s correlation coefficient (ρ) for the correlations between T_B and T_{Subs} . All comparisons were significant at $p < 0.0001$.

Habitat	<i>n</i>	T_B	T_{Subs}	Var T_B	Var T_{Subs}	ρ
flowing water	total (406)	9.3–29.1 18.0 \pm 2.1	11.3–28.9 17.9 \pm 2.1	4.6	4.6	0.992
	day (317)	14.8–29.1 18.6 \pm 1.6	14.3–28.9 18.6 \pm 1.6	2.6	2.7	0.989
	night (89)	9.3–20.0 15.7 \pm 2.2	11.3–21.0 15.7 \pm 2.3	5.0	5.1	0.99
non-flowing water	total (250)	14.4–33.5 20.6 \pm 3.8	14.4–33.7 20.5 \pm 3.8	14.2	14.1	0.995
	day (112)	16.4–33.5 22.9 \pm 4.2	16.3–33.7 22.8 \pm 4.3	18.0	18.3	0.994
	night (138)	14.4–24.6 18.7 \pm 1.8	14.4–24.6 18.7 \pm 1.7	3.2	3.1	0.988
non-aquatic	total (189)	9.5–23.3 15.9 \pm 2.5	10.1–23.1 15.9 \pm 2.5	6.1	6.2	0.965
	day (36)	16.3–23.3 19.1 \pm 1.7	15.9–23.1 19.1 \pm 1.7	3.0	3.0	0.992
	night (153)	9.5–20.8 15.1 \pm 2.0	10.1–21.0 15.2 \pm 2.0	3.8	4.0	0.947

Table 2. Body temperature (T_B) and substrate temperature (T_{Subs}) parameters (in °C) measured in three habitat types listed by season for a diverse group of frogs in Andasibe, Madagascar. Included are ranges with means \pm standard deviations, variances (Var T_B , Var T_{Subs}), and Spearman’s correlation coefficient (ρ) for the correlations between T_B and T_{Subs} . The significance of the correlations is provided as a p -value. The bullet symbol (•) indicates fields for which no calculations were possible due to low sample size.

Habitat	<i>n</i>	T_B	T_{Subs}	Var T_B	Var T_{Subs}	ρ	<i>p</i>
FEBRUARY							
flowing water	total (117)	12.4–22.8 17.3 \pm 2.2	12.4–22.6 17.2 \pm 2.2	4.8	4.9	0.995	< 0.001
	day (94)	15.0–22.8 18.0 \pm 1.6	15.0–22.6 18.0 \pm 1.6	2.5	2.5	0.992	< 0.001
	night (23)	12.4–18.3 14.2 \pm 1.7	12.4–18.2 14.1 \pm 1.6	2.7	2.6	0.972	< 0.001
non-flowing water	total (232)	14.4–31.7 20.5 \pm 3.6	14.4–31.7 20.5 \pm 3.6	13.2	12.9	0.995	< 0.001
	day (94)	16.6–31.7 23.2 \pm 4.0	16.6–31.7 23.2 \pm 3.9	15.8	15.5	0.997	< 0.001
	night (138)	14.4–24.6 18.7 \pm 1.8	14.4–24.6 18.7 \pm 1.7	3.2	3.1	0.998	< 0.001
non-aquatic	total (122)	9.5–22.2 16.2 \pm 2.6	10.1–22.0 16.2 \pm 2.6	6.8	6.8	0.975	< 0.001
	day (35)	16.3–22.2 19.0 \pm 1.6	15.9–22.0 18.9 \pm 1.6	2.5	2.6	0.992	< 0.001
	night (87)	9.5–20.8 15.1 \pm 2.0	10.1–20.8 15.1 \pm 2.1	4.1	4.3	0.959	< 0.001
NOVEMBER							
flowing water	total (18)	9.3–19.8 14.8 \pm 2.4	11.3–21.0 14.9 \pm 2.4	5.8	5.9	0.990	< 0.001
	day (1)	15.4	15.1	•	•	•	•
	night (17)	9.3–19.8 14.8 \pm 2.4	11.3–21.0 14.9 \pm 2.4	6.2	6.2	0.989	< 0.001
non-flowing water	total (7)	19.7–33.5 25.9 \pm 5.3	19.7–33.7 25.9 \pm 6.1	28.5	37.4	0.929	0.003
	day (7)	19.7–33.5 25.9 \pm 5.3	19.7–33.7 25.9 \pm 6.1	28.5	37.4	0.929	0.003
	night (0)	•	•	•	•	•	•
non-aquatic	total (1)	23.3	23.1	•	•	•	•
	day (1)	23.3	21.3	•	•	•	•
	night (0)	•	•	•	•	•	•

differed between the three habitat types and during day and night (Fig. 1C, D; Table 3).

Frogs sampled in non-flowing waters revealed the highest T_B compared with flowing water frogs ($U = 33770.5$; $z = -7.2$; $n = 656$; $p < 0.001$) and frogs from non-aquatic habitat ($U = 8413.0$; $z = -11.5$; $n = 439$; $p < 0.001$; Fig. 1C). T_B in frogs sampled in non-aquatic habitat, however, was lower compared to those sampled in flowing water habitats ($U = 21488.5$; $z = -8.64$; $n = 595$; $p < 0.001$; Fig. 1C). At night, T_B differed between all habitats significantly (flowing waters vs. non-aquatic: $U = 5745.0$; $z = -2.02$; $n = 242$; $p = 0.043$; flowing waters vs. non flowing waters: $U = 1969.0$; $z = -8.63$; $n = 227$; $p < 0.001$; non-flowing waters vs. non-aquatic: $U = 1535.0$; $z = -12.58$; $n = 291$; $p < 0.001$; Fig. 1D), whereas T_B in non-aquatic frogs was not significantly different from those

frogs sampled in flowing waters (flowing waters vs. non-aquatic: $U = 462.0$; $z = -1.83$; $n = 353$; $p = 0.067$; non-flowing waters vs. non-aquatic: $U = 911.5$; $z = -4.97$; $n = 149$; $p < 0.001$; flowing waters vs. non flowing waters: $U = 6759.0$; $z = -9.83$; $n = 430$; $p < 0.001$; Fig. 1D).

Predictors of differences between field body temperatures of Malagasy rainforest frogs and substrate temperatures. When modelling the difference between T_B and T_{Subs} , the predictive variables substrate type, substrate height, and the interaction between habitat type and diel cycle were discarded throughout the stepwise regression procedure. The final model included the variables diel cycle ($p < 0.001$), habitat type ($p < 0.001$), season ($p = 0.060$), and the interaction of diel cycle and habitat type ($p < 0.001$) as relevant predictors of the difference between T_B and T_{Subs} . However, the

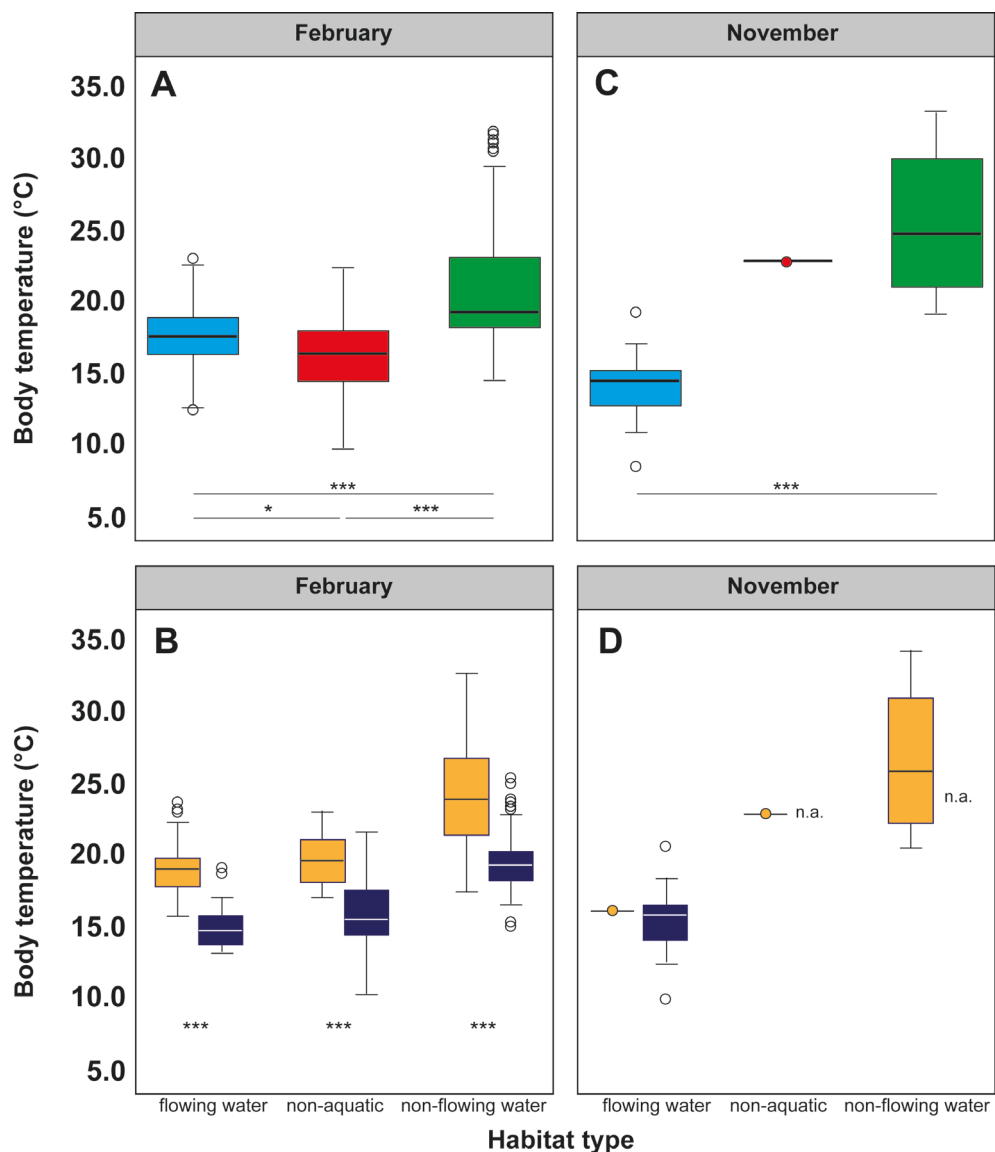


Figure 2. Seasonal differences in Malagasy rainforest frog body temperatures (°C) sampled in Andasibe in February (A, B) during the warm-wet season ($n = 471$) and in November (C, D) during the cool-dry season ($n = 26$) in or near different habitat categories (A, C) and separated by diel cycle (B, D). Colours indicate flowing water (light blue), non-aquatic habitat (red), in or near non-flowing water (green), daytime samples (orange) and night-time samples (dark blue). Asterisks indicate a significant difference at $p < 0.001$ based on a Mann-Whitney U Test with Bonferroni Correction.

coefficient of determination ($r^2 = 0.02$) was very low, indicating a low goodness of fit of this model.

Seasonal comparisons in field body temperatures of Malagasy rainforest frogs from Andasibe. Seasonal comparisons in temperature records between February and November in Andasibe were modelled based on a reduced data set of 497 samples (Table 3). All models that included substrate temperature, season, the interaction of substrate type and substrate height, and interaction between habitat type and diel cycle were excluded via backward elimination in a stepwise fashion. The final model only retained daytime ($p < 0.001$), habitat type ($p < 0.001$), substrate type ($p < 0.001$), and substrate height ($p = 0.023$) as significant predictors of T_B ($r^2 = 0.98$; Table 3). Consequently, T_B decreased significantly with increasing substrate height (Table 3).

Table 3. Retained multiple linear mixed models using the Akaike information criterion as a selection criterion, showing the influence of environmental parameters (substrate Temperature, T_{Subs}) on body temperature (T_{B}) of Malagasy rainforest frogs ($n = 845$) sampled at two sites, and at Andasibe ($n = 497$) during different seasons (February, November). Shown are the models and their predictors, with regression coefficients (RC), standard errors (SE), t -values, and the significance values of the predictors (p). While either model is significant in its own right, there are subtle differences among the predictors.

Model	Predictors	RC	SE	t	p
TWO SITES					
T_{B} T_{Subs} + daytime + habitat type + daytime : habitat type + (1 / site : species) Adjusted $r^2 = 0.987$, $p < 0.001$	(Intercept)	0.279	0.126	2.204	0.027
	Substrate temperature	0.983	0.004	226.32	< 0.001
	Daytime	−0.058	0.067	−0.873	0.382
	Habitat type	0.160	0.046	3.441	< 0.001
	Daytime: Habitat type	−0.069	0.031	−2.187	0.029
Diff daytime + habitat type + season + daytime : habitat type + (1 / site : species) Adjusted $r^2 = 0.022$, $p < 0.001$	(Intercept)	−0.303	0.097	−3.127	0.001
	Daytime	0.235	0.061	3.866	< 0.001
	Habitat type	0.183	0.043	4.218	< 0.001
	Season	0.053	0.028	1.882	0.060
	Daytime: Habitat type	−0.105	0.029	−3.602	< 0.001
ANDASIBE					
T_{B} daytime + habitat type + substrate type + substrate height + (1 / species) Adjusted $r^2 = 0.988$, $p < 0.001$	(Intercept)	12.866	0.768	16.743	< 0.001
	Daytime	−2.153	0.280	−7.675	< 0.001
	Habitat type	2.054	0.166	12.313	< 0.001
	Substrate type	4.253	0.511	8.324	< 0.001
	Substrate height	−0.117	0.051	−2.284	0.023
Diff daytime + habitat type + season + daytime : habitat type + (1 / species) Adjusted $r^2 = 0.073$, $p < 0.001$	(Intercept)	−0.458	0.139	−3.297	0.001
	Daytime	0.241	0.101	2.377	0.017
	Habitat type	0.188	0.061	3.056	< 0.001
	Season	0.212	0.064	3.311	< 0.001
	Daytime: Habitat type	−0.112	0.042	−2.639	0.008

Whereas T_{B} in frogs from flowing waters was significantly lower in November than in February ($U = 457.0$; $z = -3.85$; $n = 135$; $p < 0.001$), frogs from non-flowing waters revealed a significantly higher T_{B} in November ($U = 295.0$; $z = -2.86$; $n = 239$; $p = 0.004$; Fig. 2B).

When modelling the difference between T_{B} and T_{Subs} in the seasonal comparison, the predictive variables substrate type, substrate height, and the interaction between substrate type and substrate height were discarded throughout the stepwise regression procedure. The final model included the variables diel cycle ($p = 0.017$), habitat type ($p < 0.001$), season ($p = 0.060$), and the interaction of diel cycle and habitat type ($p = 0.008$) as relevant predictors of the difference between T_{B} and T_{Subs} in Andasibe. However, the coefficient of determination ($r^2 = 0.02$) was very low, indicating a low goodness of fit of this model.

Discussion

Measuring field body temperatures in amphibians is a valuable approach for assessing the relevance of thermal environment in the concept of niche conservatism (Kozak and Wiens, 2010), susceptibility to diseases (Lips et al., 2008; Muths et al., 2008) and climate change (Lowe, 2012). Here, frogs revealed a mean body temperature of $18.2 \pm 3.2^\circ\text{C}$ across both sites and seasons. These values fit within the T_{B} range of other amphibian species previously recorded in tropical regions (Feder and Lynch, 1982; Navas et al., 2013; Rasolonjatovo et al., 2020). Our key finding was that T_{B} of Malagasy rainforest frogs differed between habitat types and is to that effect correlated with ecology. We found that frogs from pond habitats have a higher T_{B} across seasons than those from stream or non-aquatic habitats. However, our results also highlight the need for more studies to evaluate the seasonal fluctuations of field body temperatures in rainforest frogs.

Field body temperature correlates with ecological niche in Malagasy rainforest frogs. In contrast to other ectothermic vertebrates, amphibians face a unique challenge because their skin offers little to no resistance to evaporative water loss (Shoemaker et al., 1992; Tracy and Christian, 2005; Navas et al., 2008). Behavioural thermoregulation in most amphibians is consequently limited by hydration state and depends on skin morphology. We found that frogs occupying different ecological niches show differences in field body temperatures. In contrast to pond- or stream-dwelling frogs, terrestrial or arboreal frogs are known to have a higher resistance to evaporative water loss (Wygoda, 1984; Tracy and Christian, 2005; Young et al., 2005; Mokhatla et al., 2019) allowing them to experience higher T_B and peak locomotory performance. Instead, aquatic or semi-aquatic frogs avoid evaporative water loss by surrounding water. However, their capability to frequent microclimates allowing them to behaviourally thermoregulate is often low if ponds are shallow. Here, frogs from non-flowing habitats (i.e., pond-dwelling frogs) revealed the highest T_B during both day and at night. Streams usually show a slightly lower (and more constant) water temperature than ponds and these differences were also found in frogs inhabiting these habitats. Our results confirm that both stream- and pond-dwelling frogs are thermoconformers. However, the difference between T_B and T_{Subs} as well as the variance of T_B were lower in stream-dwelling frogs, indicating that these can be considered as absolute thermoconformers with low intrapopulation variation compared to pond-dwelling frogs, which revealed the highest variance in T_B . While we have reduced sample sizes in non-flowing water habitats, variance of T_B was lower at night at these sites, possibly due to the absence of a sunlight gradient that facilitates behavioural thermoregulation and allows frogs to select microhabitats/microclimates (e.g., sun-exposed vs. shaded parts of a pond) with differing temperatures (e.g., Muñoz and Bodensteiner, 2019). Evidence suggests that nocturnal species show a reduced capacity for behavioural thermoregulation (Tracy and Christian, 2005; Beukema et al., 2021). Pond water might further act as thermoregulatory buffer at night, when temperatures of other substrates and air can drop (Vences et al., 2000, 2002). However, night T_B records were restricted to the Andasibe dataset.

In our study, we assigned habitat information to body temperature measurements based on the conditions under which each frog individual was found, rather than assigning frog species to ecological guilds. Most anuran

species in Madagascar can be seamlessly assigned to categories such as, arboreal, semi-aquatic or terrestrial based on their prevalent habitat use, or the site used for reproduction (e.g., Glaw and Vences, 2007; Bletz et al., 2017; Fig. 3). Of 406 measurements from frogs in or near flowing water, 324 correspond to species of *Mantidactylus* that would fall into the semi-aquatic ecological guild, while 236 out of 250 measurements of frogs in or near non-flowing water correspond to typical pond breeders of the genera *Aglyptodactylus*, *Blommersia*, *Guibemantis*, and *Ptychadena* (see raw data table available at <https://doi.org/10.6084/m9.figshare.14459988.v1>; Fig. 3). This suggests that the differences between habitats identified in this study may also translate into differences among species and among ecological guilds, but statistically comparing body temperatures across such guilds would require a thorough, representative sampling beyond the scope of the present study. For example, to fully understand the body temperature regime of arboreal frogs, it would be necessary to sample these resting on leaves in the canopy during the day, calling and foraging on branches at night, and reproducing in the water, considering the amount of time the individuals spend on average in each of these situations. We strongly encourage future studies that simultaneously quantify habitat use and body temperatures in Madagascar's anurans, if possible, combining these data with experimental thermophysiological assessments to determine their thermal performance and preferred temperatures.

The typical resistance to evaporative water loss in terrestrial or arboreal frogs allows them to inhabit a dehydrating habitat (Wygoda, 1984; Young et al., 2005; Tracy et al., 2010, 2014) and to regulate their T_B with less dependence on T_{Subs} by selecting favourable microhabitats to enhance performance. The lower mean body temperatures of frogs from higher substrate heights (i.e., arboreal frogs), found here in the Andasibe dataset, could either be a result of selecting lower temperatures in the height gradient or the result of greater heat loss due to evaporative cooling. Tracy et al. (2014) found that frog skin temperatures of arboreal frogs were lower than the temperature of the substrate because of evaporation. Surprisingly, we did not find a relationship between substrate height and T_B nor a difference in T_B and T_{Subs} in arboreal frogs, which left us without evidence for expected thermoregulatory behaviour, such as by selecting microclimates at different tree heights to facilitate evaporation. We hypothesize that this is due to a reduced sample size for arboreal frogs measured

during the day compared to samples at night where no sunlight gradient for microclimate selection is available (day: $n = 36$; night: $n = 153$).

Body temperatures of rainforest frogs fluctuate with season and are associated with habitat and substrate type. Because tropical climates typically exhibit much lower seasonal and daily thermal variability than

temperate climates (Janzen, 1967; Deutsch et al., 2008), the thermal regime of tropical amphibians, including field body temperatures, should be relatively stable. However, Wollenberg et al. (2008) found significant differences in substrate temperatures between the cool-dry and warm-wet season in Madagascar. Since our analyses confirmed that most amphibians are thermoconformers, field body

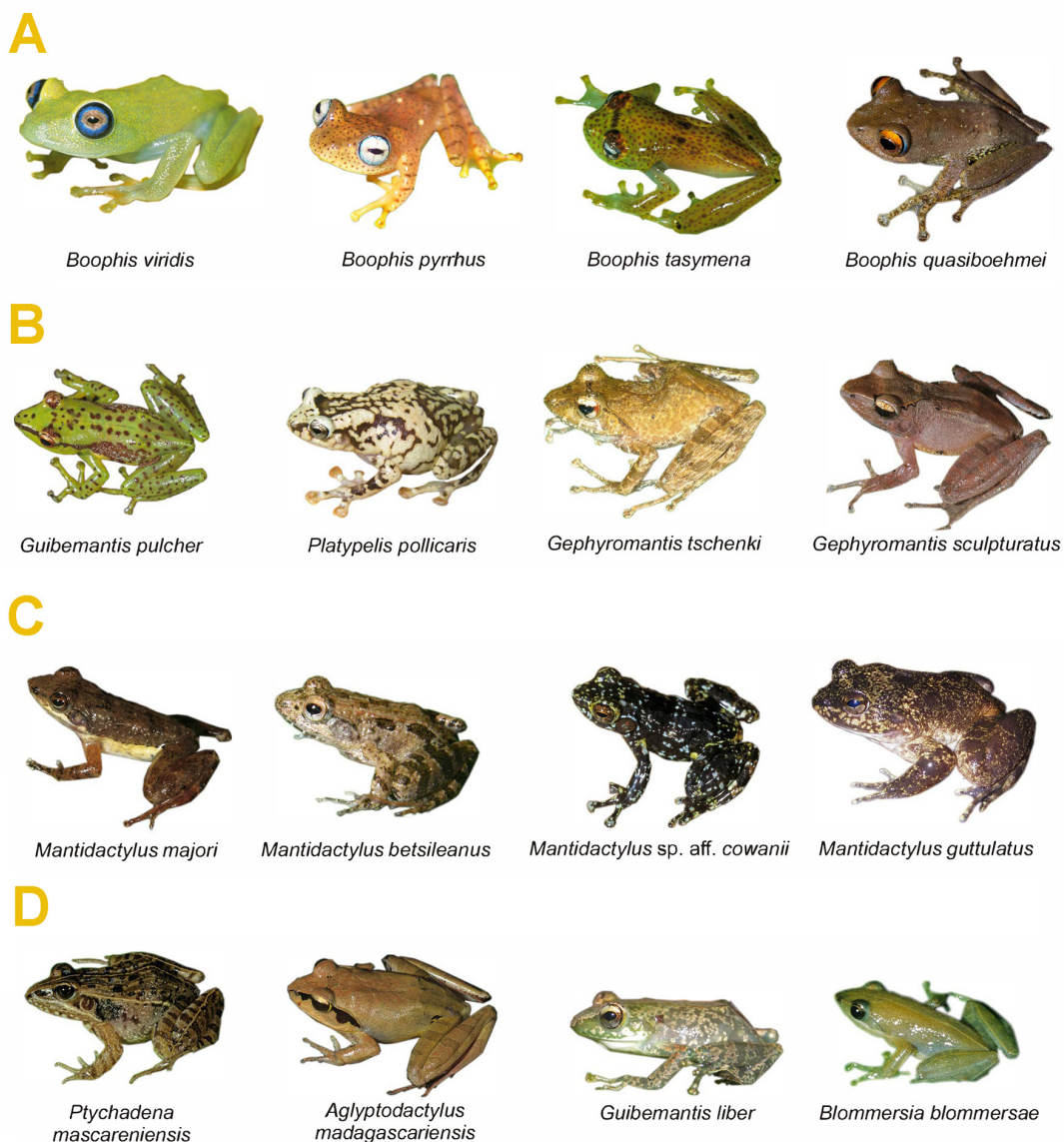


Figure 3. Examples of included Malagasy rainforest frog species categorized based on their proximity to water bodies. Note that for this study we did not categorize species, but individuals based on their proximity to water bodies and their habits (sitting in or directly near water, or in a terrestrial environment). (A) Species associated with running water but not encountered sitting in water. (B) Species typically not associated with water and not encountered in water. (C) Species associated with running water and sometimes encountered in water. (D) Species associated with standing water and sometimes encountered in water. The grouping in the plate reflects the tendency shown by most or many individuals of the illustrated species. Size of specimens not to scale.

temperatures of frogs might also vary with season in Andasibe. The present study is the first study comparing field body temperatures in tropical frogs using a multi-species approach to compare the cool-dry and warm-wet seasons in Madagascar. In agreement with the findings of Wollenberg and colleagues (2008), substrate type (i.e., aquatic vs. non-aquatic) was a relevant predictor for T_B . We found that mean T_B in pond- and stream-dwelling frogs (i.e., aquatic substrate) was significantly higher than T_B of frogs from non-aquatic habitats. For pond-dwelling frogs, we found T_B to be higher in November than in February. Instead, stream-dwelling frogs had a higher T_B in February. This pattern is confirmed by lower and higher air temperatures in November resulting in higher water temperatures of the ponds and a concomitantly higher T_B of resident frogs. Unfortunately, we lack the data to confirm this pattern for frogs from high substrate heights (i.e., arboreal frogs) but we expect the T_B to be higher in February in accordance with differences in air temperature. To confirm seasonal variation in T_B and T_{Subs} as well as the difference of both across habitats, future studies should be extended to a larger sample size in the peak of cool-dry season.

Conclusion

The severity of amphibian decline caused by chytridiomycosis is strongly associated with season, precipitation, and ambient temperature (e.g., Valencia-Aguilar et al., 2016; Scheele et al., 2019; Lambertini et al., 2021). Measuring field body temperatures in multiple amphibian species from different habitats might therefore contribute to a preliminary disease risk assessment for amphibian communities. We found that T_B of 38 frog species ranged between 9.3 and 33.5°C across habitats and diel cycle. Most of the encountered frogs had body temperatures matching the optimal temperature range for Bd growth (17–23°C; Stevenson et al., 2013; Sonn et al., 2017). Rasolonjatovo et al. (2020) confirmed this temperature range in a single-species approach with the Malagasy rainforest frog *M. belnyi*. Stevenson et al. (2020) found that thermoregulation may allow rainforest frogs to avoid Bd infections or to limit pathogen growth by selecting microhabitats with adverse conditions for Bd, but their own ecological niche may limit their ability to thermoregulate in the field. Since our key finding was that T_B differed between frogs sampled from non-aquatic, pond-, and stream habitats, we hypothesize that susceptibility to Bd might also differ between frog species specialized for such different habitats. Burrowes et al. (2017) found that Bd

prevalence was significantly reduced in arboreal frogs and hypothesized that differences in ambient temperature may play a role. In the present study, T_B of frogs sampled in Andasibe decreased with substrate height, suggesting a higher susceptibility to Bd infections in arboreal frogs occupying higher microhabitats. However, arboreal frogs are probably more likely to induce a so-called behavioural fever to cure a Bd infection by selecting microhabitats, and thus favourable microclimates, as they are known to be capable of withstanding evaporative water loss (e.g., Tracy and Christian, 2005; Young et al., 2005). In contrast, frogs from aquatic habitats are limited in their ability to thermoregulate behaviourally, and this may make them more susceptible to Bd infection. In the Neotropics, amphibian species inhabiting riparian habitats were found to decline more rapidly due to Bd infections than those found in terrestrial habitats (Lips et al., 2006). Also, Rowley and Alford (2007) showed that Bd infection was higher in Australian rainforest frogs frequently in contact with stream water. However, no further information is available on how Malagasy rainforest frogs might use behavioural fever to reduce or avoid Bd infection. Therefore, comparing the (realized) thermal niche of the frogs to that of Bd might be contributing to future disease risk assessment, but our results also highlight the need for more studies evaluating the seasonal fluctuations of field body temperatures in amphibians.

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