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Variation of body temperature of active amphibians along elevation gradients in eastern Nepal Himalaya

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ABSTRACT

Understanding the thermal ecology of active amphibians, as well as its relationship with habitat and environmental features, is a central theme in ecology. However, this topic has been poorly studied in eastern Himalaya, which is a global biodiversity hotspot. To bridge this gap, we investigated how the body temperatures of active amphibians varied along an elevation gradient in the Arun and Tamor River catchments in eastern Nepal Himalaya in the present study. Amphibian assemblages were sampled from May to July in both 2014 and 2015 using nocturnal time-constrained visual encounter surveys, and the body temperature of each individual was directly measured using a digital infrared thermometer in the field. A combination of linear regression and hierarchical partitioning analyses was used to determine the effects of elevation and environmental variables on the body temperatures of active amphibians. In total, the body temperatures of 599 amphibian individuals belonging to 28 species from six families were recorded. Our results indicated that amphibian body temperature exhibited monotonically declining trends with increasing elevations in eastern Nepal Himalaya. Interestingly, this tread was much more pronounced in subtropical (lowland) areas than in warm and cool temperate regions. Inter- and intraspecies variations in body temperature were large, which can be attributed to distinct habitat utilization among species and the change in vegetation cover in different bioclimatic zones. Among all environmental variables, substrate temperature and water temperature were the best predictors of the amphibian body temperature. Overall, this study revealed amphibian body temperature patterns along an elevation gradient in eastern Nepal Himalaya, which were principally driven by temperature-related environmental factors. We believe our results can provide important information on amphibian physiological traits, which may help ecologists predict their responses to future climate change and formulate protection strategies.

1. Introduction

Understanding amphibian body temperature patterns along an elevation gradient is one of the core questions in modern ecology. It has been widely recognized that body temperature can influence amphibian thermoregulatory systems, as well as their physiological and behavioral characteristics (Navas et al., 2008). Therefore, amphibians have their own temperature ranges that allow them to perform, disperse, and survive, which determines their potential geographic distribution (Angilletta, 2009). Accordingly, body temperature patterns provide important information for understanding amphibian vulnerability to climate change (in particular, the temperature rise) in recent decades (Deutsch et al., 2008). In addition, it is also significant to evaluate amphibian fitness based on the claims that warmer body temperatures, relative to environment, may grant individuals with elevated body temperature less susceptibility to fungal pathogens (Becker et al., 2016; Piovia-Scott et al., 2011; Rowley and Alford, 2013).

The range of activity temperatures of amphibians across the world is very large, and elevational gradients are excellent for testing relevant hypotheses in this context. This is especially true on tropical mountains, which support a high number of endemic amphibian species as well as high amphibian diversity (Navas et al., 2013). Moreover, numerous studies have demonstrated that these areas are more susceptible to climate change and extreme temperature events (Şekercioğlu et al., 2012). Although amphibian thermal ecology seems to be very important, little attention has been paid to amphibian body temperature

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patterns along an elevation gradient (but see Navas et al., 2013, Pintanel et al., 2019). This is especially true in eastern Nepal Himalaya, which is a global biodiversity hotspot (at least 29 amphibian species can be detected in this area, accounting for more than 50% of the total amphibian species in Nepal; Khatiwada et al., 2019). More importantly, eastern Nepal Himalaya has the longest elevation gradient in the world (60–8848 m asl), and it is regarded as one of the most vulnerable places to global climate change (La Sorte and Jetz, 2010; Zomer et al., 2014). All of these relevant contexts indicate that this region is an ideal system for studying the spatial patterns of amphibian body temperature.

Indeed, given their ectothermic condition and dominant small body size, most amphibians display body temperatures that are similar to macroclimate conditions, such as air, water, or substrate temperatures. One may thus expect that amphibian body temperature should exhibit a similar trend as air temperature, which decreases continuously with increasing elevation. However, since amphibians are poor dispersers, their body temperature also has a strong affinity for microhabitat features (Smith and Green, 2005). Recent studies have shown that the amphibian distribution can be determined by microhabitat (e.g., leaf litter cover, shrub cover, shrub density, and canopy cover) and microenvironmental features (e.g., air temperature, relative humidity, soil pH and temperature, water pH, water temperature, and substrate temperature; Khatiwada et al., 2019; Zhu et al., 2020), providing evidences that potential shelter, such as leaf litter, vegetation, rocks, and dead tree trunks, can also help amphibians regulate their body temperature. Therefore, habitat heterogeneity allows amphibians that live in forests to exhibit less thermal variation than individuals living in open environments (Navas et al., 2013; Pintanel et al., 2019). Consequently, we argue that the unidimensional approach cannot accurately address the spatial patterns of amphibian body temperature. Instead, both elevation and microhabitat environmental variables should be incorporated to reveal the factors determining amphibian body temperature on tropical mountains.

In the present study, we investigated the thermal ecology of active amphibians along an elevation gradient in eastern Nepal Himalaya. Based on previous studies, there is a tremendous variation in temperature along an elevation gradient (i.e., hot and humid weather at lower elevations, whereas freezing cold at higher elevations; Dobremez, 1976; Grytnes and Vetaas, 2002) in this area. The area can thus be divided into five distinct bioclimatic zonation along elevations (i.e., subtropical zone: below 1000 m; warm temperate zone: 1000–2000 m; cool temperate zone: 2000–3000 m, subalpine zone: 3000–4100 m; and alpine zone: above 4100 m; Dobremez, 1976). Specifically, we first explored how the body temperature of active amphibians varied along the whole elevation gradient we sampled. We then quantified the response of amphibian body temperature to elevations within each bioclimatic zonation (i.e., subtropical zone, warm temperate zone, and cool temperate zone; the subalpine zone and alpine zone were not included in the analyses as only five individuals of one species (*Scutiger ghunsa*) and no amphibians were detected in the two zones, respectively). After that, we tested the variation in body temperature of each species in the different bioclimatic zonation. In addition, we also revealed the environmental determinants of amphibian body temperature in eastern Nepal Himalaya. Based on previous studies (e.g., Navas et al., 2013; Rueda Solano et al., 2016), we predicted that amphibian body temperature would have a declining trend with increasing elevation in eastern Nepal Himalaya, and this tread would be stronger in subtropical zones and cool temperate zones because of the increase in habitat vegetation cover, and that the body temperatures of different species in each bioclimatic zonation would exhibit a distinct degree of variation. Microhabitat features such as water temperature, air temperature, mean annual temperature, mean annual precipitation, substrate temperature, canopy cover, shrub cover, and leaf litter cover were predicted to be equally important in determining amphibian body temperature.

2. Materials and methods

2.1. Study area

This study was conducted in the Arun and Tamor River catchments of eastern Nepal Himalaya (27.33805°–26.31893° N and 86.5994°–88.2133° E) along an elevation gradient from 78 to 4200 m asl (Fig. 1). In this area, the climate and vegetation undergo dramatic changes with increasing elevation. Specifically, the lowland subtropical forest has a hot and humid climate, while the alpine vegetation, which occurs in the higher elevation area, has a freezing cold climate (Dobremez, 1976; Grytnes and Vetaas, 2002). Rainfall is highly seasonal in the study area, and most of the precipitation can be received between June and September (monsoonal rainfall). However, short and occasional rainfall is common throughout the whole year.

2.2. Sampling design

Fieldwork was carried out from May to July in 2014 and 2015, coinciding with the rainy season and the breeding period of amphibians in Nepal (Khatiwada et al., 2016). In total, 79 transects (100 m × 4 m) were sampled after sunset (between 19:00 h and 23:00 h) using nocturnal time-constrained visual encounter surveys (Fig. 1), covering an altitudinal range from 78 m to 4200 m. All transects were randomly selected along the rugged mountain terrain and were placed near water sources if possible (three to five transects were sampled at each elevation band/locality). To reduce spatial autocorrelation, the transects were separated from each other by deep mountain gorges and streams. A detailed survey methodology has been provided in Khatiwada et al. (2019).

2.3. Environmental and microhabitat data

Nine explanatory variables (i.e., elevation, water temperature, substrate temperature, air temperature, canopy cover, shrub cover, leaf litter cover, mean annual precipitation, and mean annual temperature) were selected and measured in each transect based on their potential importance for the body temperatures of ectotherms, as shown in previous studies (Navas et al., 2013; Rueda Solano et al., 2016; Sanabria and Quiroga, 2019). Details of the measurement were as follows: we used an altimeter (a Sun altimeter) to record the elevation to the nearest meter. Air temperature, water temperature, and substrate temperature were measured using a mercury thermometer. These variables were measured in five locations (every 20 m from the starting point of each transect) in each transect, and the averaged values were used in the final data analyses. Canopy cover (%) was measured in five locations in each transect using a spherical densitometer (Forest Densitometers, Bartlesville, OK, U.S.A.). The measurements were conducted in four directions (N, S, E, W) at each location, and then we averaged the values (Lemmon, 1957). The shrub cover (%) and leaf litter cover (%) within each transect were estimated visually in five locations at each transect, and values were also averaged. We defined 0% shrub cover as all ground surface visible and 100% cover as no substrate visible. Similarly, 0% leaf litter cover was defined as all substrate visible, and 100% cover was defined as no substrate visible. The mean annual precipitation and mean annual temperature data were obtained from the WorldClim database (http://www.worldclim.org/) at a 30 arc sec (~1 km) spatial resolution (Hijmans et al., 2005).

2.4. Body temperature measurement

Amphibians were considered to be active if they were resting on the substrate (e.g., rock, floor, leaves or tree branches), waiting for the prey, or jumping when they were detected. The body temperature of each amphibian was taken from its skin, which is regarded as the most reliable method for amphibians (Navas et al., 2013). Once individuals were

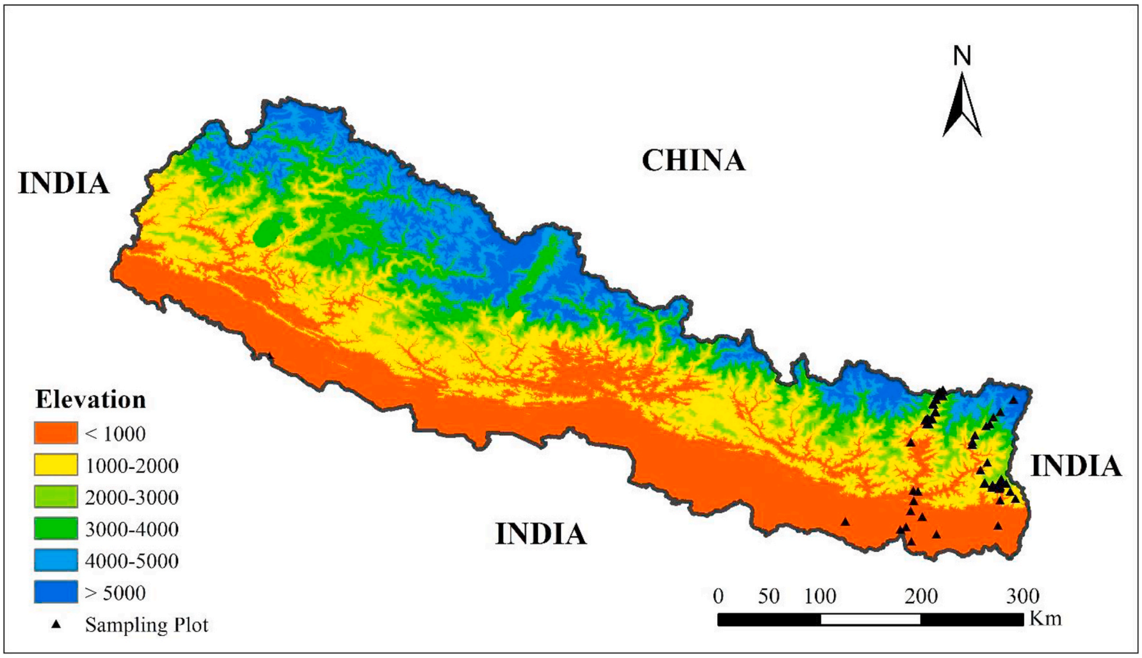

Fig. 1. The study area in eastern Nepal Himalaya. The triangles denote the transects/sampling sites selected for amphibian sampling.

detected visually or through auditory aids during the sampling events, their body temperatures were measured directly using an infrared thermometer (Oakton WD-39644-00) with the distance of the infrared sensor at an 11:1 distance-to-spot measurement ratio according to the manufacturer's instructions.

2.5. Data analyses

Linear regression analyses were first used to examine the relationships between body temperature and elevation, as well as the relationships between temperature-related environmental factors and elevation. The individuals of each species were pooled to calculate the overall coefficient of variation (CV) of body temperature for that species. When focused on each bioclimatic zone, individuals were first pooled together to calculate the overall CV of body temperature in each zone. The CV of body temperature for each species was then calculated to reveal their body temperature variation in different zones. Finally, hierarchical partitioning (Chevan and Sutherland, 1991; Mac Nally, 2002) was used to explore the relative contributions of each explanatory variable to amphibian body temperature. This method can be used to calculate goodness-of-fit measures according to all possible combinations of explanatory variables (Walsh and Mac Nally, 2013), and to identify the independent contribution of each explanatory variable (Mac Nally, 2002). Therefore, this method is highly suitable if multicollinearity issues exist among response variables (Olea et al., 2010). All statistical analyses were carried out in R v. 3.6.1 (R Development Core Team, 2019).

3. Results

The body temperatures of 599 individual amphibians belonging to 28 species from six families was recorded in total (Supplementary Material Table S1). We found that the amphibians in eastern Nepal Himalaya showed high variation in body temperature. The species with the highest body temperature was *Duttaphrynus melanostictus* (mean = 21.9 °C ± 3.7 SD, N = 105), and the species with the lowest body temperature was *S. ghunsa* (mean = 6.2 °C ± 0.23 SD, N = 5). All temperature-related variables showed linear declining trends along an elevation gradient (water temperature: $R^2 = 0.813$, $P = 0.001$; air temperature: $R^2 = 0.672$, $P = 0.00$; and substrate temperature: $R^2 = 0.851$, $P = 0.001$; Fig. 2).

3.1. Amphibian body temperature along an elevation gradient

Overall, the amphibian body temperature exhibited a monotonically declining trend with increasing elevation ($R^2 = 0.74$, $P < 0.001$; Fig. 3). At the family level, amphibian body temperature also showed an overall declining trend (Bufonidae: $R^2 = 0.749$, $P < 0.001$; Dicroglossidae: $R^2 = 0.797$, $P < 0.001$; Megophryidae: $R^2 = 0.545$, $P = 0.01$; Microhylidae: $R^2 = 0.807$, $P = 0.001$; Ranidae: $R^2 = 0.910$, $P = 0.248$ and Rhacophoridae: $R^2 = 0.450$, $P = 0.004$) with elevation (supplementary material Fig. A). However, at the species level (only considering the species with wide distribution ranges (>500 m) and more than 5 individuals), eight out of 15 species (*Amolops mahabharatensis*: $R^2 = 0.98$, $P < 0.001$; *Duttaphrynus himalayanus*: $R^2 = 0.63$, $P < 0.001$; *Duttaphrynus melanostictus*: $R^2 = 0.51$, $P < 0.001$; *Euphlyctis cyanophlyctis*: $R^2 = 0.58$, $P < 0.001$; *Fejervarya teraiensis*: $R^2 = 0.32$, $P < 0.001$; *Megophrys zhangi*: $R^2 = 0.32$, $P < 0.001$; *Microhyla nilphamariensis*: $R^2 = 0.93$, $P < 0.001$ and *Nanorana liebigii*: $R^2 = 0.43$, $P < 0.001$) showed significant declining trends with elevation (supplementary material Fig. B).

When analyzing data separately in three different bioclimatic zones, we found that amphibian body temperature also exhibited significant declines with increasing elevation in subtropical areas (range: 18.7 – 30.1 °C; mean = 24.8 °C ± 2.3 SD; $R^2 = 0.50$; $P < 0.001$), warm temperate areas (range: 11.7 – 26.7 °C; mean = 18.8 °C ± 3.5 SD; $R^2 = 0.17$; $P < 0.001$), and cool temperate zones (range: 9.9 – 22.6 °C; mean = 13.0 °C ± 3.0 SD; $R^2 = 0.37$, $P < 0.001$) (Fig. 4).

3.2. Amphibian body temperature variation

The highest CV value for body temperature was in the cool temperate zone (22.9%), followed by the warm temperature zone (18.7%) and subtropical zone (9.6%). Thirteen species exhibited CVs of body temperature of > 10%, and the top three species were *Megophrys robusta* (21.37%), *Megophrys zhangi* (20.75%), and *Polypedates himalayensis* (20.54%). The CVs of body temperature for some species were zero or were extremely low, such as for *Polypedates taeniatus* (0.00%), *Hylarana nigrovittata* (0.00%), and *Rhacophorus maximus* (0.31%). However, the same species in different bioclimatic zonation could have different CVs

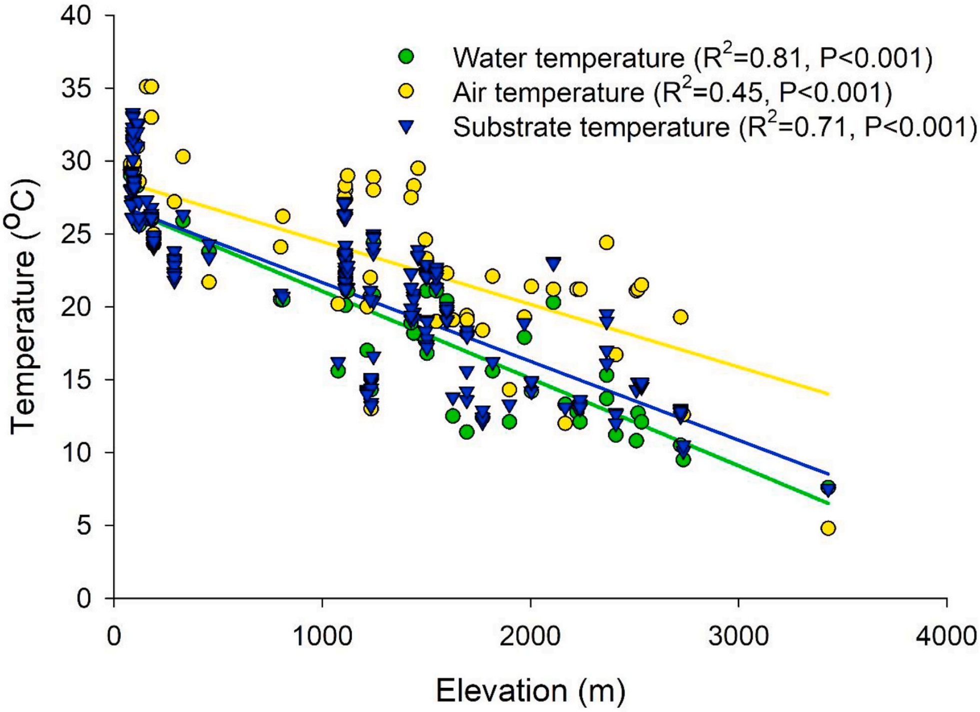

Fig. 2. Variation of water temperature, air temperature, and substrate temperature along elevation gradients in eastern Nepal Himalaya. Green circles refer to water temperature, yellow circles refer to air temperature and inverted blue triangles refer to substrate temperature.

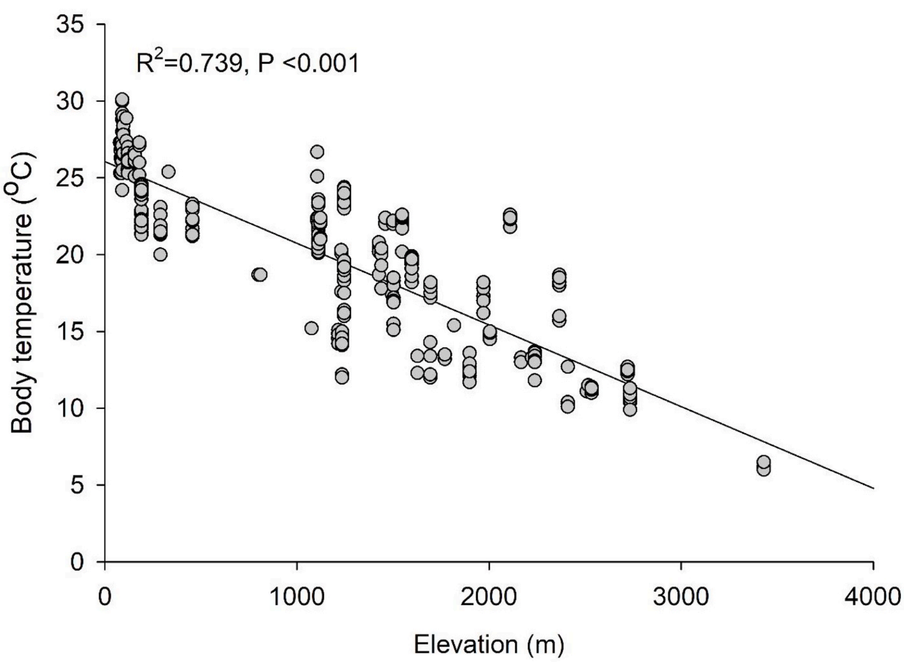

Fig. 3. Variation of active amphibian body temperature along elevation gradients in eastern Nepal Himalaya. The grey circles (N = 599) denote the body temperature of active amphibians.

of body temperature. For instance, the CVs of body temperature of *Duttaphrynus melanostictus* in subtropical, warm temperate, and cool temperature zones were 6.61%, 13.25%, and 1.63%, respectively (Table 1). In addition, different species in the same bioclimatic zones could also have distinct CVs of body temperature (subtropical zone: range = 0.00%–10.88%, mean = 4.49% ± 4.07%; warm temperature zone: range = 0.31%–20.36%, mean = 9.08% ± 7.27%; cool temperature zone: range = 0.64%–20.90%, mean = 7.17% ± 8.19%; Table 1).

3.3. Environmental factors determining amphibian body temperature

Hierarchical partitioning analyses indicated that substrate temperature and water temperature were the two most important variables determining amphibian body temperature (25.8% and 25.2%, respectively). The third and fourth important contributors were elevation and air temperature (17.0% and 15.0%, respectively). In contrast, shrub cover had the lowest effects on amphibian body temperature (0.2%;

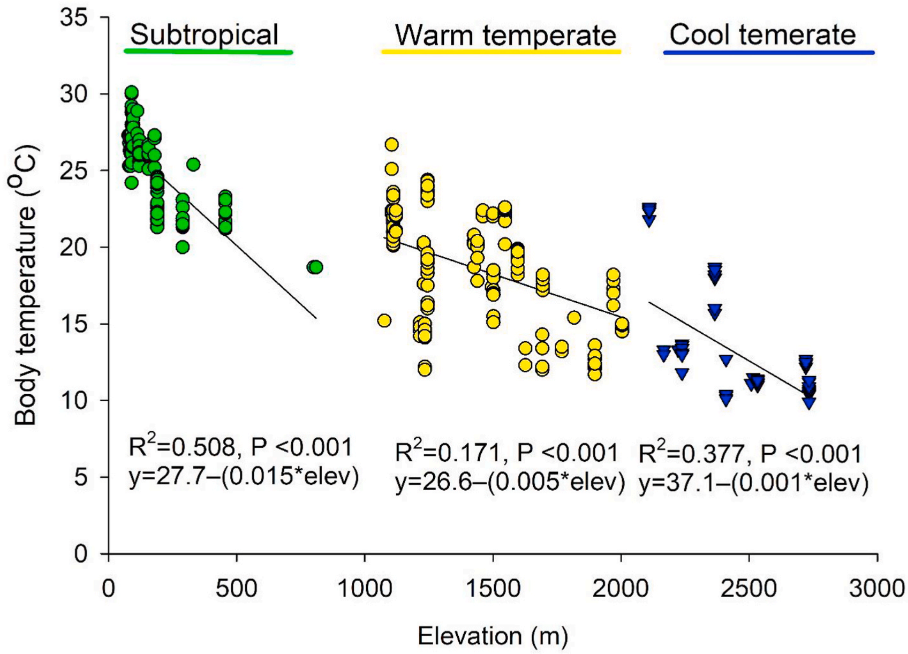

Fig. 4. Variation of amphibian body temperature along elevation gradients in different bioclimatic zones in eastern Nepal Himalaya. The green circles refer to the individual recorded in the subtropical zone, yellow circles refer to the warm temperate and inverted blue triangles refer to cool temperate zone.

Table 1
Coefficient of variation of each species body temperature across the whole sampling sites, in the subtropical zone, in the warm temperature zone, in the cool temperature zone, and in the sub-alpine zone, respectively. Number of the individuals have been provided in the brackets.

Family	Species	Overall	Subtropical	Warm temperate	Cool temperate	Sub-alpine
Bufonidae	*Duttaphrynus himalayanus*	14.41% (35)	– (0)	5.83% (8)	3.06% (27)	– (0)
	Duttaphrynus melanostictus	16.97% (105)	6.61% (24)	13.25% (75)	1.63% (6)	– (0)
	Duttaphrynus stomaticus	10.74% (27)	10.74% (27)	– (0)	– (0)	– (0)
Dicroglossidae	*Euphlyctis cyanophlyctis*	16.26% (103)	8.71% (75)	18.09% (28)	– (0)	– (0)
	Fejervarya nepalensis	18.81% (32)	0.00% (2)	19.45% (30)	– (0)	– (0)
	Fejervarya syhadrensis	11.02% (5)	– (1)	6.99% (4)	– (0)	– (0)
	Fejervarya teraiensis	11.50% (39)	10.88% (27)	9.84% (12)	– (0)	– (0)
	Hoplobatrachus crassus	2.60% (15)	2.60% (15)	– (0)	– (0)	– (0)
	Hoplobatrachus tigerinus	5.01% (6)	6.34% (4)	1.79% (2)	– (0)	– (0)
	Nanorana liebigii	14.29% (50)	– (0)	19.10% (11)	3.51% (39)	– (0)
	Ombrana sikimensis	1.85% (2)	– (0)	1.85% (2)	– (0)	– (0)
	Sphaerotheca maskeyi	– (1)	– (1)	– (0)	– (0)	– (0)
	Sphaerotheca swani	11.49% (8)	2.64% (5)	0.78% (3)	– (0)	– (0)
Megophryidae	*Megophrys monticola*	1.31% (4)	– (0)	– (0)	1.31% (4)	– (0)
	Megophrys robusta	21.37% (8)	– (0)	3.26% (7)	– (1)	– (0)
	Megophrys zhangi	20.75% (36)	– (0)	16.47% (26)	19.12% (10)	– (0)
	Scutiger ghunsa	3.68% (5)	– (0)	– (0)	– (0)	3.68% (5)
Microhylidae	*Microhyla nilphamariensis*	6.90% (26)	2.41% (25)	– (1)	– (0)	– (0)
	Microhyla taraiensis	– (1)	– (1)	– (0)	– (0)	– (0)
	Uperodon globulosus	– (1)	– (1)	– (0)	– (0)	– (0)
	Uperodon taprobanica	1.88% (3)	1.88% (3)	– (0)	– (0)	– (0)
Ranidae	*Amolops formosus*	5.34% (3)	– (0)	– (1)	0.64% (2)	– (0)
	Amolops mahabharatensis	13.75% (14)	0.20% (4)	2.48% (10)	– (0)	– (0)
	Hylarana nigrovittata	0.00% (3)	0.00% (3)	– (0)	– (0)	– (0)
Rhacophoridae	*Polypedates himalayensis*	20.54% (38)	– (0)	20.36% (34)	20.90% (4)	– (0)
	Polypedates taeniatus	0.00% (8)	0.00% (8)	– (0)	– (0)	– (0)
	Polypedates teraiensis	8.76% (19)	9.89% (13)	5.42% (6)	– (0)	– (0)
	Rhacophorus maximus	0.31% (2)	– (0)	0.31% (2)	– (0)	– (0)
Average	–	9.58%	4.49%	9.08%	7.17%	3.68%
SD	–	7.01%	4.07%	7.27%	8.19%	0

Fig. 5).

4. Discussion

Amphibians are widely distributed in eastern Nepal Himalaya. They inhabit a variety of ecosystems and provide important ecosystem services for local human societies (Khatiwada et al., 2016). In recent years, studies have increasingly started to focus on the taxa in this area in terms of taxonomy (Khatiwada et al., 2015, 2017), community (Khatiwada et al., 2019), and distribution modeling (Subba et al., 2018). However, little attention has been paid to amphibian thermal ecology in this area. In the present study, we bridged this gap and showed for the first time that amphibian body temperature decreased continuously along an elevation gradient in eastern Nepal Himalaya, which was principally

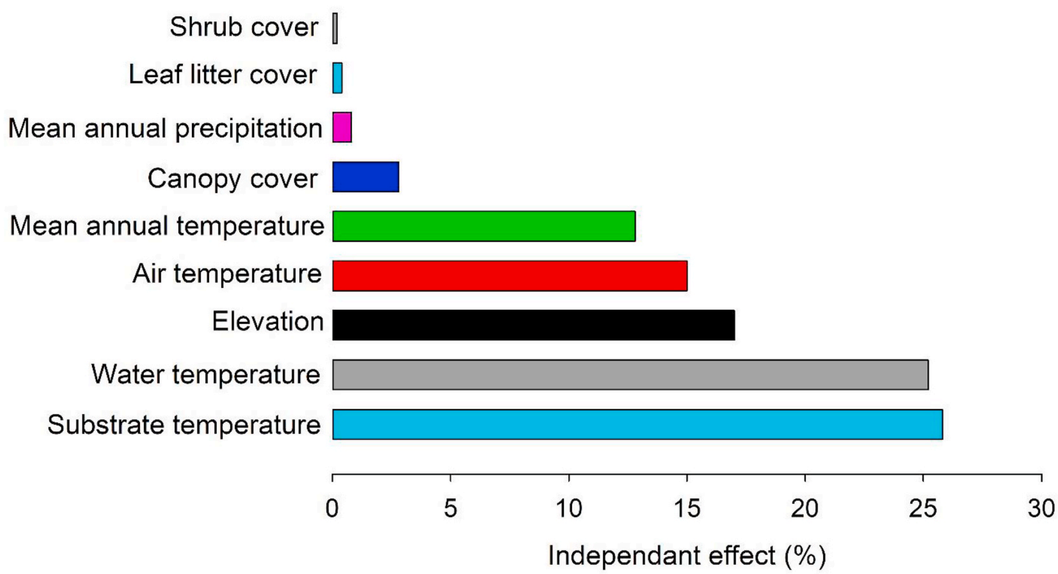

Fig. 5. Results of the hierarchical partitioning analyses showing the independent contribution of each environmental variable to the variation of body temperature of active amphibians.

driven by microhabitat environmental variables, such as substrate temperature and water temperature.

Overall, our results were consistent with those of previous studies from the tropical Andes and showed that amphibian body temperature declined significantly along an elevation gradient in eastern Nepal Himalaya. This trend was similar to the trends of the temperature-related variables exhibited with increasing elevations, which supported the claims that amphibian body temperature was strongly correlated with surrounding temperature factors (Rozen-Rechels et al., 2019). This is not surprising; as ectotherms, amphibians largely regulate their body temperatures depending on the thermal conditions of microhabitats (Angilletta, 2009). Therefore, it is difficult for amphibians to maintain normal metabolisms if environmental temperature raises beyond their thermal breadths (caused by climate change, for instance), which increases some individual or even population-level effects (Botts et al., 2013). However, at the species level, only eight species exhibited significant decreasing relationships with increasing elevation. This is because these species were elevation generalists with wider thermal tolerance ranges and were present in wide elevation distribution ranges (e.g., *Duttaphrynus melanostictus*, distributed from 78 to 2366 m). In addition, exceptional species-specific cases were also detected in both our study and the study from the tropical Andes, which indicated no significant relationships between body temperature and elevation gradient. Therefore, our study provided valuable results to infer the general patterns of thermal ecology for amphibians on mountains. These species were usually elevation specialists with very narrow thermal tolerance ranges. Therefore, these species should be more easily affected by climate change (i.e., temperature rise). This is especially true for species distributed in lowland areas, as most of them live in areas where the environmental temperature from May to September is close to their maximum thermal limits. Short-term or long-term thermal stress can profoundly reduce the fitness and survival of these species if maximum daily temperatures reach their critical thermal maximum (Nowakowski et al., 2015). In contrast, amphibian species at higher elevations may favor the increasing temperature caused by climate change, as previous studies have indicated that individuals living in colder areas were easily infected by chytrid fungus (Becker et al., 2016; Piovia-Scott et al., 2011; Rowley and Alford, 2013).

Variations in amphibian body temperature were high in all three bioclimatic zones. However, the degree of the variation was different. Our results showed that the highest CV value of body temperature was detected in the cool temperature zone, followed by that in the temperate zone and that in the subtropical zone. In Nepal Himalaya, the lowland areas (i.e., subtropical zone) are occupied by cultivated or managed land (Uddin et al., 2015), providing little shelter for amphibians. Therefore, the amphibians in this zone exhibited relatively low variation in body temperature. However, habitat heterogeneity increased with increasing elevation gradient. In high-elevation areas, considerable contrasts in vegetation cover may occur in the adjacent areas even at similar elevations because of the local climate, terrain type, and other geological/ecological factors (Seebacher and Alford, 2002). Consequently, the species in this zone may have experienced differing natural selection for thermal physiology (and may even display contrasting thermal strategies) as they can live in different areas dominated by specific biomes (Navas et al., 2013). A similar pattern was observed when focusing on intraspecies body temperature variation. Most of the species had higher variation in the warm temperature zone than in the subtropical and cool temperature zones, which was also attributed to changes in the habitat environment. When focusing on each zone, the interspecies body temperature variation was also large. Species with large body temperature variation were usually widely distributed in habitats with variable vegetation cover (e.g., *Polypedates himalayensis* was captured in forests, on rocks, and on shrubs during the sample events). Alternatively, species with low variation in body temperature may have some specific ecological or behavioral types of buffer. For instance, no body temperature variation was observed in *Polypedates taeniatus* in the subtropical zone. This is because this species was hidden only at the base of the tall *Typha angustifolia* reeds, in which the environmental (substrate) temperature was stable (Kaushik et al., 2018).

Hierarchical partitioning analyses showed that substrate temperature and water temperature were the two most important factors determining amphibian body temperature in eastern Nepal Himalaya. Previous studies have indicated that habitat thermal variation determines amphibian physiological barriers on mountains (Pintanel et al., 2019), where substrates and water-related environmental factors play more important roles in structuring the distribution of amphibian species (Khatiwada et al., 2019; Zhu et al., 2020). We thus suggested that microhabitat climates rather than macroclimates drive the mechanism of amphibian body temperature variation in eastern Nepal Himalaya. More importantly, this mechanism can help amphibians withstand thermal shocks caused by climate change, supporting the claims by previous studies that microhabitat features are important for amphibian conservation (Rittenhouse et al., 2008; Hossack et al., 2009).

In conclusion, the present study suggested that amphibian body temperature decreased continuously along an elevation gradient in eastern Nepal Himalaya. However, body temperature did not exhibit significant relationships with elevation for all species (especially elevational specialists). We believe that the spatial patterns of amphibian thermal ecology can provide the basic physiological traits of amphibians and provide important information for predicting the responses of each species to future climate change. Accordingly, different conservation strategies should be conducted for amphibian species in the near future. In addition, since amphibian body temperature is strongly determined by temperature-related microhabitat variables, such as substrate temperature and water temperature, microhabitat conservation should also be incorporated in amphibian conservation.

Ethics

All amphibians handling and processing were in accordance with the guidelines of the Department of National Park and Wildlife Conservation, Nepal.

Author contributions

JRK conceived the ideas, conducted the field work, and collected the data; JRK and TZ analyzed and interpreted the data; JRK and TZ drafted the manuscript. All authors approved the final version of the manuscript.

Declaration of competing interest

The authors declare no competing interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2020.102653.

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