# amphibian species richness and composition along an elevational gradient in chitwan, nepal

janak raj khatiwada

NORWEGIAN UNIVERSITY OF LIFE SCIENCES
Department of ecology and natural resource management
Master Thesis 60 credits 2011

# AMPHIBIAN SPECIES RICHNESS AND COMPOSITION ALONG AN ELEVATIONAL GRADIENT IN CHITWAN, NEPAL

Photo: Six-lined tree frog (*Polypedates taeniatus*)

Janak Raj Khatiwada

**Supervisor**

Torbjørn Haugaasen, PhD

Associate professor

Norwegian University of Life Sciences

# Acknowledgements

I would like to thank my supervisor Torbjørn Haugaasen, Associate professor, Norwegian University of Life Sciences, for providing guidance and support throughout the study period. His valuable supervision and feedback helped me to produce final thesis in this shape.

I am grateful to Department of Ecology and Natural Resource Management, Tropical Ecology of Norwegian University of Life Sciences (UMB) for providing travel grant to carry out this study. I am very much thankful to Columbus Zoo and Aquarium for financial support.

I am thankful to Dr. Khem Raj Bhattrai, Dilli Rijal and Lila Nath Sharma for their valuable guidance on survey method design. I thankfully acknowledge to Prof. Karan Bahadur Shah, Natural History Museum, Tribhuvan University, Soyambhu, Kathmandu, Nepal for the identification of collected specimens.

Thank goes to Devendra Paudel and Tilak Giri for invaluable field assistance. I am thankful to Bhakta Bahadur Shrestha and Nirmal Mani Dahal provided comments and support in several aspects of statistical analysis.

I am especially thankful to my wife Shanta, son Aayam and all family members for their encouragements and support throughout my study.

Sincerely,

Janak Raj Khatiwada

1430, Ås

# Abstract

Amphibian assemblages were studied along an elevational gradient from 200-1500masl in a degraded landscape in Chitwan, Nepal. The study aimed to assess patterns of amphibian species richness on the southern and northern slopes of Siraichuli hill using nocturnal and diurnal survey techniques. The effects of environmental variables (altitude, soil temperature, soil moisture, RRI and disturbance level) on amphibian species richness and composition were also explored. Additionally, a comparison between diurnal and nocturnal surveys is presented. A total of 17 species from four families were recorded during the study period. Linear regression showed a declining trend in observed amphibian species richness with increasing elevation on both the southern and northern slopes across both nocturnal and diurnal surveys. Nocturnal surveys detected a greater number of species compared to diurnal surveys. Altitude, soil moisture and RRI index had a combined effect on the observed pattern of species richness on the southern slope, whereas altitude alone was retained as the significant effect on the northern slope. Disturbance negatively impacted species richness on both slopes. More than 55% of species were recorded in the lower sampling sites (below 600m) indicating more favorable climatic conditions for amphibian assemblages at lower elevations. I conclude that amphibian portrayed narrow distribution ranges along the elevation gradients and along with altitude, soil moisture and disturbance also played significant role in the distribution pattern.

**Key words:** Elevation gradient, Amphibians, Species richness, Survey methods, Chitwan

# Introduction

Altitudinal gradients and the physical environment are prime factors that determine spatial and temporal distribution, abundance and richness patterns of organisms (Korner 2000; Nagy & Grabherr 2009). Many studies have demonstrated a relationship between species richness and elevation in a variety of taxa in different geographic locations (Grau et al. 2007; McCain 2005; McCain 2007a). Altitudinal variability causes variation in climatic, biological and geographical conditions, which ultimately affect species richness patterns (Lomolino 2001; Rahbek 1995; Rosenzweig 1995; Whittaker et al. 2001). Mountains are therefore an ideal place for researchers exploring ecological and evolutionary processes (Körner 2007). Patterns of species richness along elevational gradients generally follow three widely accepted trends: monotonic decline, mid-elevation peak or increase with elevation (Rahbek 1995). Rahbek (1995, 2005) report monotonic declines and mid-elevation peaks as the most common patterns (25% and 50% of studies examined, respectively). Fewer species towards the top of the gradient and a greater number of species at or around mid-elevation sites, is the most common pattern now documented (Colwell & Lees 2000; Colwell et al. 2004; McCain 2004; Stevens 1992; Zapata et al. 2003). This pattern appear to be relatively ubiquitous across the world and across several different taxonomic groups, such as plants (Bhattarai et al. 2004; Grau et al. 2007; Grytnes & Vetaas 2002; Klimes 2003; Oommen & Shanker 2005; Sánchez González & López Mata 2005; Vetaas & Grytnes 2002; Wang et al. 2003), mammals (Li et al. 2003; McCain 2004; McCain 2005; McCain 2007b; Mena & Vázquez Domínguez 2005), birds (Blake & Loiselle 2000; Kattan & Franco 2004; Lee et al. 2004; McCain 2009), herpetofauna (Chettri et al. 2010; Fu et al. 2007; Hofer et al. 1999; Hu et al. 2011; McCain 2010; Navas 2006) and invertebrates (Escobar et al. 2005; Sanders et al. 2003; Wilson et al. 2007).

Numerous hypotheses have been proposed to explain the patterns of species richness along altitudinal gradients. For example, Colwell & Hurtt (1994) and Colwell & Lees (2000) explain the mid-elevation peaks in species richness through the so-called mid-domain effects, where the high species richness is due to the maximum overlap of species towards the centre domain of a mountain. This is because the elevational range of a species is restricted by the highest and lowest elevations, called hard boundaries or geometrict constrains. The geometric constraint or mid-domain effect has been recognised as an important factor in shaping the unimodal pattern of species richness along an elevational gradient (Brown 2001; Colwell & Lees 2000). On the other hand, Rahbek (1995) has emphasized sampling methods and size of the study area. It is plausible that many differences in richness patterns occur because of differences in sampling effort and lack of standardization of sampling plots (Lomolino 2001). The ongoing debate about inconsistencies in diversity patterns is very often related to the lack of consensus about sampling protocols and scale effects (Grytnes & Vetaas 2002; Nogués-Bravo et al. 2008; Rahbek 1995). This is important since information about species distribution patterns across elevation gradients would provide invaluable and necessary information for the formulation of guidelines, management applications and setting conservation priorities in biodiversity conservation (Hunter Jr & Yonzon 1993; Vetaas & Grytnes 2002).

Plants are the most well studied taxonomic group in the Himalayas (Acharya et al. 2011; Acharya et al. 2008; Bhattarai & Vetaas 2003; Bhattarai et al. 2004; Grau et al. 2007; Grytnes & Vetaas 2002; Oommen & Shanker 2005). Contrastingly, the distribution pattern of amphibians along the altitudinal gradient in Nepal and elsewhere has rarely been investigated. Nevertheless, existing studies show a mixed pattern of species richness. Monotonic decline of richness with elevation is apparently the most common pattern (Hu et al. 2011). However, Naniwadekar & Vasudevan (2007) reported a linear increase of anuran species that peaked near the mountain summit (1100m) and Fu et al. (2006) found that frog species peaked at mid-elevation in the Hengduan Mountains, China.

The major goal of this study was to explore the patterns of amphibian species richness along the altitudinal gradient of Chitwan, Nepal. The province of Chitwan lies in central Nepal and it is widely recognized that its unique and valuable ecosystems are rapidly disappearing due to agricultural expansion after the eradication of malaria from lowland terai (Baral & Heinen 2007). Amphibians were sampled from the southern and northern slopes of Siraichuli hill using diurnal and nocturnal survey methods. Amphibian richness, abundance and composition patterns of the southern and northern slopes were explored and I particularly addressed the following questions: 1) Does amphibian species richness decline with increasing elevations? 2) How does species composition vary between low, mid- and high-elevational sites? 3) Do species richness, abundance and composition vary between the southern and northern slopes? 4) What is the most efficient way to assess amphibian species richness and diversity along an altitudinal gradient? 5) Is species composition and species richness affected by human disturbance?

## Materials and Methods

### Study Area

The current study took place in the Northern part of Chitwan district, Mahabharat hill. The area lies in the Sub-Himalayan mountain range of central Nepal (27˚45'N to 27˚31'E; Figure, 1). The study area is called Siraichuli hill and encompasses an elevation range of 200-1600 meter above sea level (m.a.s.l). Amphibian surveys were conducted on the southern and northern slopes of Siraichuli hill. The southern slope is connected to the Barandabhar Community Forest, the Buffer Zone area of Chitwan National Park and under the jurisdiction of the Shaktikhor Village Development Committee. The Northern slope ends by the Trisuli River and is under the jurisdiction of the Kaule Village Development Committee. The southern slope is drained by the Kayara Khola (stream) and the Northern slope is drained by the Hugdi Khola (stream). The natural vegetation mainly consists of Sal (*Shorea robusta)* forest below 1000 m. The Sal forest is replaced by broadleaved forests dominated by *Schima wallichii* and *Castanopsis indica* above 1000 m. The subtropical climate predominates below 1000m. January is the coldest month ($2^{o}$C-$22^{o}$C) and the warmest months are April and May. Monsoonal rainfall occurs between June-September. The area receives the majority of annual rainfall during the monsoon. The average annual rainfall for 2002-2007 was 2400 mm for the nearby Bharatpur Airport (Department of Hydrology and Meteorology, Government of Nepal, 2002-2007). Monsoon rains cause dramatic floods and changes the character and courses of local rivers and streams (Laurie 1978). Temperate climate prevails above 1000 m (Bhattarai et al. 2004). This area falls within the zone of temperate monsoon climate where approximately 80% of the precipitation falls between June and September. However, short showers are common throughout the year. During January and February, occasional snowfall is common above 1500 m.

The landscape is heavily fragmented and degraded with scattered villages and agricultural fields. The remaining undisturbed parts are steep and mainly covered by mature broad leaved forest and scrub. Due to recent road construction on both slopes, many amphibian breeding ponds have been completely destroyed.

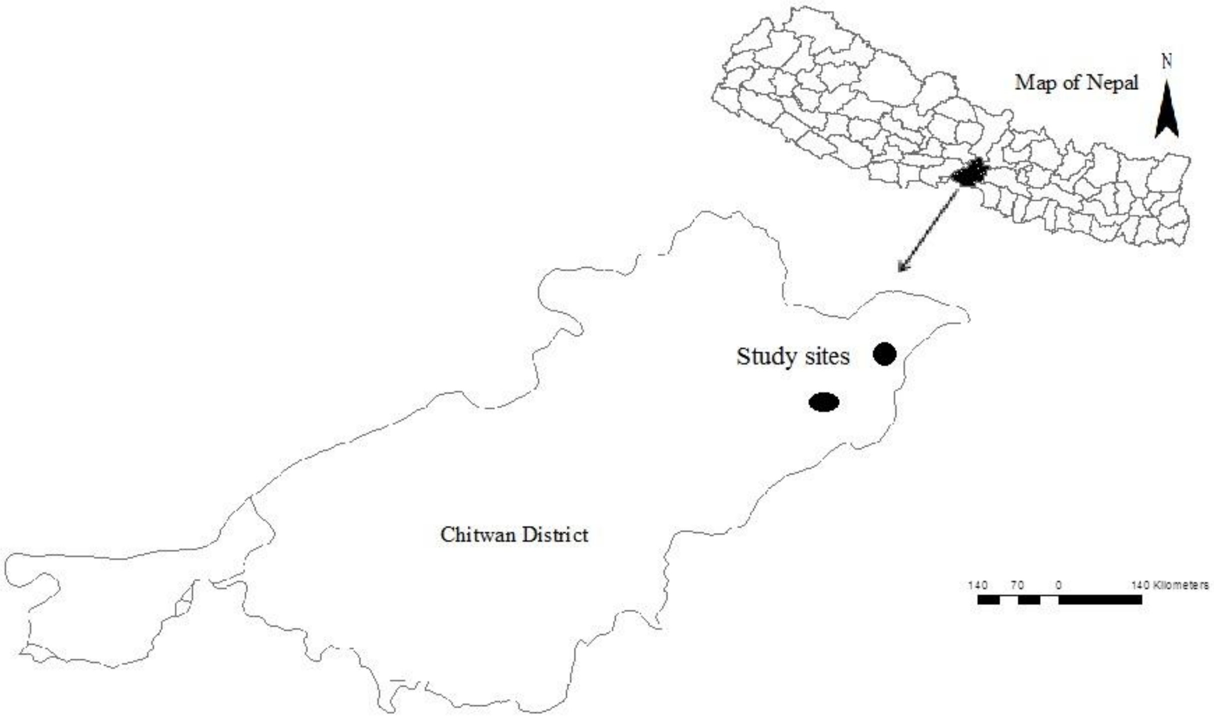


Figure 1 Map of Nepal showing the study area in the Chitwan district with the northern slope (upper right circle) and southern slope (lower left circle) indicated.

## Amphibian-human interactions

People of different castes and ethnic groups reside in the study area. The Chepang people (tribal people in the mid-hills of Nepal who are highly dependent upon natural resources) are the main inhabitants and subsistence farming is the major occupation in this area. The Chepang people mostly engage in animal husbandry, wild food gathering, and wage laboring, which are important aspects of their economic activities (Sharma & Bastakoti 2010). Moreover, fishing and frog collection for consumption are common practices by Chepang communities in the rainy season. Adult frogs are preferred, but tadpoles may also be collected. *Chaparana sikimensis*, *Amolops marmortus* and *Paa liebigii* are heavily exploited frog species in the area. *Paa liebigii* also has a high medicinal value. Shah and Tiwari (2004) have reported over-exploitation of this species for food and medicinal purposes in other parts of Nepal.

## Sampling design and data collection

Amphibian surveys were carried out every 100 m on the southern and northern slopes along an elevation gradient from 200-1600 m during July and August 2010. This coincides with the monsoonal rainfall and breeding time of amphibians (Schleich and Kästle 2002). Amphibians were sampled from diurnal and nocturnal surveys in order to maximize the species capture in each 100 m elevational band. The same level of effort was employed for the two survey methodologies to compare their effectiveness.

## Diurnal and nocturnal surveys

Four diurnal transect of 50 × 4 m were searched by four persons in each 100m elevational band for 30 minutes. Transects were searched from 6.00-9.00 hr and 15.00-18.00 hr. Active searches in leaf litter, under logs and stones were performed. Similarly, tree canopies and bushes near water sources were also searched for arboreal amphibians. The majority of amphibians often aggregate near water sources (Naniwadekar & Vasudevan 2007).Transects were thus placed as near the streams as possible.

Two 100 × 4 m nocturnal transects were searched by four people for 1 hr using torches, whilst the observers walked with a slow pace. These surveys were carried out from 19.00 to 22.00 hr. The number of species and individuals encountered in each transect was recorded. There was often some degree of altitudinal variation between the start and end point of transects due to the rugged mountain terrain. In these cases, the altitude at the beginning of transect was considered for the statistical analysis.

All captured animals were released in the same habitat after being measured (snout-vent length) and photographed. Amphibians were identified in the field with the help of field guide books (Schleich & Kästle 2002; Shah & Tiwari 2004). Unidentified specimens were fixed with 10% neutral-buffered formalin and deposited at the Natural History Museum, Tribhuvan University, Kathmandu, for further identification.

## Habitat variables

At each transect, longitude, latitude, and elevation was measured using a global positioning system (GPS, Garmin-vista). Elevation was cross-checked with an altimeter. The altimeter was calibrated each day before sampling by using a topographic map to verify the altitude.. Soil moisture and temperature was recorded using a Soil Moisture Meter with a scale of 1 to 100 for both parameters at 15m intervals along each transect. The slope of each transect was recorded using a clinometers to calculate relative radiation index (RRI; Oke 1987), which gives a relative value of how much solar radiation a particular spot receives at noon at the equinox. The value range from +1 to -1 and is calculates as:

$$RRI = \cos(180° - \Omega).\sin\beta .\sin Ø + \cos\beta. \cos Ø$$

Where $\Omega$ is aspect, $\beta$ is the slope, and Ø is the latitude of each transect.

Livestock grazing, presence of dung and location in relation to human settlements and agriculture lands were combined to evaluate the disturbance level. Disturbance level was graded on a 0-3 scale with 0 for no sign of disturbance. Level 1 was assigned if the transect was situated away from settlements and in or near agricultural land, but with a conspicuous absence of livestock signs/dung. Areas having moderate grazing and away from human settlement were assigned level 2 and areas near human settlement and intensive grazing are considered as disturbance level 3.

## Statistical Analyses

Species accumulation curves and species rank-abundance tests were performed for nocturnal and diurnal surveys from the northern and southern slopes using BiodiversityR package in R version 2.13.1 (Kindt & Coe 2011; R Development Core Team 2009). Species accumulation curves were constructed to verify the completeness of the survey. The difference in amphibian species composition on the southern and northern slope was analyzed from vegan community ecology package in R 2.13.1 (R Development Core Team 2009; Rossi 2011) with Bray–Curtis similarity metrics in 1000 randomizations (Oksanen et al. 2011).

The number of species recorded in each 100m elevational band was considered the observed species richness and total number of individual in each sampling plot (transect) was considered abundance.

The observed species pattern was compared with the Mid-Domain Null program with predictions generated by a Monte Carlo simulation procedure (McCain 2004). This program generates prediction curves (95%) based on 50,000 simulations without replacement from empirical range sizes (Colwell & Lees 2000; McCain 2003).

Linear regression was used to examine the species richness along the altitudinal gradient. Spearman rank order correlation was used to compare species richness patterns between the southern and northern slopes. Chi-square test was performed to analyze the effect of disturbance pattern between southern and northern slopes. Mann-Whitney U-test was used to explore the relationship between observed species richness and abundance and disturbance level. These analyses were performed in Minitab version 16.0.

A Generalized linear model (GLM) was used elucidate the effect of environmental variables (altitude, soil temperature, soil moisture, disturbance level, RRI) on species richness along the elevational gradient. The model was selected using a "step-down" approach and ranked according to Akaike Information Criterion (AIC); (Burnham & Anderson 2002). The model with the lowest AIC value was selected as the best model. The statistical program R 2.13.1 (R Development Core Team 2009) was used for regression analyses and graphical representations.

The amphibian species composition in three major elevational strata, low (200-600m), mid (600-1000m) and high (above 1000m) was analyzed with a non-metric multidimensional scaling (NMDS) based on Bray-Curtis similarities. This analyses was performed using the software Primer 5.2 (Clarke & Primer 2001) and all analyses were carried out without transformations.

Multivariate analysis was used to examine the effect of environmental variables on amphibian species composition using CANOCO for Windows version 4.5. The results showed that the gradient length of the ordination axis for the southern slope was 4.398 and therefore a canonical correspondence analysis (CCA) with default options was selected to relate amphibian species composition with environmental variables. However, the gradient length for the northern slope was 2.912, and therefore, RDA was considered a suitable method for the analysis of this data (Ter Braak & Smilauer 2002). All five environmental variables were tested through a forward selection procedure. The statistically significant variables ($P < 0.05$) were assessed by Monte Carlo permutation tests and included in further analysis.

## Results

### Amphibian Species richness

In total, 448 man-hours were spent searching 11,200m of transects across the elevational gradients. A total of seventeen species of amphibians, representing four families, were recorded on the southern and northern slopes combined (Table 1). Among the four amphibian families, family Ranidae comprised 70% of total species recorded followed by Bufonidae (12%), Microhylidae (6%) and Rhacopphoridae (12%). The Rhacopphoridae family was only recorded from the southern slope.

Table 1. Amphibian species recorded along an elevational gradient in Chitwan, Nepal.

| **Family** | **Species** | **Common Name** | **Local Name** |
|---|---|---|---|
| BUFONIDAE | *Bufo melanostictus* | Black-spined toad | Khasre Bhyaguto |
| | *Bufo stomaticus* | Assam toad | Khasre Bhyaguto |
| MICROHYLIDAE | *Microhyla ornata* | Oronate rice frog | Bhyaguto |
| RANIDAE | *Amolops marmortus* | Burmese spadefoot toad | Dyang paha |
| | *Chaparana sikimensis* | Sikkimese frog | Pahelo paha |
| | *Euphlyctis cyanophlyctis* | Skittering frog | Tik tike paha |
| | *Hoplobatrachus crassus* | Jerdon's bull frog | Sirke paha |
| | *Hoplobatrachus tigerinus* | Indian bull frog | Sirke paha |
| | *Limnonectes pierrei* | Pierre's criket frog | Kithre Bhyaguto |
| | *Limnonectes syhadrensis* | Syhadre frog | Ahale Bhyaguto |
| | *Limnonectes teraiensis* | Terai cricket frog | Tik tike paha |
| | *Megopyrus parva* | Burmese spadefoot toad | Dyang paha |
| | *Paa liebigii* | Liebig's paha | Man paha |
| | *Sperotheca brevicep* | Maskey's burrowing frog | Rani Bhyaguto |
| | *Sperotheca maskeyi* | Indian burrowing frog | Rani Bhyaguto |
| RHACOPPHORIDAE | *Polypedates maculatus maculates* | Common Indian tree frog | Rukh Bhyaguto |
| | *Polypedates taeniatus* | Common Indian tree frog | Rukh Bhyaguto |

Species rank-abundance pattern revealed that *Bufo melanostictus* had the highest overall rank abundance and *Polypedates maculates* had lowest overall species abundance in the area (Table 2). Similarly, *Bufo melanostictus* showed the highest rank abundance on the southern slope for both nocturnal and diurnal surveys and also for diurnal survey on the northern slope. *Amolops marmortus* had the highest rank abundance on the northern slope for nocturnal surveys. *Polypedates taeniatus* and *Polypedates maculates* showed the lowest rank-abundance on both slopes (Table 2).

Table 2. Species rank-abundance recorded during nocturnal and diurnal surveys on the southern and northern slopes. The species are ranked from the most abundant to the least abundant. Rank 1 denotes the highest species rank and 17 denotes the lowest. P-values were calculated from the BiodiversityR package in R. The results are based on 100 randomizations. The bold numbers refer to species ranked first, second and third.

| **Name of species** | | **South** | | **North** | |
|---|---|---|---|---|---|
| | Overall | Nocturnal | Diurnal | Nocturnal | Diurnal |
| *Bufo melanostictus* | **1** | **1** | **1** | **3** | **1** |
| *Amolops marmortus* | **2** | 4 | 11 | **1** | 11 |
| *Euphlyctis cyanophlyctis* | **3** | **2** | **2** | 5 | **3** |
| *Meophrys parva* | 4 | 5 | 10 | **2** | 10 |
| *Bufo stomaticus* | 5 | 7 | **3** | 4 | 5 |
| *Limnonectes syhadrensis* | 6 | 6 | 9 | 7 | **2** |
| *Hoplobatrachus tigerinus* | 7 | **3** | 6 | 9 | 4 |
| *Limnonectes teraiensis* | 8 | 12 | 4 | 6 | 7 |
| *Sphaerotheca maskeyi* | 9 | 16 | 5 | 8 | 5 |
| *Chaparana sikimensis* | 10 | 11 | 12 | 10 | 12 |
| *Sphaerotheca brevicep* | 11 | 8 | 7 | 15 | 17 |
| *Limnonectes pierrei* | 12 | 9 | 14 | 12 | 8 |
| *Paa liebigii* | 13 | 13 | 15 | 11 | 14 |
| *Microhyla ornata* | 14 | 10 | 8 | 14 | 9 |
| *Hoplobatrachus crassus* | 15 | 14 | 13 | 13 | 13 |
| *Polypedates taeniatus* | 16 | 15 | 16 | 16 | 15 |
| *Polypedates maculatus* | 17 | 17 | 17 | 17 | 16 |

The amphibian species richness and evenness were highest from the nocturnal data of the southern slope. The steeper slope of the nocturnal data from the northern slope indicated less evenly distributed species in this area. In contrast, diurnal survey data showed a higher richness on the northern slope compared to the southern slope (Fig. 2).

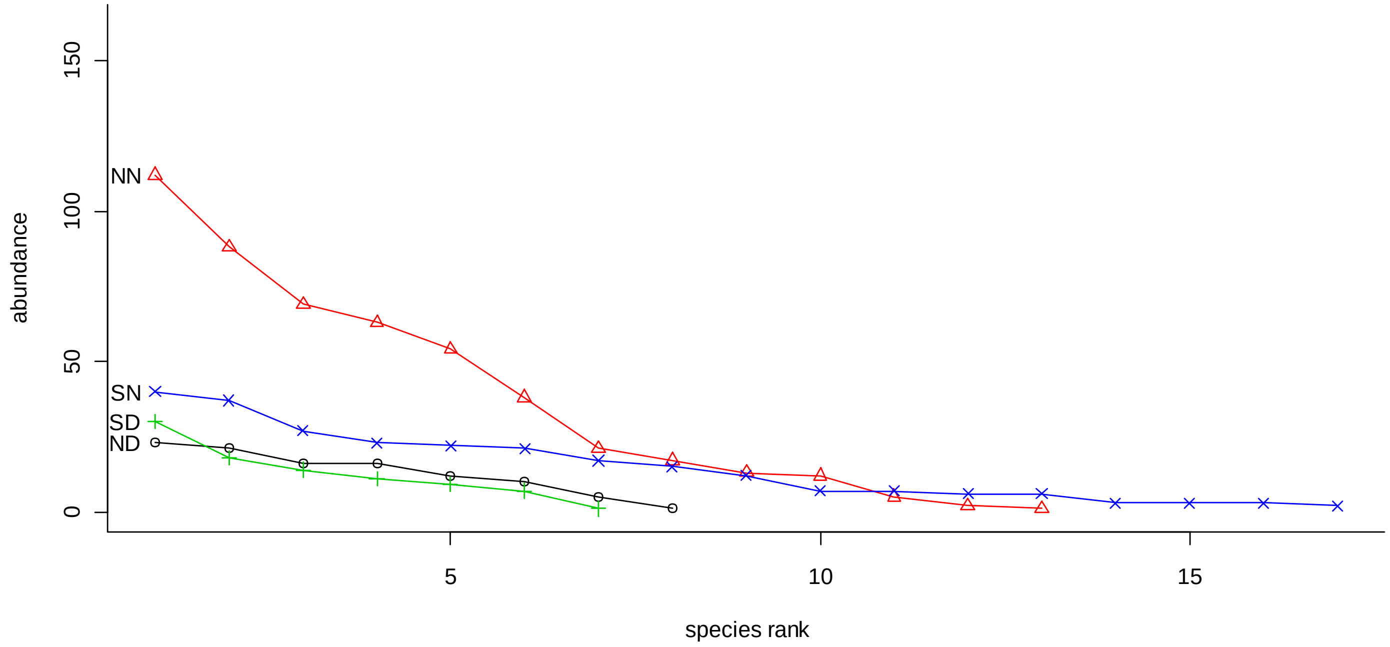


Figure 2. Comparison of species rank abundance curves for the southern and northern slopes from nocturnal (SN=south nocturnal, NN= north nocturnal) and diurnal (SD=south diurnal, ND= north diurnal) surveys.

The rarefaction curves from diurnal and nocturnal surveys on both the southern and northern slopes clearly reached an asymptote. The sample-based rarefaction curve showed that nocturnal sampling was distinctly better than diurnal surveys (Fig. 3). Among the 17 species observed, all were encountered on the southern slope while only 13 species were encountered on the northern slope during nocturnal surveys. Seven species were detected during diurnal surveys on the southern slope and eight were detected on the northern slope (Fig. 3).

The individual-based rarefaction curve clearly showed that the highest abundance was recorded from nocturnal surveys. Nocturnal surveys from both the northern and southern slopes performed better than diurnal surveys (Fig. 4). Diurnal surveys from the southern slope had the lowest recorded abundance.

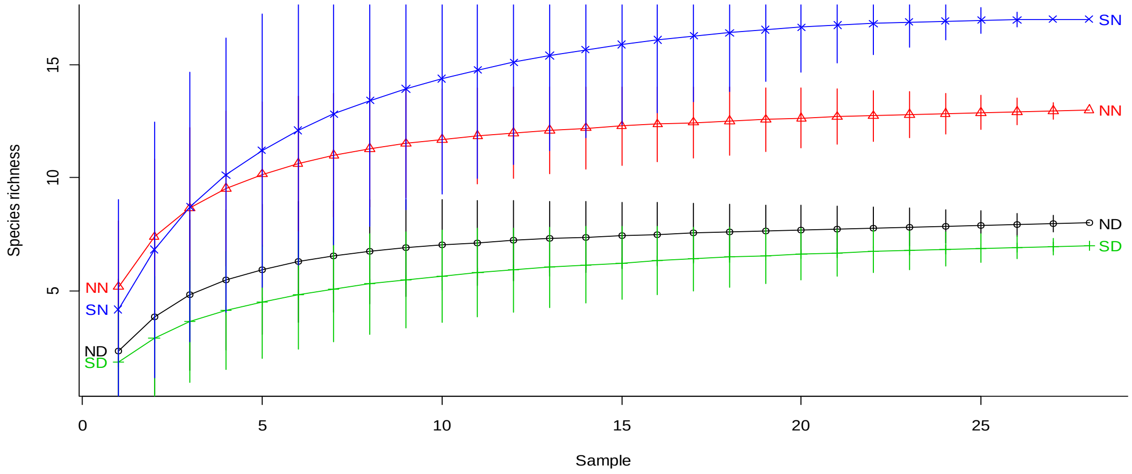


Figure 3. Sample-based species rarefaction curves for nocturnal (SN = south nocturnal, NN = north nocturnal) and diurnal (south diurnal =SD, ND = north diurnal) surveys on the southern slope and northern slopes. The bars indicate the 95% confidence interval (CI) based on standard deviation.

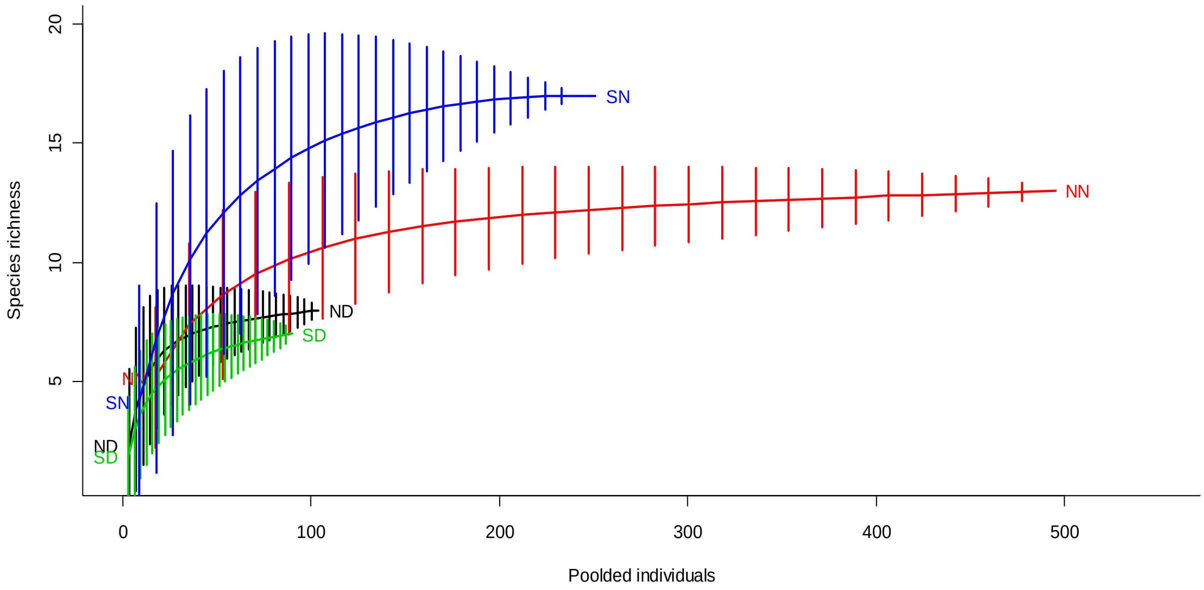


Figure 4. Individual-based species rarefaction curves for nocturnal (SN=south nocturnal, NN= north nocturnal) and diurnal (south diurnal =SD, ND = north diurnal) surveys on the southern slope and northern slopes. The bars indicate the 95% confidence interval (CI) based on standard deviation.

Nocturnal surveys gained the highest mean species richness for both slopes and the difference between mean and cumulative species richness from nocturnal and diurnal surveys was significant (Table 3). Mean amphibian species richness was higher on the northern slope than on the southern slope (Table 4). Species richness observed from nocturnal surveys was markedly higher than diurnal surveys for both slopes (Table 4).

Table 3. Comparison of mean and cumulative species richness for nocturnal and diurnal surveys on the southern and northern slopes. The estimated mean and cumulative species richness and P-values were calculated from the Rich package in R. The results are based on 999 randomizations.

| **Statistic** | **Slope** | **Survey method** | | **Difference** | **P- value** |
|---|---|---|---|---|---|
| | | Nocturnal | Diurnal | Nocturnal-Diurnal | |
| Mean species richness | Southern | 4.17 | 1.85 | 2.32 | 0.01 |
| | Northern | 5.17 | 2.35 | 2.82 | 0.01 |
| Cumulative species richness | Southern | 17 | 7 | 10 | 0.001 |
| | Northern | 13 | 8 | 5 | 0.001 |

Table 4. Comparison of mean and cumulative species richness from the southern and northern slopes. The estimated mean, cumulative number and P-values were calculated from the Rich package in R. The results are based on 999 randomizations.

| **Statistical method** | **Slope** | | **P- value** |
|---|---|---|---|
| | Southern | Northern | |
| Mean species richness | 4.92 | 6.00 | 0.040 |
| Cumulative species richness | 17 | 13 | 0.046 |

## Altitudinal zonation and amphibian species richness

Overall species richness declined with increasing altitude (Fig. 5). More than 55% of species were recorded in the 200-300m elevational zones. There was a continued gradual declining trend of species richness from 300m to 700m. However, species richness increased from 700m to 900m, after which it again decreased with increasing altitude. The observed species richness on the southern slope showed a steeply declining curve from 300m to 600m, a gradual increment from 600m to 1000m, and then a subsequent decline. Contrastingly, the northern slope had a gradual declining trend with altitude (Fig. 5).

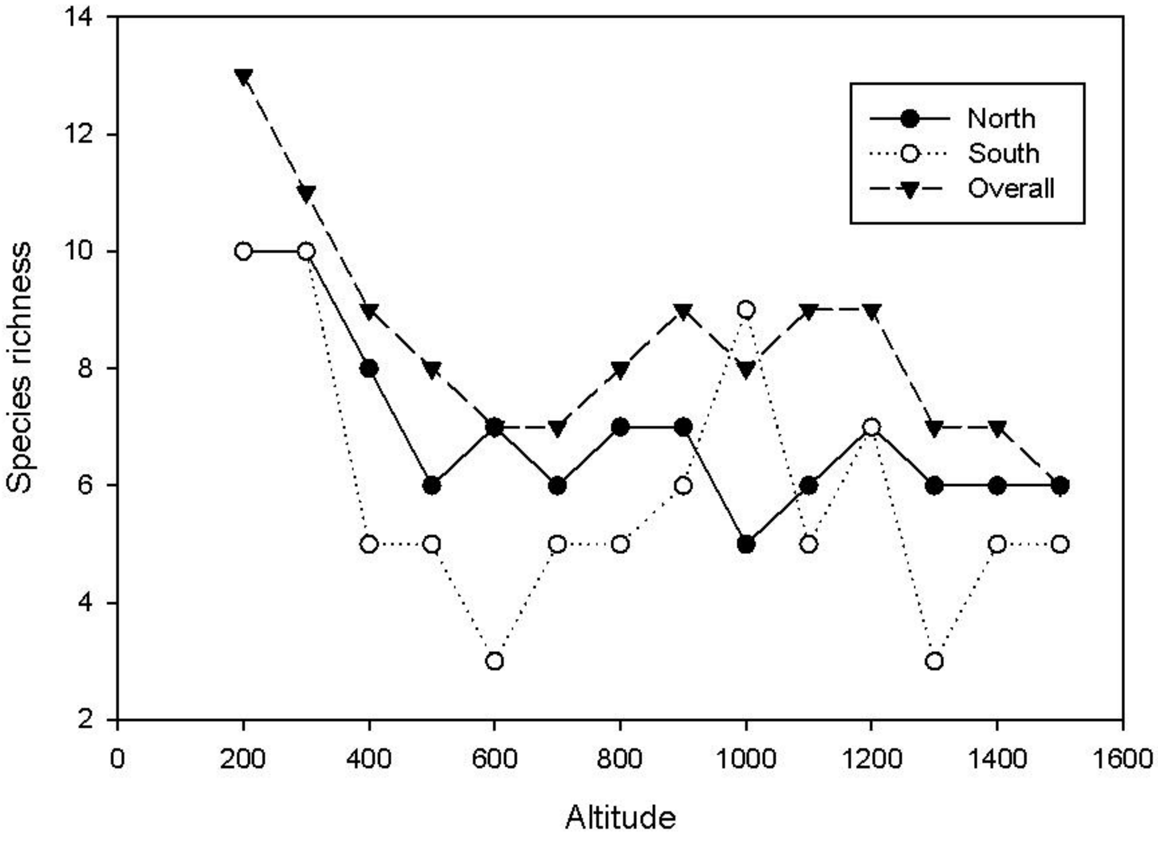


Figure 5. Observed amphibian species richness from nocturnal surveys along the elevational gradient in Chitwan, Nepal.

Based on Mid-Domain Null model, the simulated amphibian species richness did not peak at mid-elevations (Fig. 6). The species richness pattern showed a gradual decline from 200m to 500m and a weak peak at 1000m to 1100m. However, 95% upper and lower prediction curves with 50,000 simulations showed a mid-elevation peak and a fit with the prediction of the null model. The regression analysis also showed that simulated species richness was highly significant with overall (F = 29.354, P <0.001), southern (F = 17.310, P= 0.001) and northern (F = 18.887, P <0.001) amphibian species richness.

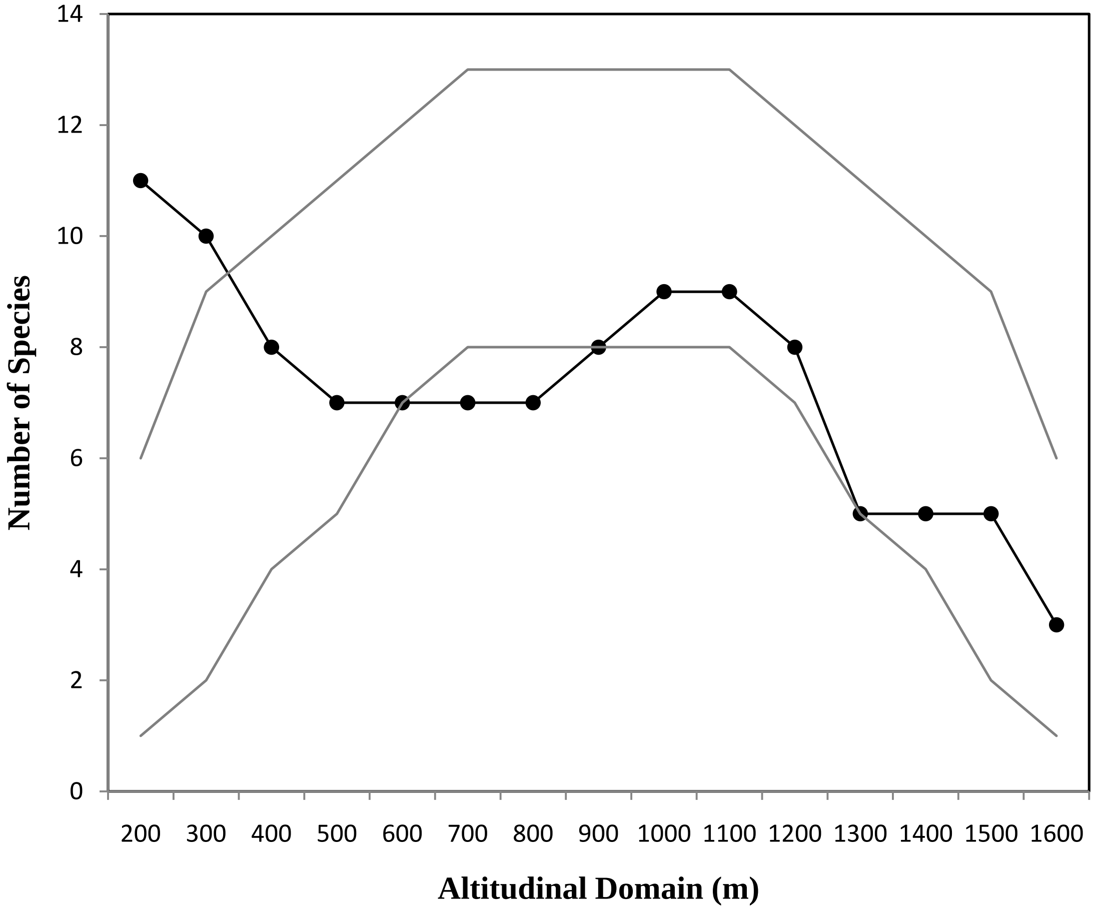


Figure 6. Simulated species richness (black line with closed circle) pattern of amphibian observed in Chitwan, Nepal, and 95% prediction curves with upper and lower limit (smooth lines) generated from the mid-domain null model (McCain 2004).

A declining trend in amphibian species richness and abundance with altitude was also detected on both the southern and northern slopes when analyzed individually (Fig. 7 a, b and 8 a, b). The observed species richness and abundance differed between the southern and northern slopes ($U = 174.0$, $P = 0.001$ and $U = 229.0$, $P = 0.007$, respectively). Nevertheless, the observed species richness in each 100m elevational band was positively correlated between slopes ($r_s = 0.69$, $N = 14$, $p < 0.001$). Linear regression showed that overall species richness declined with elevation ($r^2 = 0.50$, $F = 12.11$, $df = 12$, $P = 0.005$; (Fig. 9).

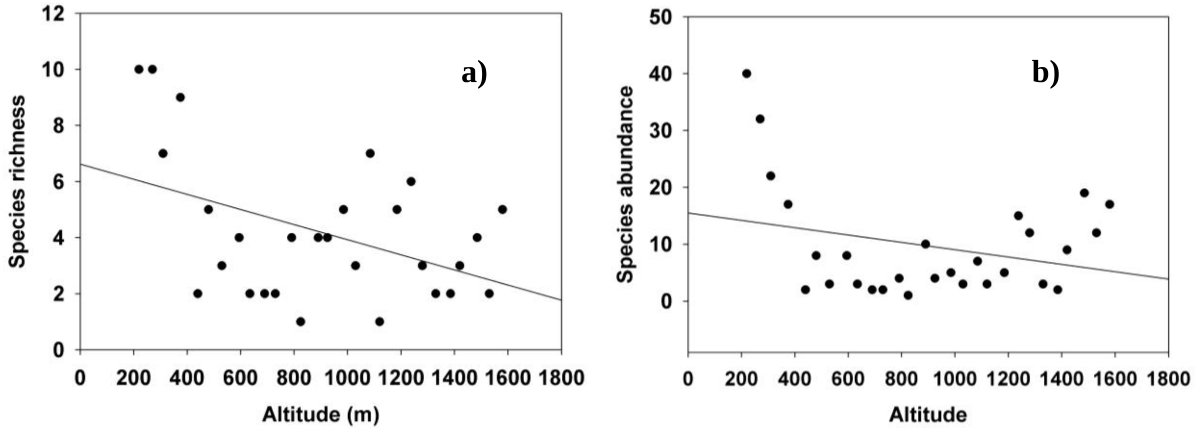


Figure 7. Linear regression model showing the effect of altitude on amphibian a) observed species richness ($y = -0.0027x + 6.6194$, $R^2 = 0.194$) and b) species abundance ($y = 14.102 - 0.00443x$, $R^2 = 0.0447$) on the southern slope.

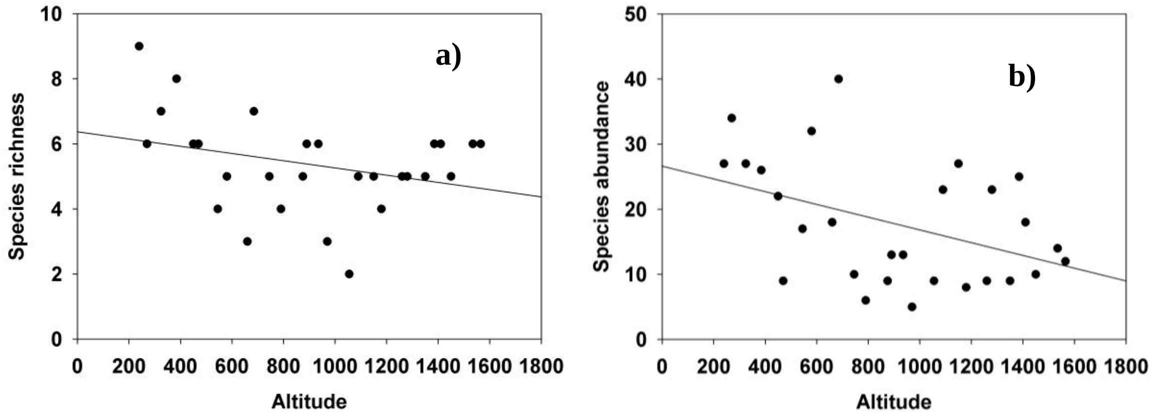


Figure 8. Linear regression model showing the effect of altitude on amphibian a) observed species richness on ($y = -0.0011x + 6.37$, $R^2 = 0.0953$) and b) species abundance ($y = 26.610 - 0.00980x$, $R^2 = 0.180$) on the northern slope.

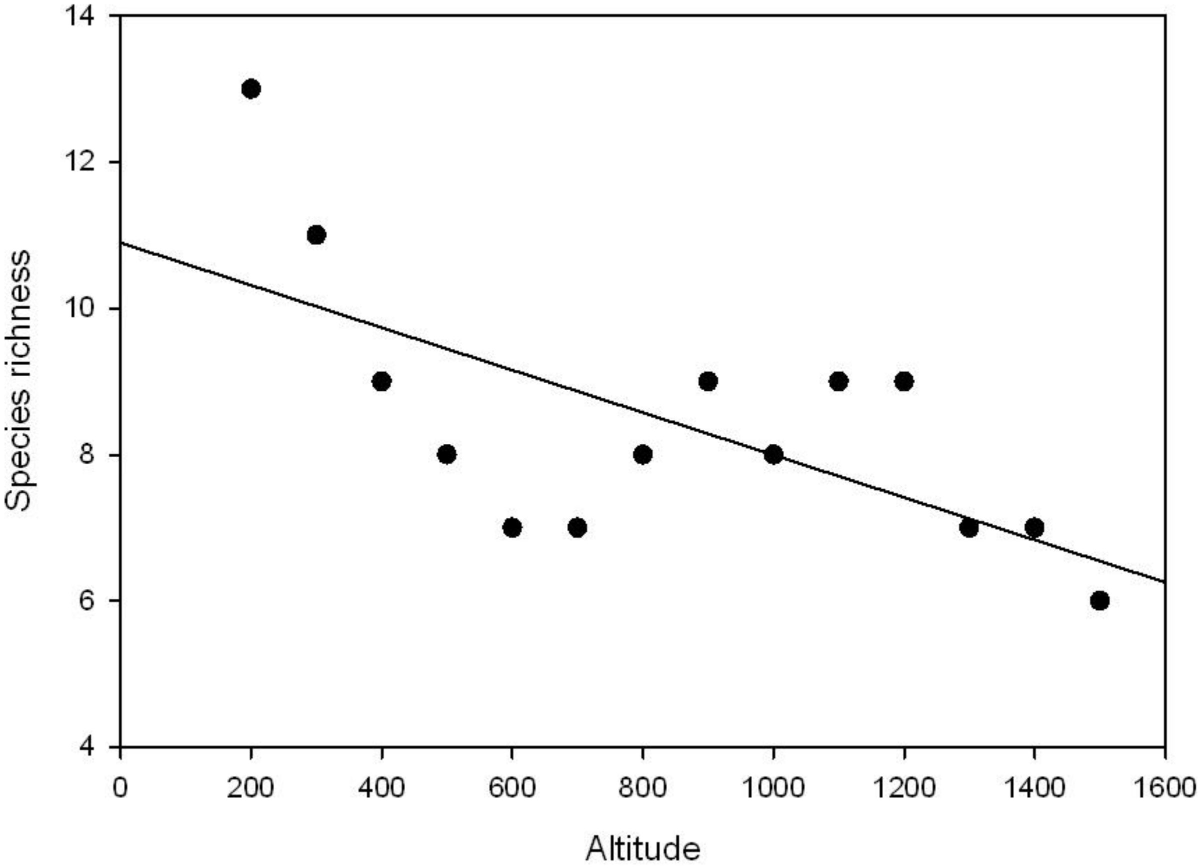


Figure 9. Relationship between overall species richness (combined species richness of southern and northern slopes) and altitude ($y=-0.002929x + 10.8982$, $R^2= 0.4409$).

## Effects of environmental variables on species richness and abundance

The results from the Generalized Linear Model (GLM) showed that altitude, soil moisture and disturbance level had a combined effect on observed species richness on the southern slope for data from both nocturnal and diurnal surveys (Table 5). However, on the northern slope, the model retaining only altitude had the lowest AIC value (Table 6).

Table 5. Summary of generalized linear models (GLM) showing the effect of environmental variables (altitude, soil temperature, soil moisture, disturbance level, RRI) on amphibian species richness on the southern slope for the nocturnal and diurnal surveys. Bold writing signifies the model with the lowest AIC value.

| **Survey method** | **Variables included** | **AIC** |
|---|---|---|
| **Nocturnal** | Altitude, Soil temperature, Soil moisture, Disturbance level, RRI | 264.73 |
| | Altitude, Soil moisture, Disturbance level, Soil temperature | 262.77 |
| | **Altitude, Soil moisture, Disturbance level** | **260.78** |
| | Altitude, Disturbance level | 262.75 |
| | Altitude | 266.64 |
| **Diurnal** | Altitude, Soil temperature, Soil moisture, Disturbance level, RRI | 163.62 |
| | Altitude, Soil temperature, Soil moisture, Disturbance level | 161.68 |
| | **Altitude, Soil moisture, Disturbance level** | **160.04** |
| | Altitude, Disturbance level | 162.12 |
| | Altitude | 164.2 |

Table 6. Summary of generalized linear models (GLM) showing the effect of environmental variables (altitude, soil temperature, soil moisture, disturbance level, RRI) on amphibian species richness on the northern slope for the nocturnal and diurnal surveys. Bold writing signifies the model with the lowest AIC value.

| **Survey method** | **Variables included** | **AIC** |
|---|---|---|
| **Nocturnal** | Altitude, Soil temperature, Soil moisture, Disturbance level, RRI | 119.27 |
| | Altitude, Soil moisture, Disturbance level, Soil temperature | 117.90 |
| | Altitude, Soil moisture, Disturbance level | 115.98 |
| | Altitude, Disturbance level | 114.42 |
| | **Altitude** | **112.70** |
| **Diurnal** | Altitude, Soil temperature, Soil moisture, Disturbance level, RRI | 203.59 |
| | Altitude, Soil temperature, Soil moisture, Disturbance level | 201.78 |
| | Altitude, Soil moisture, Disturbance level | 199.86 |
| | Altitude, Disturbance level | 198.31 |
| | **Altitude** | **196.85** |

## Amphibian community structure across the altitudinal gradient

A non-metric multi-dimensional scaling (NMDS) plot based on Bray-Curtis similarities of species composition and abundance showed a distinct clustering of low-altitude plots compared with mid and high altitude plots (Fig. 10). Low-altitude plots mainly clustered at the right end of the ordination diagram. The high-altitude plots appear to cluster more to the left side of the diagram, whereas mid-elevation plots are more scattered and distributed amongst and between low and high altitude plots (Fig. 10).

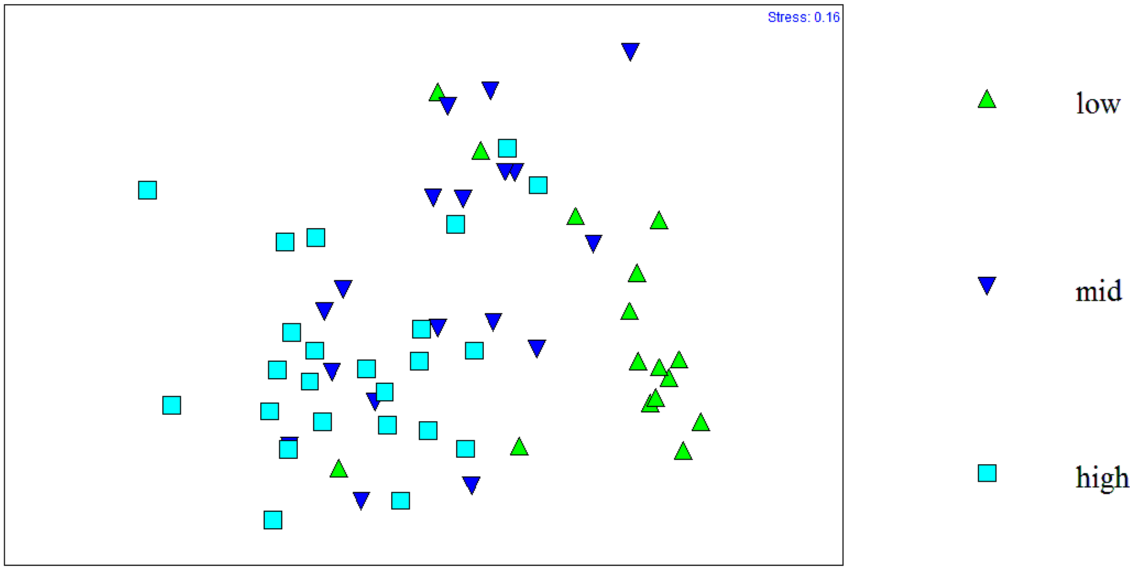


Figure 10. Non-metric multi-dimensional scaling (NMDS) ordination plot based on Bray-Curtis similarities of amphibian species composition and abundance in the low, mid and high elevation plots of Chitwan, Nepal. A low stress value of 0.16 indicates a reasonable goodness of fit in the 2D configuration.

## Influence of environmental variables on species composition and distribution patterns on the southern slope

The first three ordination axes of the CCA explained 100% of the total variance in amphibian assemblages on the southern slope (Table 8). The result showed that altitude had highest correlation with first CCA axis (r = 0.994). Likewise, the higher eigenvalues (0.735) of the first axis compared to the second axis (0.191) indicate that there is one major gradient which corresponds to altitude. Soil moisture (-0.7968) and disturbance level (-0.3507) had highest correlation with axis 2 (Fig. 11b).

The CCA ordination portrayed clear clusters in the distribution pattern of high, medium and low elevation sampling plots (Fig. 11a). In general, low elevation sampling plots were characterized by moisture loving species, mid-elevation sites by disturbance tolerant species and high elevation sites by species restricted to this altitude. However, there was some intermixing of low and high elevation sampling plots with mid-elevation sites, seemingly more disturbed than other low and high elevation sampling plots.

Canononical Correspondence Analysis (CCA) with forward selection showed that altitude (F=11.39, P=0.002), soil moisture (F=2.33, P=0.03) and distrubance level (F=3.05, P=0.002) had a significant effect on amphibian asamblages on the southern slope (Monte-Carlo Permutation $P < 0.05$, Table 9 & 10). *Paa liebigii*, *Meophrys parva*, *Amolops marmortus* and *Chaparana sikimensis* were restricted to higher elevations and strongly associated with altitude (Fig. 11b). *Microhyla ornata*, *Hoplobatrachus crassus*, *Limnonectes pierrei*, *Limnonectes teraiensis*, *Rana nigrivitta*, *Euphlystis cynophlyctis*, *Hoplobatrachus tigerinus*, *Polypedates maculatus* and *Polypedates taeniatus* were found at lower elevations and corresponded well with high soil moisture. These species were therefore strongly correlated with CCA axis 2 (Fig. 11b). *Limnonectes syhadrensis*, *Sphaerotheca maskeyi* and *Sphaerotheca brevicep* were recorded at mid-elevation sites. *Bufo melanostictus* and *Bufo stomaticus* were recorded in all elevational bands, but were more abundant at lower elevations. These species were correlated with distrubance (Fig.11b).

Table 7. Canonical correspondence analysis (CCA) of amphibian assemblages and environmental variables derived from Monte Carlo Permutation ($P < 0.05$) on the southern slope. Total inertia was 2.389. This table corresponds to figures 11 a & b.

| Statistics | **Axis 1** | **Axis 2** | **Axis 3** | **Axis 4** |
|---|---|---|---|---|
| Eigenvalues | 0.735 | 0.191 | 0.113 | 0.355 |
| Species-environment correlations | 0.968 | 0.721 | 0.648 | 0.000 |
| Cumulative percentage variance of | | | | |
| species data | 30.8 | 38.8 | 43.5 | 58.4 |
| species-environment relation | 70.7 | 89.1 | 100.0 | 0.0 |

Table 8. Correlation matrix between the environmental variables and the CCA axes for the southern slope.

| **Environmental variables** | **Axis 1** | **Axis 2** | **Axis 3** | **Axis 4** |
|---|---|---|---|---|
| Altitude | 0.9942 | -0.0495 | 0.0958 | 0.0000 |
| Soil moisture | -0.7968 | 0.5626 | 0.2204 | 0.0000 |
| Disturbance level | -0.3507 | -0.8488 | 0.3957 | 0.0000 |

Table 9. Summary of Monte Carlo Permutations test of environmental variables. Bold numbers signify the significant P-values.

| **Environmental variables** | **F-ratio** | **P-value** |
|---|---|---|
| Altitude | 11.39 | **0.0020** |
| Soil moisture | 2.33 | **0.0300** |
| Soil temperature | 0.18 | 0.9920 |
| Disturbance level | 3.05 | **0.0020** |
| RRI | 1.48 | 0.1560 |

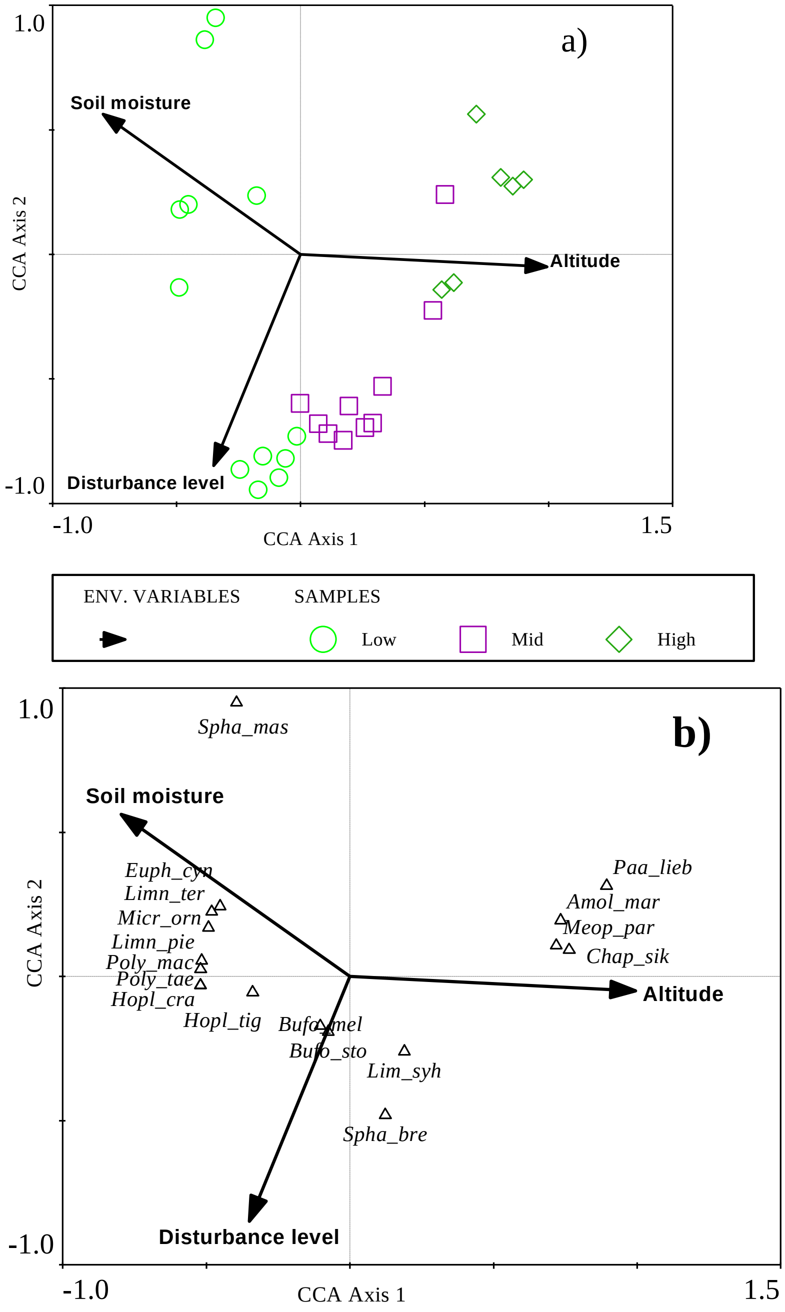


Figure 11. Canonical correspondence analysis (CCA) biplot of amphibian species-environment relations from the southern slope; a) between samples and significant environmental variables and b) between species and significant environmental variables. Five environmental variables were tested by forward selection using the Monte Carlo permutation test. Soil temperature and RRI were not significant ($P > 0.05$) and therefore not included in further analysis. The species abbreviations are given in Appendix 1.

## Influence of environmental variables on species composition and distribution pattern on the northern slope

Correlations between species distribution and environmental variables are shown in Table 11, 12 and 13. The RDA statistics portrayed that the first axis (0.713) and second axis (0.562) had positive species-environmental correlation (Table 11). The first two ordination axes explained 100% of the species-environment variation on the northern slope (Table 11). The first two axes of eigenvalues were relatively weak and the total variance explained by both axes accounted for only 32.7%. A Monte Carlo Permutation with forward selection revealed that altitude (F=6.27, P=0.004) and RRI (F=4.49, P=0.01) had a significant effect on pattern of species distribution (Table 13).

There is clear distribution pattern of sampling plots along the environmental gradient (Fig. 12a). Low elevation sites clustered on the right, mid elevation sites clustered in the middle and high elevation sites clustered to the left and the ordination diagram clearly portrayed the distribution of species along the elevational gradient (Fig. 12b). *Chaparana sikimensis, Paa liebigii* and *Meophrys parva* were recorded in the high altitude sampling sites and showed strong association with altitude, while *Limnonectes syhadrensis*, *Hoplobatrachus crassus*, *Limnonectes pierrei*, *Sphaerotheca maskeyi*, *Hoplobatrachus tigerinus*, *Euphlystis cynophlyctis* and *Limnonectes teraiensis* showed a negative correlation with altitude (Fig. 12b). *Bufo melanostictus* were recorded in most of the elevational bands and its confinement to the mid-section of the ordination plot reflected the association with all environmental gradients.

Table 10. Redundancy analysis (RDA) of the amphibian assemblages and environmental variables derived from Monte-Carlo Permutation test (P< 0.05) on the northern slope. This table corresponds to Figures 12 a & b.

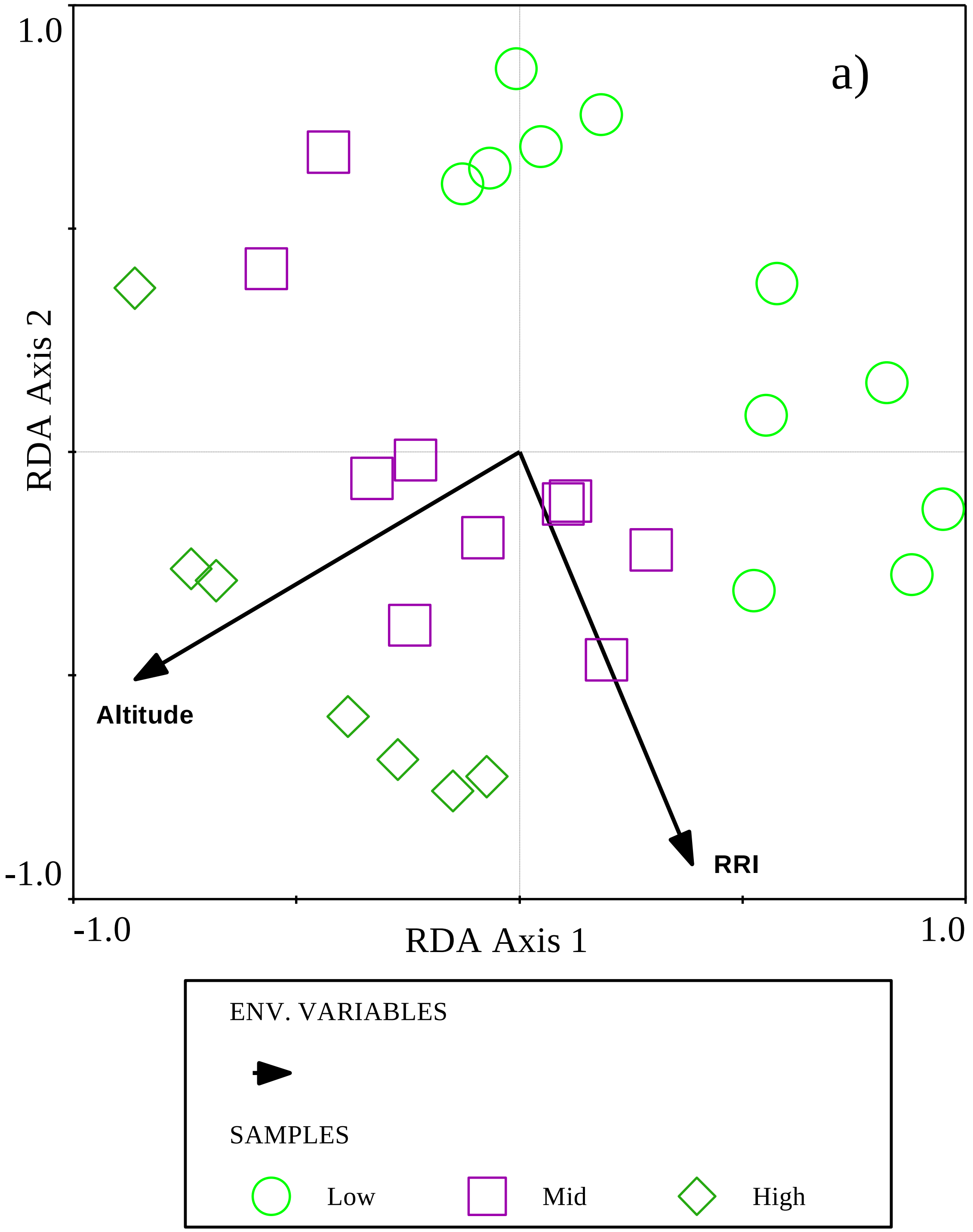
| Statistics | **Axis 1** | **Axis 2** | **Axis 3** | **Axis 4** |
|---|---|---|---|---|
| Eigenvalues | 0.233 | 0.084 | 0.329 | 0.132 |
| Species-environment correlations | 0.713 | 0.562 | 0.000 | 0.000 |
| Cumulative percentage variance of | | | | |
| species data | 23.3 | 31.7 | 64.6 | 77.8 |
| species-environment relation | 73.4 | 100.0 | 0.0 | 0.0 |

Table 11. Correlation matrix between the environmental variables and the RDA axes for the northern slope.

| **Environmental variables** | **Axis 1** | **Axis 2** | **Axis 3** | **Axis 4** |
|---|---|---|---|---|
| Altitude | -0.8610 | -0.5087 | 0.0000 | 0.0000 |
| RRI | 0.3865 | -0.9223 | 0.0000 | 0.0000 |

Table 12. Summary of Monte-Carlo Permutations test of environmental variables. Bold numbers signify the significant P-values.

| **Environmental variables** | **F-ratio** | **P-value** |
|---|---|---|
| Altitude | 6.27 | **0.0040** |
| RRI | 4.49 | **0.01** |
| Soil moisture | 2.25 | 0.0640 |
| Disturbance level | 1.16 | 0.3200 |
| Soil temperature | 0.68 | 0.5840 |


1.0
a)
RDA Axis 2
Altitude
RRI
-1.0
-1.0
RDA Axis 1
1.0
ENV. VARIABLES
SAMPLES
Low
Mid
High

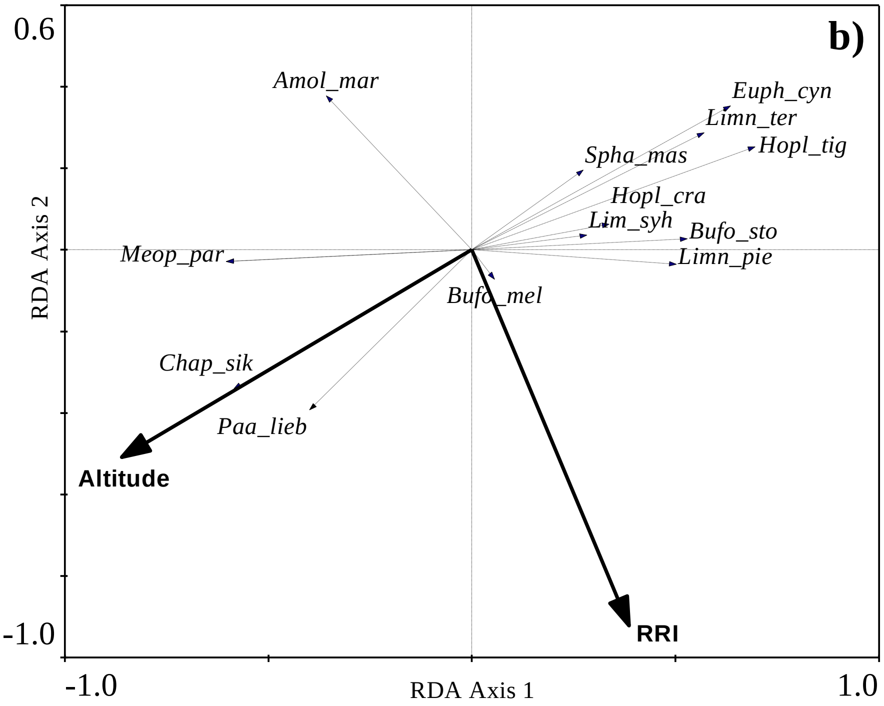


Figure 12. . Redundancy analysis (RDA) of amphibian species-environment relations from the northern slope; a) between sample and significant environmental variables and b) between species and significant environmental variables. Five environmental variables were tested by forward selection using the Monte-Carlo permutation test. Soil temperature, soil moisture and disturbance level were not statistically significant ($P > 0.05$) and therefore not included for the further analysis. The species abbreviations are given in Appendix 1.

## Effect of disturbance on species richness and abundance

Anthropogenic disturbance had a significant effect on amphibian observed species richness and abundance on both the southern and northern slopes (Table 9). However, the disturbance pattern did not vary between slopes ($\chi2 = 7.402$, df = 9, $p = 0.595$).

Table 13. Summary of Mann-Whitney (U) statistics of amphibian observed species richness and abundance with disturbance.

| | Mann-Whitney statistics (U) | | |
|---|---|---|---|
| | Southern slope | Northern slope | P-value |
| Species richness | 78.5 | 31.5 | 0.001 |
| Species abundance | 45 | 29.5 | 0.001 |

## Discussions

This study was carried out in highly disturbed and degraded landscape of Chitwan, Nepal, has high herpetofaunal diversity (Schleich & Kästle 2002; Shah & Tiwari 2004) but it remains is poorly known and information is generally known from very old and outdated literature (Leviton et al. 1956; Mitchell & Zug 1995; Nanhoe & Ouboter 1987; Shah & Gruber 1994; Smith & Battersby 1951; Smith & Battersby 1953; Zug & Mitchell 1995). The current study should therefore be a valuable addition to the literature.

The study revealed that sampling effort on both the southern and northern slopes was complete, as the rarefaction curves attained a long and stable plateau. *Bufo melanostictus* had the highest overall rank abundance among the 17 species recorded although *Amolops marmortus* had the highest observed rank abundance for nocturnal surveys on the northern slope. Schleich & Kästle (2002) reported that *B. melanostictus* is the most common frog in Nepal and is regarded as a perianthropic species generally found below 1800m. Likewise, *A. marmortus* is also a common frog species, often heavily exploited for food and medicinal purposes (Shah & Tiwari 2004). *A. marmortus* is mainly found near rapid flowing streams with a rich vegetation cover. The greater abundance of this species on the northern slope is therefore explained by the prominence of fast flowing streams with rich vegetation on this slope, making the area a very a suitable habitat for this species (Schleich & Kästle 2002). However, interestingly, the current study recorded *A. marmortus* as low as 545m on the northern slope. This is extremely low compared to other places in Nepal where this clearly is a high altitude species mainly found above 1100m (Shah & Tiwari 2004).Species restricted to low and high altitude sampling sites had the lowest rank abundance. *Bufo melanosticuts* and *Euphlyctis cyanophlyctis* were most abundant species in the area. These species are extremely adaptable and are regarded as habitat generalists and can even tolerate pollution (Schleich & Kästle 2002).

## Species richness and elevational gradients

The present study revealed a declining trend in amphibian species richness and abundance with altitude for both the southern and northern slopes. A similar trend was observed for amphibian assemblage in Western Ghats (Naniwadekar & Vasudevan 2007). Many herpetofaunal studies have shown a monotonic decline of species richness along elevational gradients on tropical mountains (Fauth et al. 1989; Gifford & Kozak 2011). In the present study, 55% of species recorded below 600 m and a slight peak was noticed around 1100 m. The species richness subsequently decreased at high elevation sampling sites (Fig. 3). Based on linear regression analysis, altitude was the major factor that significantly influenced the species richness pattern. Higher species richness at lower elevation sites may be due to more favorable climatic conditions for amphibian assemblages at lower elevations. This includes higher average temperatures, evapo-transpiration, productivity and precipitation, which are widely recognized as important for the spatial and temporal distribution pattern of amphibians (Buckley & Jetz 2007) and other taxa (Lomolino 2001) . Chetri et al. (2010), for example, found a seven-fold decline in reptiles species richness along an elevation gradient in eastern Himalaya. Nevertheless, some herpetofaunal studies have shown a hump-shaped relationship between species richness and elevation (Fischer & Lindenmayer 2005; Fu et al. 2006; Gifford & Kozak 2011; Hu et al. 2011; McCain 2010).

Surface area is also a major component which is reduced on the mountains with increasing elevation (Brown 2001; Korner 2000; Körner & Paulsen 2004; Körner 2007; Lomolino & Weiser 2001). Area, interpolation and the mid-domain effect have therefore been emphasized as the major non-environmental variables to be considered in elevational studies (Grytnes & Vetaas 2002; Korner 2000; Lomolino 2001). In this study, amphibian assemblages of Chitwan did not follow the prediction of mid-domain null model, suggesting that they were not constrained by lower geographical hard boundaries. Perhaps explained by the fact that the lower elevations of the study area have a linear connection with alluvial flood plains of India and is more than ca. 800km away from the nearest sea.

## Effects of environmental variables on distribution patterns of amphibians

The NMDS plots (Fig. 10) suggests that low, mid and high elevation sites have relatively distinct amphibian asseblages. However, the intermediate positioning of mid-elevation sites clearly shows that these sites contain species from both low and high elevation sites– perhaps supporting the mid-domain effect hypothesis. In addition, some low and high elevation sites appear to be more of intermediate character. This is due to the fact that *Bufo melanostictus* and *Bufo stomaticus* and *Euphlyctis cyanophlyctis* were recorded in most of the elevational bands on the southern and northern slopes. The clear distinction of lower elevation sampling plots is due to the family Rhachoforidae, which was only recorded at lower elevation sampling sites.

Both GLM and CCA statistics revealed that altitude, soil moisture and disturbance level had a significant effect on species composition on the southern slope. This result also suggested that amphibians species compostion is greatly influences by abiotic factors. On the southern slope, species recorded at lower elevation sites were spread along the soil moisture gradient (Fig. 11b). Species recorded at high elevation sites were spread along the altitudinal gradient. On the southern slope, species like *Hoplobatrachus crassus*, *Polypedates taeniatus* and *Polypedates maculates* were only recorded from two sampling sites below 300m. Contrastingly, *Paa liebigii* was recorded from three sampling sites above 1480m.

The RDA ordination diagram for the northern slope (Fig. 12b) revealed that altitude and RRI had a significant effect on species composition on the northern slope. There might me other environmental variable affecting species richness pattern along the elevational gradients. The southern slope is connected to Chitwan National Park, which has high levels of biodiversity (Bhattarai & Kindlmann 2011). On the other hand, the northern slope is completely isolated from the southern alluvial plain and may explain the fact that this study failed to record species of the Rachophoridae family on the northern slope.

## Comparison of sampling techniques

Amphibians are generally nocturnal, but some species may be seen during daytime hours (Schleich & Kästle 2002). Of the 17 species recorded, only eight species were recorded during diurnal surveys, even though the same level of effort was employed during both diurnal and nocturnal surveys. The current study therefore suggests that all of the recorded species were nocturnal, but that some species may be active during cloudy and rainy days (see Appendix 1 for activity patterns of species). Nocturnal surveys were thus the most robust and effective method for investigating amphibian assemblages in my study area, which is consistent with past studies (Hsu et al. 2005; Parris et al. 1999; Pearman et al. 1995).

## Disturbance and amphibian assemblages

The current study showed that mid-elevation sampling sites were more disturbed than low and high elevational sites (Fig. 11). This is probably due to heavier settlements in these regions and contrasts significantly with Nogués-Bravo et al. (2008) who argued that anthropogenic disturbance is higher in the lowlands and highlands compared to the mid-elevations. However, other disturbances such as road construction, deforestation, pollution of water bodies and adult frog and tadpole collection were also prominent in the landscape. .

Studies have shown that anthropogenic disturbances are a major factor in local and global amphibian declines (Gray et al. 2004). This is consistent with findings reported here, where amphibian species richness was negatively affected by disturbance on both the southern and northern slopes. The northern slope was more disturbed than the southern slope, mainly because it was more densely populated and all the sampling sites were thus close to agricultural fields. The Chepang people are the major inhabitants of the area and completely rely on natural resources for their livelihood (Sharma & Bastakoti 2010). People of all ages, but mainly children and young males, engaged in fishing. They also collected adult frogs and even tadpoles due to their delicious taste and presumed health benefits. During the survey period, fishing activities were noticed in all the streams surveyed. Studies have shown that frogs are an important source of livelihood for these people (Gonwouo & Rödel 2008; Jenkins et al. 2010) and remain an integral part of local medicinal heritage (Mohneke et al. 2011).

A separate survey revealed that *Amolops marmortus, Chaparana sikimensis, Hoplobatrachus crassus, Hoplobatrachus tigerinus* and *Paa liebigii* were the most collected frog species by the local people for food and medicinal purposes. Of these, *Paa liebigii* appear to be the most exploited frog species in the study area and other mountainous parts of Nepal (Shah & Tiwari 2004). This heavy exploitation may also lead to local or global declines and even extinctions through unsustainable collection (Warkentin et al. 2009) Monitoring of these species and collection activities is therefore a conservation priority in the study area and elsewhere in Nepal.

## Summary

This study in degraded landscape of Chitwan, Nepal, demonstrated a decreasing trend in species richness and abundance of amphibians along an elevational gradient. A monotonic decline in species richness along the elevational gradient was noticed on both the southern and northern slopes for both nocturnal and diurnal survey techniques. Nocturnal sampling proved the most robust technique to detect amphibians in this landscape.

Altitude, soil moisture and disturbance were major environmental factors shaping the abundance and distribution pattern of amphibians on the southern slope whereas altitude and RRI significantly affected patterns on the northern slope. Disturbance alone also showed a significant effect on species richness and abundance of amphibians. Frogs are easily available in the local streams and are collected by locals due to high protein values, delicious taste and presumed health benefits. There should therefore be a proper regulation for amphibian harvesting and further research on amphibian-human interactions in the area.

## References

Acharya, B. K., Chettri, B. & Vijayan, L. (2011). Distribution pattern of trees along an elevation gradient of Eastern Himalaya, India. *Acta Oecologica*, 37: 329-336.

Acharya, K. P., Vetaas, O. R. & Birks, H. (2008). Orchid species richness along Himalayan elevational gradients. *Journal of Biogeography*, 38: 1821–1833.

Baral, N. & Heinen, J. T. (2007). Resources use, conservation attitudes, management intervention and park-people relations in the Western Terai landscape of Nepal. *Environmental conservation*, 34 (1): 64-72.

Bhattarai, B. P. & Kindlmann, P. (2011). Habitat heterogeneity as the key determinant of the abundance and habitat preference of prey species of tiger in the Chitwan National Park, Nepal. *Acta Theriologica*: 1-9.

Bhattarai, K. R. & Vetaas, O. R. (2003). Variation in plant species richness of different life forms along a subtropical elevation gradient in the Himalayas, east Nepal. *Global Ecology and Biogeography*, 12 (4): 327-340.

Bhattarai, K. R., Vetaas, O. R. & Grytnes, J. A. (2004). Fern species richness along a central Himalayan elevational gradient, Nepal. *Journal of Biogeography*, 31 (3): 389-400.

Blake, J. G. & Loiselle, B. A. (2000). Diversity of birds along an elevational gradient in the Cordillera Central, Costa Rica. *The Auk*, 117 (3): 663-686.

Brown, J. H. (2001). Mammals on mountainsides: elevational patterns of diversity. *Global Ecology and Biogeography*, 10 (1): 101-109.

Brown, W. C. & Alcala, A. C. (1961). Populations of amphibians and reptiles in the submontane and montane forests of Cuernos de Negros, Philippine Islands. *Ecology*, 42 (4): 628-636.

Buckley, L. B. & Jetz, W. (2007). Environmental and historical constraints on global patterns of amphibian richness. *Proceedings of the Royal Society B: Biological Sciences*, 274 (1614): 1167.

Burnham, K. P. & Anderson, D. R. (2002). *Model selection and multimodel inference: a practical information-theoretic approach*: Springer Verlag.

Chettri, B., Bhupathy, S. & Acharya, B. K. (2010). Distribution pattern of reptiles along an eastern Himalayan elevation gradient, India. *Acta Oecologica*, 36 (1): 16-22.

Clarke, K. & Primer, G. R. N. (2001). v5: User Manual/Tutorial. *Plymouth, UK: PRIMER-E*.

Colwell, R. K. & Hurtt, G. C. (1994). Nonbiological gradients in species richness and a spurious Rapoport effect. *American Naturalist*, 144 (4): 570-595.

Colwell, R. K. & Lees, D. C. (2000). The mid-domain effect: geometric constraints on the geography of species richness. *Trends Ecology and Evolution*, 15: 70-76.

Colwell, R. K., Rahbek, C. & Gotelli, N. J. (2004). The mid-domain effect and species richness patterns: what have we learned so far. *American Naturalist*, 163 (3): E1-E23.

Escobar, F., Lobo, J. M. & Halffter, G. (2005). Altitudinal variation of dung beetle (Scarabaeidae: Scarabaeinae) assemblages in the Colombian Andes. *Global Ecology and Biogeography*, 14 (4): 327-337.

Fauth, J. E., Crother, B. I. & Slowinski, J. B. (1989). Elevational patterns of species richness, evenness, and abundance of the Costa Rican leaf-litter herpetofauna. *Biotropica*: 178-185.

Fischer, J. & Lindenmayer, D. B. (2005). The sensitivity of lizards to elevation: A case study from south-eastern Australia. *Diversity and distributions*, 11 (3): 225-233.

Francesco Ficetola, G. & De Bernardi, F. (2004). Amphibians in a human-dominated landscape: the community structure is related to habitat features and isolation. *Biological Conservation*, 119 (2): 219-230.

Fu, C., Hua, X., Li, J., Chang, Z., Pu, Z. & Chen, J. (2006). Elevational patterns of frog species richness and endemic richness in the Hengduan Mountains, China: geometric constraints, area and climate effects. *Ecography*, 29 (6): 919-927.

Fu, C., Wang, J., Pu, Z., Zhang, S., Chen, H., Zhao, B., Chen, J. & Wu, J. (2007). Elevational gradients of diversity for lizards and snakes in the Hengduan Mountains, China. *Biodiversity and Conservation*, 16 (3): 707-726.

Gifford, M. E. & Kozak, K. H. (2011). Islands in the sky or squeezed at the top? Ecological causes of elevational range limits in montane salamanders. *Ecography*, 34: 001–011.

Gonwouo, L. & Rödel, M. (2008). The importance of frogs to the livelihood of the Bakossi people around Mount Manengouba, Cameroon, with special consideration of the Hairy Frog, Trichobatrachus robustus. *Salamandra*, 44: 23-34.

Grau, O., Grytnes, J. A. & Birks, H. (2007). A comparison of altitudinal species richness patterns of bryophytes with other plant groups in Nepal, Central Himalaya. *Journal of Biogeography*, 34 (11): 1907-1915.

Gray, M. J., Smith, L. M. & Brenes, R. (2004). Effects of agricultural cultivation on demographics of Southern High Plains amphibians. *Conservation Biology*, 18 (5): 1368-1377.

Grytnes, J. A. & Vetaas, O. R. (2002). Species richness and altitude: a comparison between null models and interpolated plant species richness along the Himalayan altitudinal gradient, Nepal. *American Naturalist*: 294-304.

Hofer, U., Bersier, L. F. & Borcard, D. (1999). Spatial organization of a herpetofauna on an elevational gradient revealed by null model tests. *Ecology*, 80 (3): 976-988.

Hsu, M. Y., Kam, Y. C. & Fellers, G. M. (2005). Effectiveness of amphibian monitoring techniques in a Taiwanese subtropical forest. *The Herpetological Journal*, 15 (2): 73-79.

Hu, J., Xie, F., Li, C. & Jiang, J. (2011). Elevational patterns of species richness, range and body size for spiny frogs. *PloS one*, 6 (5): 1-10.

Hunter Jr, M. L. & Yonzon, P. (1993). Altitudinal distributions of birds, mammals, people, forests, and parks in Nepal. *Conservation Biology*: 420-423.

Jenkins, R. K. B., Rabearivelo, A., Wai Mine Andre, C. T. C., Randrianavelona, R. & Randrianantoandro, J. C. (2010). The harvest of endemic amphibians for food in eastern Madagascar. *Tropical Conservation Science*, 2 (1): 25-33.

Kattan, G. H. & Franco, P. (2004). Bird diversity along elevational gradients in the Andes of Colombia: area and mass effects. *Global Ecology and Biogeography*, 13 (5): 451-458.

Kindt, R. & Coe, R. (2011). BiodiversityR: GUI for biodiversity and community ecology analysis. Version 1.6.

Klimes, L. (2003). Life-forms and clonality of vascular plants along an altitudinal gradient in E Ladakh (NW Himalayas). *Basic and Applied Ecology*, 4 (4): 317-328.

Korner, C. (2000). Why are there global gradients in species richness? Mountains might hold the answer. *Trends in Ecology and Evolution*, 15 (12): 513-514.

Körner, C. & Paulsen, J. (2004). A world-wide study of high altitude treeline temperatures. *Journal of Biogeography*, 31 (5): 713-732.

Körner, C. (2007). The use of altitude in ecological research. *Trends in Ecology & Evolution*, 22 (11): 569-574.

Laurie, W. A. (1978). *The ecology and behaviour of the greater one-horned rhinoceros*. The PhD thesis: University of Cambridge, Cambridge.

Lee, P. F., Ding, T. S., Hsu, F. H. & Geng, S. (2004). Breeding bird species richness in Taiwan: distribution on gradients of elevation, primary productivity and urbanization. *Journal of Biogeography*, 31 (2): 307-314.

Leviton, A., Myers, G. & Swan, L. (1956). Zoological results of the California Himalayan expedition to Makalu, Eastern Nepal. I. Amphibians and reptiles. *Nat. Hist. Mus. Stanford University, Stanford*.

Li, J. S., Song, Y. L. & Zeng, Z. G. (2003). Elevational gradients of small mammal diversity on the northern slopes of Mt. Qilian, China. *Global Ecology and Biogeography*, 12 (6): 449-460.

Lomolino, M. & Weiser, M. (2001). Towards a more general species-area relationship: diversity on all islands, great and small. *Journal of Biogeography*: 431-445.

Lomolino, M. V. (2001). Elevation gradients of species-density: historical and prospective views. *Global Ecology and Biogeography*, 10: 3-13.

McCain, C. M. (2003). North American desert rodents: a test of the mid-domain effect in species richness. *Journal of Mammalogy*, 84 (3): 967-980.

McCain, C. M. (2004). The mid domain effect applied to elevational gradients: species richness of small mammals in Costa Rica. *Journal of Biogeography*, 31 (1): 19-31.

McCain, C. M. (2005). Elevational gradients in diversity of small mammals. *Ecology*, 86 (2): 366-372.

McCain, C. M. (2007a). Area and mammalian elevational diversity. *Ecology*, 88 (1): 76-86.

McCain, C. M. (2007b). Could temperature and water availability drive elevational species richness patterns? A global case study for bats. *Global Ecology and Biogeography*, 16 (1): 1-13.

McCain, C. M. (2009). Global analysis of bird elevational diversity. *Global Ecology and Biogeography*, 18 (3): 346-360.

McCain, C. M. (2010). Global analysis of reptile elevational diversity. *Global Ecology and Biogeography*, 19 (4): 541-553.

Mena, J. L. & Vázquez Domínguez, E. (2005). Species turnover on elevational gradients in small rodents. *Global Ecology and Biogeography*, 14 (6): 539-547.

Mitchell, J. C. & Zug, G. R. (1995). Keys to the known amphibians and reptiles of the Royal Chitwan National Park, Nepal. *Smithsonian-Herpetological-Information-Service*.

Mohneke, M., Onadeko, A. B. & Rödel, M. O. (2011). Medicinal and dietary uses of amphibians in Burkina Faso. *African Journal of Herpetology*, 60 (1): 78-83.

Nagy, L. & Grabherr, G. (2009). *The biology of alpine habitats*: Oxford University Press, USA.

Nanhoe, L. M. R. & Ouboter, P. E. (1987). The distribution of reptiles and amphibians in the Annapurna-Dhaulagiri region (Nepal). *Zoologische Verhandelingen*, 240 (1): 1-100.

Naniwadekar, R. & Vasudevan, K. (2007). Patterns in diversity of anurans along an elevational gradient in the Western Ghats, South India. *Journal of Biogeography (J. Biogeogr.)*, 34: 842-853.

Navas, C. A. (2006). Patterns of distribution of anurans in high Andean tropical elevations: Insights from integrating biogeography and evolutionary physiology. *Integrative and Comparative Biology*, 46 (1): 82.

Nogués-Bravo, D., Araújo, M., Romdal, T. & Rahbek, C. (2008). Scale effects and human impact on the elevational species richness gradients. *Nature*, 453 (7192): 216-219.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., O'Hara, R., Simpson, G. L., Solymos, P., Stevens, M. H. H. & Wagner, H. (2011). vegan: Community Ecology Package. *R package version*: 1.17-10.

Oommen, M. A. & Shanker, K. (2005). Elevational species richness patterns emerge from multiple local mechanisms in Himalayan woody plants. *Ecology*, 86 (11): 3039-3047.

Parris, K. M., Norton, T. W. & Cunningham, R. B. (1999). A comparison of techniques for sampling amphibians in the forests of south-east Queensland, Australia. *Herpetologica*, 55 (2): 271-283.

Pearman, P. B., Velasco, A. M. & López, A. (1995). Tropical amphibian monitoring: a comparison of methods for detecting inter-site variation in species' composition. *Herpetologica*, 51 (3): 325-337.

R Development Core Team. (2009). *R: a language and environment for statistical computing. R foundation for Statistical Computing, Vienna.* http://www.R-project.org.

Rahbek, C. (1995). The elevational gradient of species richness: a uniform pattern? *Ecography,* 18 (2): 200-205.

Ribeiro-Júnior, M. A., Gardner, T. A. & Ávila-Pires, T. C. S. (2008). Evaluating the effectiveness of herpetofaunal sampling techniques across a gradient of habitat change in a tropical forest landscape. *Journal of Herpetology*, 42 (4): 733-749.

Rosenzweig, M. L. (1995). *Species diversity in space and time*: Cambridge Univ Pr.

Rossi, J. P. (2011). rich: An R Package to Analyse Species Richness. *Diversity*, 3 (1): 112-120.

Sánchez González, A. & López Mata, L. (2005). Plant species richness and diversity along an altitudinal gradient in the Sierra Nevada, Mexico. *Diversity and Distributions*, 11 (6): 567-575.

Sanders, N. J., Moss, J. & Wagner, D. (2003). Patterns of ant species richness along elevational gradients in an arid ecosystem. *Global Ecology and Biogeography*, 12 (2): 93-102.

Schleich, H. H. & Kästle, W. (2002). *Amphibians and reptiles of Nepal*: ARG. Ganther Verlag KG. Ruggell.

Shah, K. & Gruber, U. (1994). *Bufo microtympanum* Boulenger, 1882, a bufonid toad new for Nepal. *Spixiana*, 17: 57-61.

Shah, K. B. & Tiwari, S. (2004). *Herpetofauna of Nepal: A conservation companion*: IUCN Nepal.

Sharma, L. N. & Bastakoti, R. (2010). Ethnobotany of Dioscorea L. with emphasis on food value in Chepang communities in Dhading district, central Nepal. *Botanica Orientalis: Journal of Plant Science*, 6 (0): 12-17.

Smith, M. & Battersby, J. (1951). On a collection of Amphibians and Reptiles from Nepal. *Ann. Mag. Nat.*, 4: 726-728.

Smith, M. & Battersby, J. (1953). On a collection of amphibians and reptiles from Nepal. *The Annals & Magazine of Natural History*, 6 (69): 702-704.

Stevens, G. C. (1992). The elevational gradient in altitudinal range: an extension of Rapoport's latitudinal rule to altitude. *American Naturalist*, 140 (6): 893-911.

Ter Braak, C. J. F. & Smilauer, P. (2002). *Canoco 4.5: Reference Manual and Canodraw for Windows. User's Guide: Software Form Canonical Community Ordination (version 4.5)*: Microcomputer Power.

Vetaas, O. R. & Grytnes, J. A. (2002). Distribution of vascular plant species richness and endemic richness along the Himalayan elevation gradient in Nepal. *Global Ecology and Biogeography*, 11 (4): 291-301.

Wang, G., Zhou, G., Yang, L. & Li, Z. (2003). Distribution, species diversity and life-form spectra of plant communities along an altitudinal gradient in the northern slopes of Qilianshan Mountains, Gansu, China. *Plant Ecology*, 165 (2): 169-181.

Warkentin, I. G., Bickford, D., Sodhi, N. S. & Bradshaw, C. J. A. (2009). Eating frogs to extinction. *Conservation Biology*, 23 (4): 1056-1059.

Whittaker, R. J., Willis, K. J. & Field, R. (2001). Scale and species richness: towards a general, hierarchical theory of species diversity. *Journal of Biogeography*, 28 (4): 453-470.

Wilson, R. J., GutiERrez, D., GutiERrez, J. & Monserrat, V. J. (2007). An elevational shift in butterfly species richness and composition accompanying recent climate change. *Global Change Biology*, 13 (9): 1873-1887.

Zapata, F., Gaston, K. & Chown, S. (2003). Mid domain models of species richness gradients: assumptions, methods and evidence. *Journal of Animal Ecology*, 72 (4): 677-690.

Zug, G. R. & Mitchell, J. C. (1995). Amphibians and Reptiles of the Royal Chitwan National Park, Nepal. *Asiatic Herpetological Research*, 6: 172-180.

## Appendices

Appendix 1. Overall rank, abundance, species code and activity times of amphibians recorded on the both southern and northern slope by both nocturnal and diurnal methods.

| **Name of species** | **Rank** | **Abundance** | **Species code** | **Activity times** |
|---|---:|---:|---|---|
| *Bufo melanosticuts* (Schneider 1799) | 1 | 162 | *Bufo_mel* | Nocturnal, occasionally diurnal |
| *Amolops marmortus* (Blyth 1855) | 2 | 135 | *Amol_mar* | Nocturnal, diurnal during rain |
| *Euphlyctis cyanophlyctis* (Schneider 1799) | 3 | 125 | *Euph_cyn* | Nocturnal |
| *Meophrys parva* (Schneider 1799) | 4 | 110 | *Meop_par* | Nocturnal |
| *Bufo stomaticus* (Lutken 1862) | 5 | 106 | *Bufo_sto* | Nocturnal |
| *Limnonectes syhadrensis* (Annandale 1991) | 6 | 63 | *Lim_syh* | Diurnal, feed at night |
| *Hoplobatrachus tigerinus* (Daudin 1802) | 7 | 63 | *Hopl_tig* | Nocturnal |
| *Limnonectes teraiensis* (Dubois 1984) | 8 | 60 | *Limn_ter* | Nocturnal |
| *Sphaerotheca maskeyi* (Schleich &Anders 1799) | 9 | 39 | *Spha_mas* | Nocturnal |
| *Chaparana sikimensis* (Jerdon 1870) | 10 | 19 | *Chap_sik* | Nocturnal |
| *Sphaerotheca brevicep* (Schneider 1799) | 11 | 16 | *Spha_bre* | Nocturnal |
| *Limnonectes pierrei* (Dubois 1975) | 12 | 15 | *Limn_pie* | Nocturnal |
| *Paa liebigii* (Gunther 1860) | 13 | 11 | *Paa_lieb* | Nocturnal |
| *Microhyla ornata* (Dumeril & Bibron 1841) | 14 | 7 | *Micr_orn* | Nocturnal, diurnal on cloudy days |
| *Hoplobatrachus crassus* (Jerdon 1870) | 15 | 4 | *Hopl_cra* | Nocturnal |
| *Polypedates taeniatus* (Boulenger 1906) | 16 | 3 | *Poly_tae* | Nocturnal |
| *Polypedates maculates* (Grey 1834) | 17 | 2 | *Poly_mac* | Nocturnal |

Appendix 2. Species rank and abundance of amphibians on the southern slope recorded during nocturnal surveys.

| **Name of species** | **Rank** | **Abundance** |
|---|---|---|
| *Bufo melanosticuts* | 1 | 40 |
| *Euphlyctis cyanophlyctis* | 2 | 37 |
| *Hoplobatrachus tigerinus* | 3 | 27 |
| *Amolops marmortus* | 4 | 23 |
| *Meophrys parva* | 5 | 22 |
| *Limnonectes syhadrensis* | 6 | 21 |
| *Bufo stomaticus* | 7 | 17 |
| *Sphaerotheca brevicep* | 8 | 15 |
| *Limnonectes pierrei* | 9 | 12 |
| *Microhyla ornata* | 10 | 7 |
| *Chaparana sikimensis* | 11 | 7 |
| *Limnonectes teraiensis* | 12 | 6 |
| *Paa liebigii* | 13 | 6 |
| *Hoplobatrachus crassus* | 14 | 3 |
| *Polypedates taeniatus* | 15 | 3 |
| *Sphaerotheca maskeyi* | 16 | 3 |
| *Polypedates maculatus* | 17 | 2 |

Appendix 3. Amphibian species rank and abundance for the southern slope recorded during diurnal surveys.

| **Name of species** | **Rank** | **Abundance** |
|---|---|---|
| *Bufo melanosticuts* | 1 | 30 |
| *Euphlyctis cyanophlyctis* | 2 | 18 |
| *Bufo stomaticus* | 3 | 14 |
| *Limnonectes teraiensis* | 4 | 11 |
| *Sphaerotheca maskeyi* | 5 | 9 |
| *Hoplobatrachus tigerinus* | 6 | 7 |
| *Sphaerotheca brevicep* | 7 | 1 |

Appendix 4: Species rank and abundance of amphibians on the northern slope recorded during the nocturnal surveys.

| **Name of species** | **Rank** | **Abundance** |
|---|---|---|
| *Amolops marmortus* | 1 | 112 |
| *Meophrys parva* | 2 | 88 |
| *Bufo melanosticuts* | 3 | 69 |
| *Bufo stomaticus* | 4 | 63 |
| *Euphlyctis cyanophlyctis* | 5 | 54 |
| *Limnonectes teraiensis* | 6 | 38 |
| *Limnonectes syhadrensis* | 7 | 21 |
| *Sphaerotheca maskeyi* | 8 | 17 |
| *Hoplobatrachus tigerinus* | 9 | 13 |
| *Chaparana sikimensis* | 10 | 12 |
| *Paa liebigii* | 11 | 5 |
| *Limnonectes pierrei* | 12 | 2 |
| *Hoplobatrachus crassus* | 13 | 1 |

Appendix 5: Species rank and abundance of amphibians on the northern slope recorded during the diurnal surveys.

| **Name of species** | **Rank** | **Abundance** |
|---|---:|---:|
| *Bufo melanosticuts* | 1 | 23 |
| *Limnonectes syhadrensis* | 2 | 21 |
| *Euphlyctis cyanophlyctis* | 3 | 16 |
| *Hoplobatrachus tigerinus* | 4 | 16 |
| *Bufo stomaticus* | 5 | 12 |
| *Sphaerotheca maskeyi* | 6 | 10 |
| *Limnonectes teraiensis* | 7 | 5 |
| *Limnonectes pierrei* | 8 | 1 |

Appendix 6. Summary of the total number of individuals and species (in brackets) encountered in during diurnal (50 x 4 m) and nocturnal (100 x 4 m) surveys in each 100m elevational bands.

| **Altitudinal band** | **Southern Slope** | | | **Southern slope** | | |
|---|---|---|---|---|---|---|
| | **Diurnal** | **Nocturnal** | **Total species** | **Diurnal** | **Nocturnal** | **Total species** |
| 300 | 22(5) | 72(10) | 10 | 19(8) | 61(10) | 10 |
| 400 | 14(6) | 39(10) | 10 | 8(5) | 53(10) | 10 |
| 500 | 7(3) | 10(5) | 5 | 10(4) | 31(8) | 8 |
| 600 | 5(4) | 11(5) | 5 | 11(6) | 49(6) | 6 |
| 700 | 2(4) | 5(3) | 3 | 7(3) | 58(7) | 7 |
| 800 | 2(2) | 6(5) | 5 | 5(4) | 16(6) | 6 |
| 900 | 4(2) | 11(5) | 5 | 11(6) | 22(7) | 7 |
| 1000 | 4(1) | 9(6) | 6 | 9(3) | 18(7) | 7 |
| 1100 | 6(3) | 9(9) | 9 | 4(3) | 32(5) | 9 |
| 1200 | 8(3) | 8(5) | 5 | 4(3) | 35(6) | 6 |
| 1300 | 4(3) | 26(7) | 7 | 7(5) | 32(7) | 7 |
| 1400 | 2(2) | 4(3) | 3 | 7(6) | 34(6) | 6 |
| 1500 | 4(2) | 23(5) | 5 | 3(2) | 28(6) | 6 |
| 1600 | 3(2) | 18(5) | 5 | 0(0) | 26(6) | 6 |