DIVERSITY AND DISTRIBUTION PATTERN OF AMPHIBIANS IN MAHAKALI RIVER BASIN, FAR-WESTERN NEPAL

Madan Raj Mishra

T.U. Registration No: 5-2-0061-0007-2012

T.U. Examination Roll. No: Zoo 427/073

Batch: 2073

A Dissertation submitted in partial fulfilment of the requirements for the award of the Degree of Master of Science in Zoology with special paper "Ecology and Environment"

Submitted to
Central Department of Zoology
Institute of Science and Technology
Tribhuvan University
Kirtipur, Kathmandu
Nepal
September, 2019

DECLARATION

I hereby declare that the work presented in this dissertation has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to author(s) or institution(s).

Date: 2076/05/20

....

Madan Raj Mishra

TRIBHUVAN UNIVERSITY ☏ 01-4331896
CENTRAL DEPARTMENT OF ZOOLOGY
Kirtipur, Kathmandu, Nepal.

Ref.No.:

Central Department of Zoology T.U., Kirtipur

RECOMMENDATION

This is to recommend that the dissertation entitled "**DIVERSITY AND DISTRIBUTION PATTERN OF AMPHIBIANS IN MAHAKALI RIVER BASIN, FAR-WESTERN NEPAL**" has been carried out by Mr. Madan Raj Mishra for partial fulfilment of the requirement for Master's Degree in Zoology with the special paper of Ecology and Environment. This is his original work and has been carried out under my supervision. To the best of my knowledge, this work has not been submitted for any other degree in any institutions.

…………….

Co-Supervisor

Dr. Janak Raj Khatiwada

Post- Doctoral Fellow

Chengdu Institute of Biology

Chinese Academy of Sciences, China

…………….

Supervisor

Asst. Prof. Dr. Bishnu Prasad Bhattarai

Central Department of Zoology

Tribhuvan University, kirtipur

Kathmandu, Nepal

Date: 2076/05/20

TRIBHUVAN UNIVERSITY ☏ 01-4331896
CENTRAL DEPARTMENT OF ZOOLOGY
Kirtipur, Kathmandu, Nepal.

Ref.No.:

LETTER OF APPROVAL

On the recommendation of Supervisor "Asst. Prof. Dr. Bishnu Prasad Bhattarai" this dissertation submitted by Mr. Madan Raj Mishra entitled "**DIVERSITY AND DISTRIBUTION PATTERN OF AMPHIBIANS IN MAHAKALI RIVER BASIN, FAR-WESTERN NEPAL**" is approved for the examination [illegible] for partial fulfilment of the requirements for Master's Degree of Science in Zoology with special paper Ecology and Environment.

Date: 2076/05/20

....
Prof. Dr. Tej Bahadur Thapa
Head of Department
Central Department of Zoology
Tribhuvan University
Kritipur, Kathmandu, Nepal

TRIBHUVAN UNIVERSITY 01-4331896
CENTRAL DEPARTMENT OF ZOOLOGY
Kirtipur, Kathmandu, Nepal.
Ref.No.:

CERTIFICATE OF ACCEPTANCE

This dissertation work submitted by Mr. Madan Raj Mishra "**DIVERSITY AND DISTRIBUTION PATTERN OF AMPHIBIANS IN MAHAKALI RIVER BASIN, FAR-WESTERN NEPAL**" has been accepted as a partial fulfilment of the requirements for Master's Degree of Science in Zoology with special paper Ecology and Environment.

EVALUATION COMMITTEE

Supervisor
Asst. Prof. Dr. Bishnu Prasad Bhattarai
Central Department of Zoology
Trbhuvan University, Kirtipur
Kathmandu, Nepal

Head of Department
Prof. Dr. Tej Bahadur Thapa
Central Department of Zoology
Tribhuvan University, Kirtipur
Kathmandu, Nepal

Internal Examiner
Asst. Prof. Dr. Laxman Khanal
Central Department of Zoology
Tribhuvan University, Kirtipur
Kathmandu, Nepal

External Examiner
Asst. Prof. Dr. Man Kumar Dhamala
Central Department of Environmental Science, Tribhuvan University, Kirtipur
Kathmandu, Nepal

Date: 2076/06/08

ACKNOWLEDGEMENTS

I would like to express my deep gratitude to my supervisor Asst. Prof. Dr. Bishnu Prasad Bhattarai, Central Department of Zoology for his continuous guidance and encouragement in carrying out this dissertation work.

I would like to thank my co-supervisor Dr. Janak Raj Khatiwada, Chengdu Institute of Biology, Chinese Academy of Sciences, China, for his valuable guidance on survey method design, financial support and guidance to management and analysis of data as well as interpretation of results.

I am grateful to Prof. Dr. Tej Bahadur Thapa, Head of Central Department of Zoology, T.U, for his kind support and encouragement.

I am thankful to Department of National Parks and Wildlife Conservation (DNPWC) and office of Api Nampa conservation area for providing permission for research in Mahakali River basin.

Similarly, my special thank goes to my friend Mr. Suman Sapkota for his continuous help on field for data collection as well as encouragement to complete this research.

Finally, I would like to express my sincere thanks to my parents, family members for their continuous help and encouragement throughout my study.

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviations
Asl	Above Sea Level
CCA	Canonical Correspondence Analysis
DNPWC	Department of National Parks and Wildlife Conservation
GPS	Global Positioning System
Km	Kilometre
M	Metre
Mm	Millimitre
SVL	Snout-Vent length

ABSTARCT

Amphibian diversity along elevation gradient in Nepal has rarely been studied. This is the first study in Mahakali River basin along elevation gradient for amphibian diversity. This study aimed to explore diversity, distribution pattern and body size variation of amphibians along elevation gradient, in Mahakali River basin. Time-constrained visual encounter survey was conducted for data collection. Different environmental variables were measured along each transects. Body size of individuals were measured on the field and released at the site of capture. A total of 17 species of amphibians belonging to six families and one order i.e., Anura were recorded and Dicroglossidae family was dominant among them. Shannon wiener diversity index of overall study area; H = 2.436 and evenness E = 0.672 was found. Study showed that amphibians were not uniformly distributed along elevation gradient. Linear regression model showed both species richness and species abundance was declined monotonically along elevation gradient. Similarly, diversity and mean abundance of different habitat was calculated. Highest species diversity was found in forest habitat. Influence of environmental variables on amphibian species distribution was examined; main factors responsible for amphibian species distribution were elevation, temperature and humidity. Body size of two families; Bufonidae and Dicroglossidae along elevation gradient increased with increasing elevation; followed Bergmann's rule. Body size of individuals belonging to Microhylidae was decreased with increasing elevation. That means all amphibians do not follow Bergmann's rule. It is concluded that amphibians portrayed narrow distribution range along elevation gradient and with environmental variables such as temperature and elevation.

1. INTRODUCTION

1.1 Background

Amphibians are the diverse group of vertebrates which comprises three orders; Anura (frogs and toads), Caecilians and Caudata (Salamanders and Newts) (Shah and Tiwari 2004). There are more than 7,664 species of amphibians in world (Frost 2017) and 54 species in Nepal (Shah and Tiwari 2004).

Along latitudinal and elevational gradient, various kind of research have been done and their results shows that both animal and plant species are not uniformly distributed (Brhem et al. 2007, Dobremez 1976, Gemi et al. 2017, Koirala et al. 2019). Different environmental variables like climatic, biological, geographical and anthropogenic activities are responsible for species richness pattern along elevation gradient (Rahbek 1995, Khatiwada and Haugaasen 2015, Khatiwada et al. 2019). In community ecology, previous literatures revealed that the distribution, abundance, species composition and assemblages are strongly influenced by environmental gradients or contagious biotic processes such as predation, competition, dispersal and disease (Parris 2004, Hofer et al. 1999, Navas 2006, Fu et al. 2007, Chettri et al. 2010, McCain 2010, Hu et al. 2011).

Himalayan region of Nepal is rich in biodiversity as it encompasses the highest elevational variations in the world with diverse climatic conditions from Tropical to Nival zone within a relatively short horizontal distance (Dobremez 1976). Moreover, physical and climatic heterogeneity of the mountain harbours the high biodiversity and endemism (Vetaas and Grytnes 2002). Elevational variability causes variation in environmental conditions on mountains and makes them ideal for exploring ecological and evolutionary adaptation of biota to environmental influences over short spatial distances (Korner 2007). The impact of elevation on species richness and composition has been assessed for a variety of taxa at various geographic locations (Rahbek 1995, Gemi et al. 2017). Patterns of species richness along elevational gradients generally follow three widely accepted trends: monotonic decline, mid-elevation peak or increase with elevation (Rahbek 1995).

A wide variety of amphibians relies on and occupies fresh water and other variety of ecosystems. Most of the amphibians are often aggregated nearby water resources because their life histories include an aquatic larval stage before metamorphosis into semi-aquatic adults (Duellman and Trueb 1994). Unlike amphibians, water resource is very important to reptiles in many ways, for example, by providing primary habitat (e.g., crocodiles and turtles), for refuge, and for foraging ground (e.g., water snakes).

Despite their ecological and economic importance amphibians are under pressure across local to global scales. They are facing serious threats under multiple disturbances; globally, many recently known extinct taxa come from amphibians (Baillie et al. 2004). Furthermore, a number of ecosystems are being fragmented and degraded by climate change (Araujo et al. 2006) and environmental pollution (Davidson 2004), resulted in a negative influence on species distribution and community composition of amphibians. Importantly, these changes in community structure (e.g. taxonomic diversity, species composition and functional traits) can strongly affect ecosystem functioning as amphibian species play key functional roles between fresh water and terrestrial ecosystem (Ernst et al. 2006, Whiles et al. 2006).

Body size is a fundamental morphological trait, important in a physiological, ecological and social context it varies in different geographical regions and it is common to all group of vertebrates (Avise 2000, Schauble 2003). Large-scale systematic patterns of body size are a basic concern of evolutionary biology. By studying body size; it helps to researchers; to understand how life history of animals is being under evolutionary pattern, predator- prey interactions, extinction of species (Gouveia and Correia 2016, Gaeta et al. 2018, Cooper et al. 2008, Lou et al. 2012). Morphological as well as physiological changes in amphibians like body size, growth rate, age structure, population characteristics and life span are their adaptive features. Similarly induced by surrounding environments and are influenced by elevation and latitude. Life history of both endotherms and ectotherms is dependent on environmental variables, elevation as geographical gradient creates variation in temperature and season length (Lio and Lu 2010). Body size of amphibians is also dependent on available food resources, competition, predation, habitat loss, fragmentation and anthropogenic activities (Tocher et al. 1997, Johanna and Carla 2007). Disease, pathogens also reduces the body size of amphibians as well (Lips 1999). Disturbed habitat leads to reduction in body size and undisturbed and healthy habitat leads to increase in body size of amphibians (Mazerolle 2001).

Because of altitudinal variation within short geographical distance facilitation of gene flow from nearby habitats is possible. So, life-history comparisons across elevations can be more valuable (Stevens 1992). Rapport's rule states that "there is a positive relationship between the latitudinal/altitudinal geographical range of an organism and latitude/elevation" (Stevens 1992, and Keil 2009). Rapport's altitudinal rule was explained in terms of the differential ability of species to attain large range sizes. Bergman rule is also supportive to this rule, species which inhabit cold habitat they tend to attain larger body size than warm habitat occupying species (Freckleton and Watkinson 2003).

Various studies about amphibians body size variation along latitudinal gradient and altitudinal gradient have been done to find out whether this group of animal follows Bergmann's rule or not and they concluded this rules is only applicable for endotherms (Birds and Mammals) and some other group of animal species like amphibians. However, this rules are debated by amphibian researchers (Hu et al. 2011, Adams and Church 2008, Ashton 2002, Mousseau 1997).

Information about amphibian species from Nepal is not documented well. Moreover study about diversity and body size variation along elevation gradient is necessary in far western region of Nepal. This type study will be helpful to document information about amphibians in whole Nepal. The sensitivity of amphibians to environmental change and their role in ecosystems suggest that they are a group in need of additional research and conservation attention. Amphibians play important roles in ecosystems functioning because of their different roles as prey and predators. Their occurrence in transition of aquatic and terrestrial system makes them ecologically interesting. Amphibians have high economic importance as they play key role in controlling crop pests.

1.2 Rationale

Amphibian species are not well studied in Nepal in comparison of large group of animals like birds and mammals (Schleich and Kastle 2002, Vetaas and Grytnes 2002) this group of animals are not focused in conservation priority. Biodiversity of Western Himalayan region of Nepal is not explored in new studies and some studies have poorly known and information is generally known from very old and outdated literature (Smith and Battersby 1953, Leviton et al. 1956, Nanhoe and Ouboter 1987, Mitchell and Zug 1995). It is due to lack of detail information and less interests of researchers toward this small sized group of animals (Vitt et al. 2003). This study is expected to give detail information about the diversity and distribution pattern of amphibians along elevation gradients in Mahakali River basin far-western Nepal.

Researchers have found that many amphibian species have been declined from high altitudinal habitats without human impact. However, the cause of declining is not clear. (Pliasu et al.

2010) this study covers the large elevation ranges i.e. 170 m to 4200 m asl and helps to add new information on literatures about amphibians of this area.

1.4 Objectives

1.4.1 General objective

The general objective of this study was to explore the diversity and distribution pattern of amphibians along elevation gradient in Mahakali River basin, Far-Western Nepal.

1.4.2 Specific objectives

- To know diversity and distribution pattern of amphibians in Mahakali River basin, Far-Western Nepal
- To investigate amphibian body size variation along elevation gradient in Mahakali River basin, Far-Western Nepal

2. LITERATURE REVIEW

2.1 Diversity and distribution pattern of amphibians

Nepal supports a high unrecognized amphibian and reptile's diversity (Schleich and Kastle 2002). It harbors more than 54 species of amphibians and 134 reptiles (Shah and Tiwari 2004), but information about them is generally derived from relatively old studies (Smith and Battersby 1953, Nanhoe and Ouboter 1987, Mitchell and Zug 1995, Zug and Mitchell 1995). These records are primarily based on the species already discovered from neighboring countries like India and Tibet in China. However, these numbers are not based on detailed field studies of population densities within Nepal but are either from presence-only surveys or from anecdotal information. This further confirmed that Nepalese herpetofauna are poorly understood and many of them are incorrectly classified (Khatiwada 2015, Khatiwada et al. 2019). In western Nepal, in the late 1970s, the French herpetologist A. Dubois made a significant contribution to the biology of Nepalese amphibians. He and other collaborators collected more than 15,000 amphibian specimens from Nepal (Dubois 1999) and described more than 10 new species (Dubois and Matsui 1983, Dubois 1984, 1987). In mean time, Nanhoe and Ouboter (1987) surveyed the amphibians and reptiles of Annapurna-Dhaulagiri region of western Nepal and compiled an updated checklist of herpetofauna based on both field observation and museum specimens.

Along elevational gradient not enough studies have been done. Although studies showed that different patterns of species diversity, distribution pattern and species richness along elevational gradient (Rahbek 19950, Brehm et al. 2007). In Bhutan, Koirala et al. (2019) studied the species diversity and spatial distribution of amphibian fauna along the altitudinal gradients in Jigme Dorji National Park, western Bhutan; 16 species of amphibians were recorded by visual encounter survey. Diversity was declined with increasing elevation, similarly species diversity was higher in forest habitat than agro ecosystem. By using encounter survey and pitfall trap methods (Foerster and Conte 2018) recorded a total 33 species comprising nine families from 21 breeding sites in Brazil. The greatest alpha diversity was found in forest interior. In case of beta diversity all three habitat categories differ, which may be due to presence of exclusive species in each category requiring specific habitat and reproductive methods. Amphibian community structure showed monotonic decline of species richness with increasing elevation in Nepal (Khatiwada et al. 2017, Khatiwada and Haugaasen 2015, Khatiwada et al. 2019). They have recorded 29 species of amphibians from eastern Nepal and 17 species from central Nepal. Fifty one herpetofaunal species were recorded from Parsa Naional Park, Nepal (Bhattarai et al. 2018) Nepal. Among all 51 species three amphibians, two Gecko species, one Agamid, two Skinks, thirteen snakes, and one crocodile were new records to Parsa National Park.

Wagh et al. (2017) studied amphibian diversity in different habitats in Maharashtra, India. Amphibian diversity increased with increasing elevation in some places and mid domain effect was also observed (Naniwadekar and Vasudevan 2007) in Western Ghats, South India. Total of 11 species belonging to 4 families were recorded. Forty-four species of amphibians belonging to eight families and three orders reported in Sikkim from 1864 to 2015 (Subba et al. 2016). In United States (U.S.) climatic factors in northern slopes limits the distribution range of amphibians and climatic factors limits the distribution range of reptile species in southern slopes (Cunningham et al. 2015). Species richness decreased as elevation increased and distribution of species separated spatially between lowlands and highlands. Furthermore presence of water bodies has positive significant effect with amphibian richness and no relation with other environmental variables (Zancolli 2014). Ortiy Y. et al. (2013) explored the spatial distribution patterns of amphibian species richness in Antioquia, Their result revealed that temperature has most significant effect on species richness and elevation range has least effect on species richness. Species richness and distribution pattern varied significantly along elevational gradient, but diversity of species remained same at each elevation band. Negative correlation between leaf litter level and number of amphibians was observed (Wiafe and Agyei 2013). Malonza and Veith (2012) concluded that amphibian species richness increased with increased habitat disturbance from forests to streams and dams but decreased with increasing elevation in the Taita Hills, Kenya. Riparian zone had higher amphibian's richness than non-riparian zones. Further River width has also significant effect on species richness (Ribeiro et al. 2012). Keller et al. (2009) investigated the importance of environmental heterogeneity for species diversity and assemblage structure in Bornean stream frogs. The major finding was that different frog communities follow different assemblage rules in different habitat types. Canopy cover is most dominant environmental variable its positive effect (70%) was correlated with nesting. Biotic factors were less effective like hydro period, invertebrate predators and caudate biomass all accounted for 9-16% of the independent effects (Werneret et al. 2007).

2.3 Body size variation along altitudinal gradient

Bergmann's rule for amphibians is still controversial. Some regional amphibian species followed the Bergmann's rule (Asthon 2002, Hu et al. 2011) whereas others did not (Laugen et al. 2005, Adams and Church 2008). Along elevation gradient in eastern Himalaya Nepal, amphibian species showed increasing pattern of body size with increasing elevation; followed Bergmann's rule (Khatiwada et al. 2019). In Taiwan Sauter's frog *Rana sauteri* did not followed Bergmann's rule (Hsu et al. 2014). A positive correlation between body size and elevation was reported for *Rana swinhoana* (Lai et al. 2005), *Rana chensinensis* (Lu et al. 2006; Ma et al. 2009), *Pleurodema thaul* (Iturra-Cid et al. 2010), *Hyla annectans chuanxiensis* (Liao and Lu 2010), and Rana limnocharis (Liu et al. 2012), whereas a converse Bergmann cline was reported for *Rana muscosa* (Matthews and Miaud 2007), *Nanorana parkeri* and *Rana nigromaculata* (Liao et al. 2010). However, *N. parkeri* showed a steeper elevational decrease in female size, whereas no clear elevational relationship was detected for males (Zhang et al. 2012). In this study, the different trends of body size in relation to elevations between the two sexes of *R. sauteri* suggested that the temperature gradient was not only the factor affecting the body size variation in amphibians (Cvetkovic et al. 2009).

Lou et al. (2012) investigated altitudinal variation in age and body size in Yunnan pond frog (*Pelophylax pleuraden*) did not follow Bergmann's rule. But in male average SVL was significantly different but not in females. Body size of females was significantly larger than males and latitudinal variation in body size of *Rana chensinensis* across different temperature environment do not follow Bergmann's rule (Chen et al. 2011). Olalla-Tarraga and Rodri-guez (2007) concluded that anurans follow a marked Bergmann's rule pattern and urodeles are the opposite. Pliasu et al. (2010) studied the body size variation in *Rana temporaria* populations inhabiting extreme environments between two populations of Finland and Retezat. No significant difference in SVL between Retezat National Park and Finland - Kilisjarvi populations but there was significant difference in mean body size indices between three stations of same population in Finland. Their result indicated that various environmental and factors are responsible for body size variation and long term study needs to be conducted for further clarification.

Acevedo and Restrepo (2007) studied the contribution of habitat loss to changes in body size, allometry, and bilateral asymmetry in two Eleutherodactylus frogs from Puerto Rico. Result indicated that habitat destruction leads to decrease in body size of frogs. Body size was decreased from forest, low level of disturbance to high level of disturbance. Schauble (2003) identified the potential factors responsible for geographically structured morphological variation within the widespread Australian frogs *Limnodynastes tasmaniensis* Günther and *L. peronii* Duméril and Bibron. Climate was an important explanation for SSD variation in *L. peronii*, while latitude was most important for *L. tasmaniensis.*

Studying different literatures through different sources shows that there is lacking of information about amphibian's in far western region of Nepal. Still there is lacking of information about amphibians diversity distributions. There is only one article; body size variation in amphibians along elevation gradient is studied in eastern Himalayan region of Nepal before this. In western part of Nepal this type of study is new and this gap will be helpful to gather information about amphibians of Mahakali River basin.

3. MATERIALS AND METHODS

3.1 Study area

This study was conducted in catchment of Mahakali River basin along elevation gradient. Study area covers four districts of Mahakali River basin such as Darchula, Baitadi, Dadeldhura and Kanchanpur located on the southern slope of the Himalayas in the Far-Western Province, Nepal (28.487198 to 29.683765º N and 81.166073 to 81.854328º E). The study also covers some parts of the Api Nampa Conservation Area. The western Nepal is characterized by rugged terrain and high seasonal climatic variability. The spatial change in climatic variation is strongly influenced by elevation gradient. Summer monsoonal rainfall from the Bengal Bay strongly affect the seasonal temperature and precipitation in this region (Bookhagen and Burbank 2010). The mean annual temperature varies between 7 °C and 26 °C, and precipitation mostly occurs (about 80%) during May to October (Vetaas 2000). Climatic factors also vary along the elevation gradient, with mean annual temperature declining linearly from 23.4°C to 7°C with elevation.

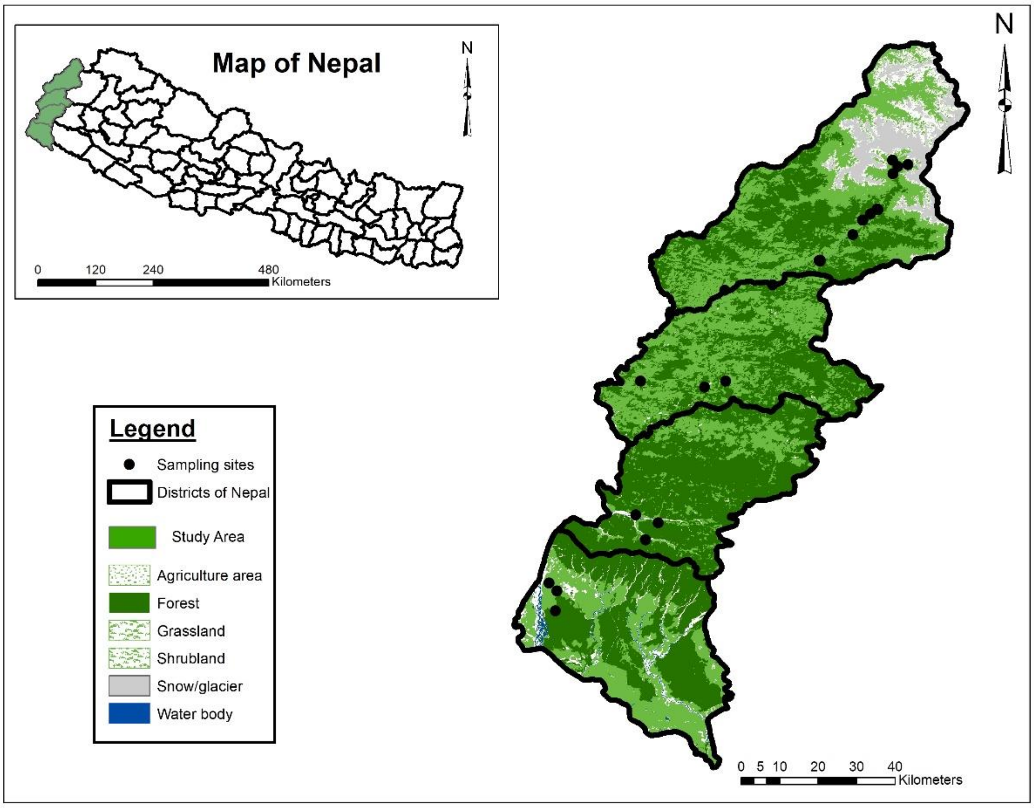

Figure 1. Map of Study Area

3.2 Materials

Following materials were used on field for data collection:

- GPS; Garmin Etrex 10
- Field guide books (Schleich and Kastle 2002, Shah and Tiwari 2004)
- Vernier calliper; DIN862
- Torch light
- Ethanol 70 %
- Weighting instrument; TOP500
- Preservation box
- pH meter; PC60
- Infrared thermometer; ST652
- Conductivity meter; EC331
- HTC-2 digital indoor/outdoor thermo-hygrometer temperature humidity meter tester

3.3 Research design

Preliminary survey was conducted in May 2018 in study area. The sampling sites were designed wherever possible. Information about geographical structure, dense forest, available resources during field survey were obtained from local people also. Field research design was made using, Google Earth, searching information through other sources and field preliminary survey. Study area was designed to survey line transects (100× 4 m) on each 200 m elevation bands from 170 m of elevation to 4, 200 m of elevation in catchment area of Mahakali River basin.

3.4 Methods

3.4.1 Amphibians survey

Surveys were conducted along marshes, temporary pools, seasonal streams and nearby forests, croplands, covering all lotic and lentic habitats. Field surveys were carried out during the monsoonal rainfall and breeding season of frogs. Nocturnal, time-constrained visual encounter surveys were conducted (Campbell and Christman 1982). Data were collected in August to September 2018 and June 2019. Amphibians were surveyed at night along transects (100 x 4 meter) between 7:30 pm to 9:30 pm. Transects were searched by four people for 30 minutes using torches, walking at a slow pace. Three transects on each elevation bands were surveyed wherever possible, over streams, forests and croplands. Transects were placed at least 250m apart from the other. A total of 22 transects were having the presence of amphibians and above

2050 m asl no amphibians were recorded and transects above this elevation are not included in data analysis.

The number of species and individuals encountered in each transect were recorded. Specimens were identified in the field with the help of field guide books (Schleich and Kastle 2002, Shah and Tiwari 2004). The first individual of each species and specimens difficult to identify in the field were euthanized 70% ethanol and preserved) and deposited to Central Department of Zoology, Tribhuvan University, Kirtipur Kathmandu, Nepal.

3.4.2 Environmental variables

Table 2 Environmental variables and collection methods

Parameters	Variables	Description
Habitat variables	Habitat types	Forest, Stream, Cropland
	Canopy cover	Habitat covered by tree (%)
	Distance to water resource	Euclidean distance measured from sampling point to the nearest waterhole with the help of Google Earth Pro
Topographic variables	Elevation	Elevation (meter) above sea level measured by using GPS
Climatic variables	Temperature	Substrate temperature and water temperature (1 meter inside from bank of water body) was measured by infrared thermometer.
	Humidity	Humidity measured in each transect by using HTC-2 digital indoor/outdoor thermo-hygrometer temperature humidity meter tester
	Conductivity	Conductivity of water nearby water resource measured by conductivity meter
Disturbance variables	Distance to road	Euclidean distance measured from sampling points to nearest road or track with the help of Google Earth Pro

	Distance to human settlements	Euclidean distance measured from sampling points to nearest human settlements with the help of Google Earth Pro

3.4.3 Measurement of Body size

To determine body size variation along elevation gradient, snout- vent lengths (SVL) of all adult individuals were measured following (Olalla-Tarraga and Rodriguez 2007) using a Vernier calliper with an accuracy of 0.1mm. All individuals were captured by hand at night using a flashlight. The sex of each individual was confirmed by direct observation of the secondary sexual characteristics (i.e., the vocal sacs in adult males and the ova in adult females). All species and individuals encountered in different habitats, forest, stream, and cropland were recorded separately.

3.5 Data Analysis

All the collected data were entered in a excel sheet and analysed by using following statistical tools.

Shannon Weiner diversity index was used to calculate the species diversity of study area which is calculated as:

$H' = -\Sigma (ni /N) \log (ni/N)$

Where,

H = Index of species diversity

Pi = the proportion of individuals in the ith species = ni N

ni = Importance value for each species (number of individuals)

N = Total importance value (Total number of individuals)

Evenness index was calculated to determine distribution patterns of amphibian species; whether species were distributed evenly across elevation gradient. It was determined by the equation

$E= H'/ \log S$

Where, H' = Shannon-Wiener's diversity index.

S= Species richness is the total number of species

Abundance and diversity of species in three habitat types; cropland, forest and streams were determined by using PAST 3.25 (Hammer et al. 2001).

Canonical correspondence analysis was done by using CANOCO 4.5 version to examine influence of environmental variables on amphibian distribution (terBraak 2009). Final result was presented in the form of biplot with Monte- Carlo permutation test by using 499 permutations under reduced model to identify which variables have significant effect on amphibian species distribution in study area. For every ordination down weighting of rare species was done.

Linear regression was used to analyze effect of elevation on species richness and effect of elevation on species abundance was analyzed by polynomial regression model in PAST 3.25 (Hammer et al. 2001). Similarly, body size variation of amphibian species along elevation gradient was also analyzed by linear regression in MS –Excel.

4. RESULTS

4.1. Diversity and distribution pattern of amphibians

A total of 17 species of amphibians belonging to six families and one order i.e. Anura were recorded from the study area. Dicroglossidae was dominant family; seven species were recorded from this; followed by Bufonifae and Microhylidae, and three species were recorded from both of them. Family Rhacophoridae has two species, family Megophryidae and Ranidae has one-one species respectively (Table 2). Species of Dicroglossidae were recorded from lower elevation to higher elevation (Figure 2). However turnover of species was observed along elevation gradient.

Table 2. Amphibian species recorded along an elevational gradient in Mahakali River basin.

S.N.	Family Name	Species Name	Species code
1.	Bufonidae	1. *Duttaphrynus himalayanus* 2. *Duttaphrynus melanostictus* 3. *Duttaphrynus stomaticus*	1. *Dut_him* 2. *Dut_melo* 3. *Dut_sto*
2.	Dicroglossidae	4. *Hoplobatrachus tigerinus* 5. *Hoplobatrachus crassus* 6. *Minervarya teraiensis* 7. *Euphlyctis cyanophlyctis* 8. *Sphaerotheca maskeyi* 9. *Sphaerotheca braviceps* 10. *Nanorana liebigii*	4. *Hop_tig* 5. *Hop_cra* 6. *Min_ter* 7. *Eup_cya* 8. *Sph_mas* 9. *Sph_bra* 10. *Nan_lei*
3.	Megophryidae	11. *Megophrys parva*	11. *Mego_pr*
4.	Microhylidae	12. *Microhyla teraiensis* 13. *Uperodon globulosus* 14. *Uperodon systomus*	12. *Mic_ter* 13. *Up_glo* 14. *Upe_sys*
5.	Ranidae	15. *Amolops marmoratus*	15. *Amo_mar*
6.	Rhacophoridae	16. *Polypedates taeniatus* 17. *Polypedates maculatus*	16. *Poly_tae* 17. *Pol_mac*

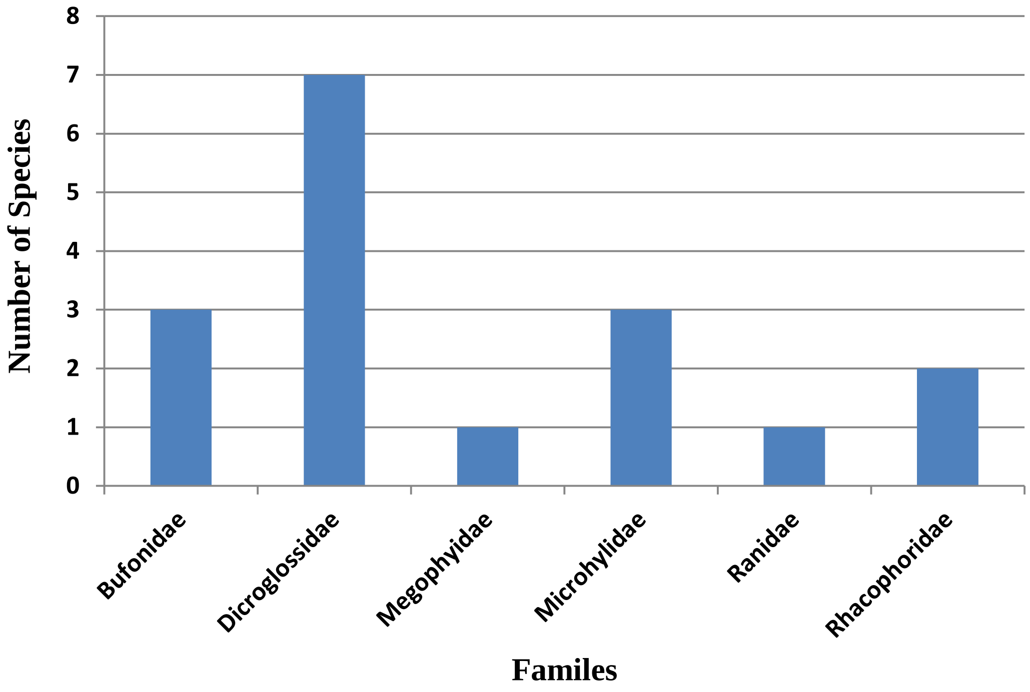

Figure 2. Number of amphibian species in different families

Shannon wiener diversity index and evenness for overall study area was found H = 2.436 and E = 0.672, respectively. Amphibians were not uniformly distributed along elevation gradient. Most of the species restricted in lower elevations. Only few species were recorded in higher elevations. Abundance of *Hoplobatrachus tigerinus* was the highest, followed by *Euphlyctis cyanophlictis, Hoplobatrachus crassus, Duttaphrynus melonistictus, Duttaphrynus himalaynus*. *Uperodon systomus, Ploypedates taeniatus, Sphaerotheca braviceps* had lowest abundance only one individuals was recorded from each of these three species.

Diversity and mean abundance of amphibians in different habitat types; forest, cropland and stream was calculated. The highest species diversity was observed in forest habitat i.e. H = 2.374. Diversity value of cropland and stream were H = 1.983 and H = 1.973 respectively. Evenness index value E = 0.7674 was found in forest, E = 0.7264 and E = 0.7988 were observed in cropland and stream respectively. Mean abundance of species was the highest in cropland habitat and followed by streams and forest habitat (Figure 3).

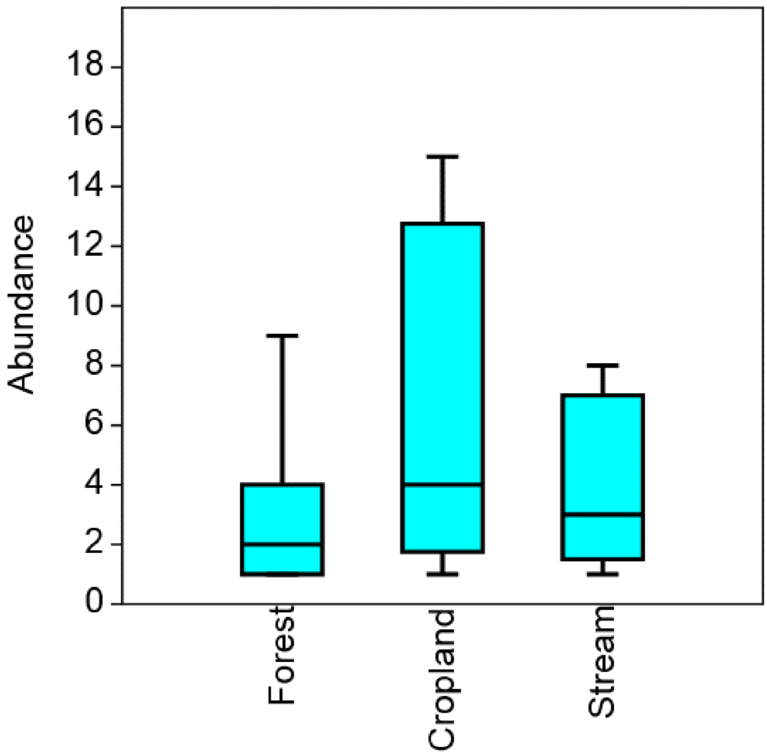

Figure 3. Abundance (Mean ± SD) of species in three habitats at 95 % confidence interval

4.1.1 Species richness and abundance along elevation gradient

There was a continued gradual declining trend of both species richness and species abundance from 170 m to 2050 m (Figure 4 and Figure 5). Linear regression analysis showed that species richness is declined with increasing elevation. Number of individuals were more in lower elevation and decreased with increasing elevation and slightly increased in higher elevations. (Figure 5). Ten species were recorded from the lowest elevation i.e. 170 m of elevation. *Hoplobatrachus tigerinus* and *Hoplobatrachus crassus* were dominant species of lower elevation. Eight species from 415 m elevation, seven species from 630 m elevation, six species from 980 m elevation, and four species from 1190 m elevation, *Nanorana liebigii* and *Duttaphrynus himalayanus* were dominant species of higher altitudinal regions. Three species from 1420 m elevation, two species from 1700 m elevation, and only one species *Duttaphrynus himalayanus* was recorded from 2050 m elevation. There was no presence of amphibian species above 2050 m elevation.

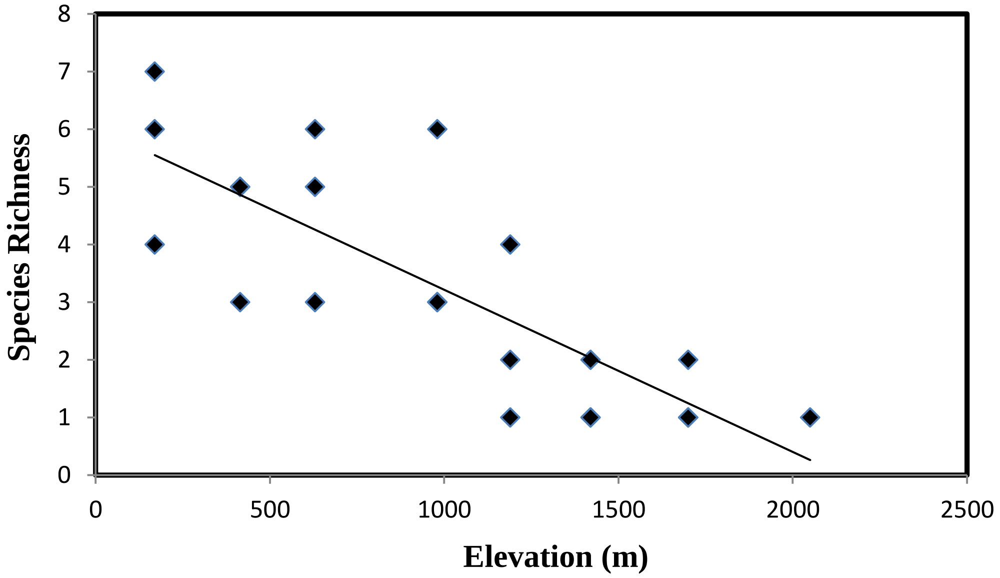

Figure 4. Linear regression model showing the effect of altitude on amphibian species richness in Mahakali River basin ($y = -0.0028x + 6.028$, $R^2 = 0.65$, $P<0.01$)

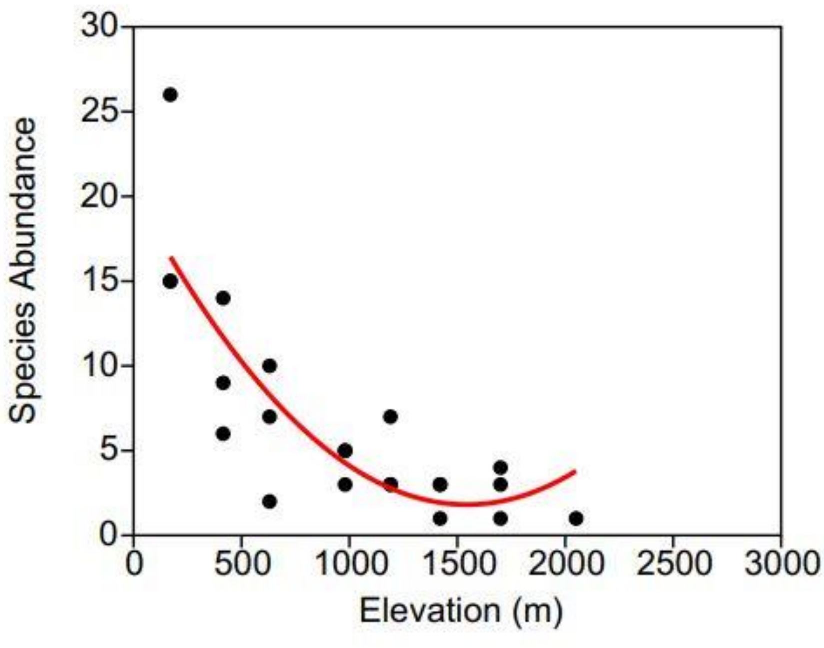

Figure 5. Polynomial regression model showing the effect of altitude on amphibian species abundance in Mahakali River basin. Equation: 7.751E-06x2-0.02393x+20.28$R^2 = 0.72$, $p<0.01$.

4.1.2. Influence of environmental variable on distribution pattern of amphibians

Effect of environmental variable on amphibian distribution indicated that substrate temperature and water temperature have same significant effect on species distribution so only one of them is interpreted here Species code are in (Table 1). Environmental variables are given code as: Elev = Elevation, Sub_temp = Substrate temperature, Conduct = Conductivity of water, PH = PH of water, Can_cov = canopy cover) First two axes are displayed. The first axis accounts for 38.5.9% and the second axis 21.3 % of the variability. *Amolops marmoratus, Duttaphrynus himalaynus, Nanorana liebigii* and were recorded in the high elevation sampling sites and showed strong association with elevation, while *Polypedates taeniatus*, *Hoplobatrachus crassus*, *Uperodon globulossus, Uperodon systomus*, *Hoplobatrachus tigerinus* and *Hoplobatrachus crassus* showed a negative correlation with elevation (Figure 6). *Duttaphrynus melanostictus* and *Euphlyctis cyanophlyctis* were recorded in most of the elevation bands. Similarly, substrate temperature has reverse effect on distribution of amphibians because of while elevation increases substrate temperature decreases. In this way those species which are found in higher elevation bands they were recorded from those transects where is low substrate temperature and those species which were recorded from lower elevation, they were recorded from those transects where is high substrate temperature. Other variables like habitat type and disturbance variable were also analysed with species data in CANOCO but their effect on distribution of amphibians was not significant (P> 0.05) that’s why these results are not interpreted in the figure.

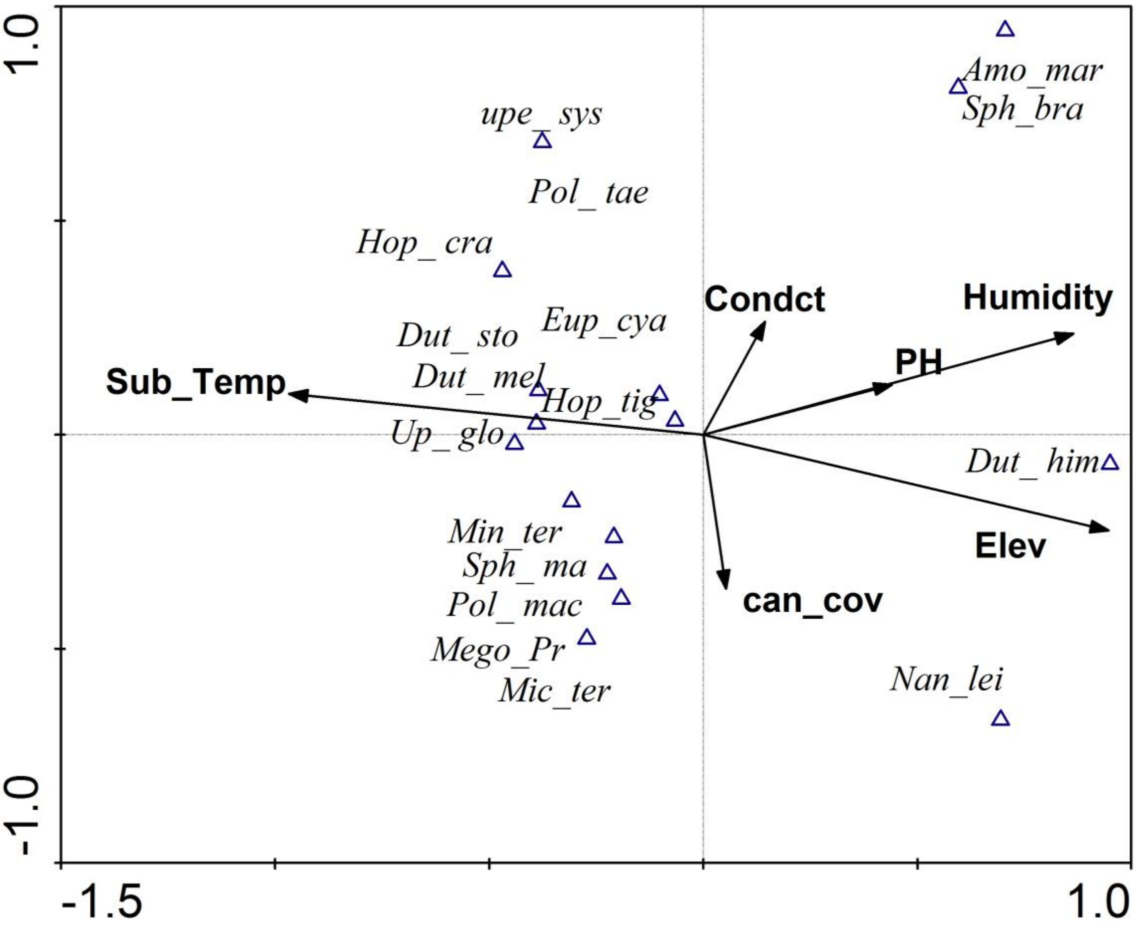

Figure 6. CCA ordination diagram (biplot) showing effect of different environmental variable on distribution of amphibians in Mahakali River basin. MonteCarlo permutation test of significance of all canonical axes: Trace= 1.926, F= 1.606, P =0.004, with 499 permutations.

4.2 Body Size variation along elevation gradient

Body size or snout-vent lengths (SVL) of all individuals from three families; Bufonidae, Dicroglossidae and Microhylidae were analysed by linear regression to test effect of elevation on body size of these frog species. In these families, individuals were collected from more than one elevation bands. Species and individuals from remaining three familes; Ranidae has only one species and recorded in only one elevation band. Megophryidae has one species and two individuals. Rhacophoridae has two species and only 3 individuals recorded from one elevation band only. So individuals of these families were not used to analyse body size variation along elevation gradients. Linear regression model showed that body size of individuals from two families; Bufonidae and Dicroglossidae were increased with increasing elevation (Figure 7, Figure 8). Individuals from lower elevation have larger body size than those individuals which were recorded from higher elevations. In this way, effect of elevation on body size was positively correlated as elevation increased body size reduced in both sexes followed Bergmann's rule.

Body size or snout-vent lengths (SVL) of all individuals of Microhylidae did not showed positive correlation with elevation. Body size of individuals belonging this family was declined with increasing elevation (Figure 9). Effect of elevation on body size was negatively correlated as elevation increased body size reduced in both sexes. Body size of these frog species was larger in lower elevation bands and declined with increasing elevation.

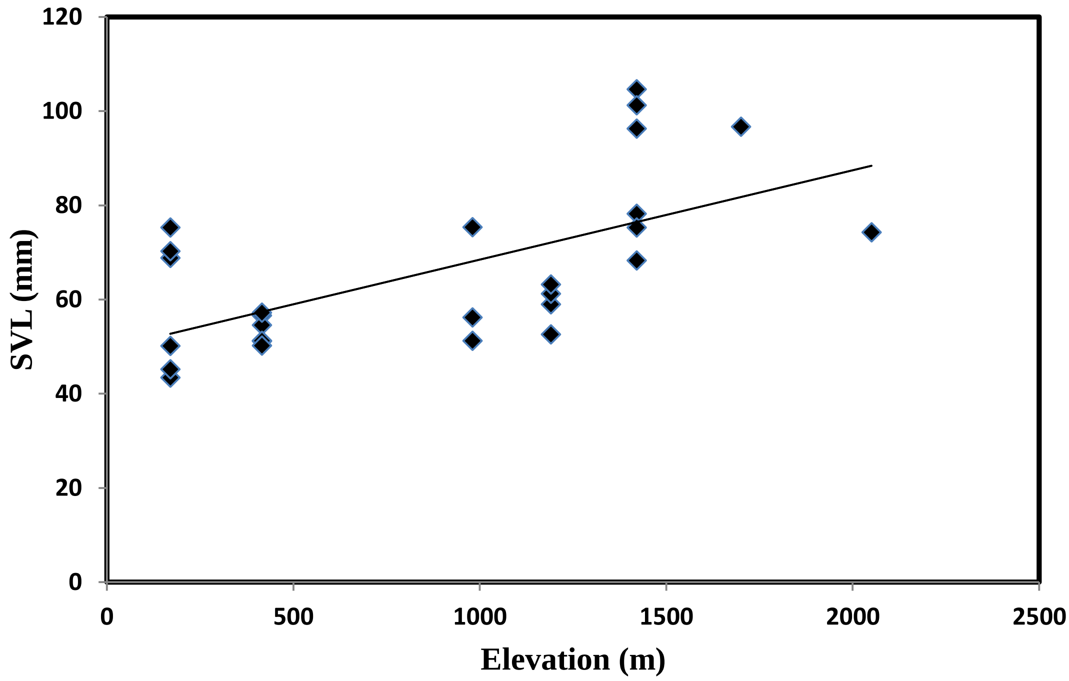

Figure 7. Linear regression showing effect of elevation on body size of Bufonidae family in Mahakali River basin (y = 0.019x + 49.481 R^2 = 0.39, p<0.01).

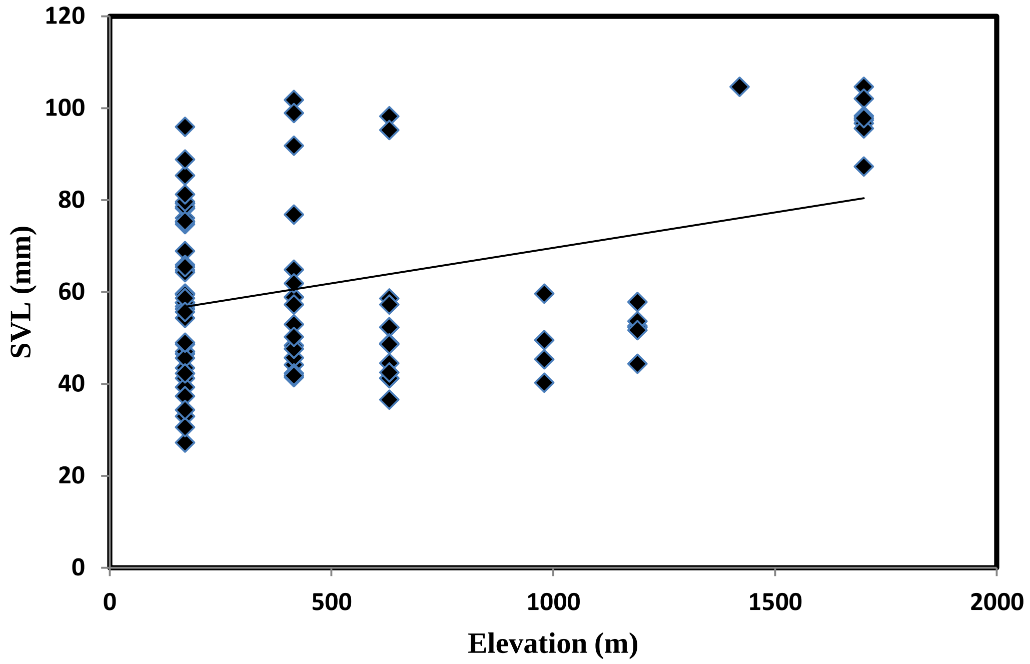

Figure 8. Linear regression showing the effect of elevation on body size of Dicroglossidae family in Mahakali River basin ($y = 0.0155x + 54.104$, $R^2 = 0.1327$, $p<0.01$).

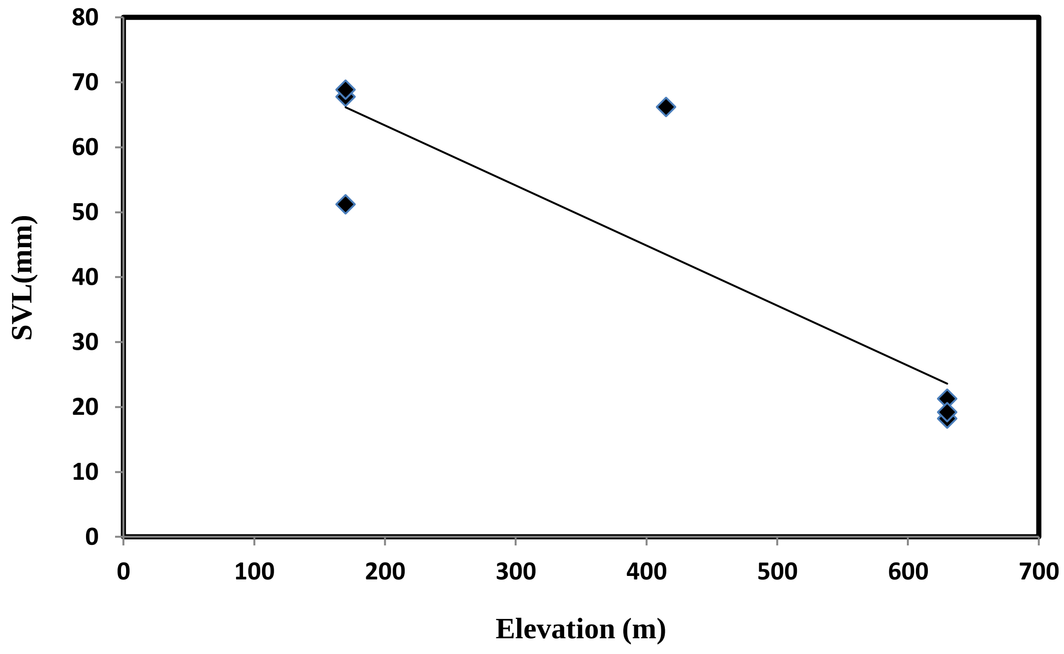

Figure 9. Linear regression showing effect of elevation on body size of Microhylidae family in Mahakali River basin ($y = -0.0925x + 81.902$ $R^2 = 0.78$, $p= 0.0317$).

5. DISCUSSION

This study was conducted in Mahakali River basin, far western Nepal. Study carried out with the objective of determining diversity and distribution pattern of amphibians along elevational gradient in Mahakali River basin. Body size variation of amphibians along elevation gradient has been assessed. The current study would therefore be a valuable addition to the literature.

5.1 Diversity and distribution pattern of amphibians

This study recorded 17 species of amphibians in Mahakali River basin. Species diversity was H= 2.436 and evenness, E= 0.672. The present study revealed a declining trend in amphibian species richness with increasing elevation. A similar trend was observed for amphibian assemblage in Jigme Dorji National Park, western Bhutan, Western Ghats, India, along elevation gradient in eastern Himalaya, and along elevation gradient in Chitwan Nepal. (Koirala et al. 2019, Naniwadekar and Vasudevan 2007, Khatiwada et al. 2019, Khatiwada and Haugaasen 2015). Many other studies related to amphibians have shown a monotonic decline of species richness along elevational gradients (Zancolli 2014, Fu et al. 2007, Malonza and Veith 2012). Species richness was observed higher in lower elevation ten species were recorded from lowest elevation band. This high species richness in lower region is because of high average temperature, productivity, annual precipitation; these are widely recognised as important factors for spatial distribution pattern of amphibians (Buckley and Zetz 2007). *Euphlyctis cyanophyctis* was recorded from most elevation bands. This species has larger distribution range than other species (Shah and Tiwari 2004, Bhattarai et al. 2017). Similar result was found in the altitudinal gradient in Chitwan (Khatiwada and Haugaasen 2015). Species turn-over was seen this study some species are restricted to lower elevation, *Hoplobatrachus crassus, Hoplobatrachus tigerinus, Uperodon globulossus, Uperodon systomus, Polypedates taeniatus*. This is because of adaptation of these species in terai region and some species are restricted to higher elevation, these species are adapted to cold climates, *Nanorana liebigii, Duttaphrynus himalayanus, Amolops marmoratus* (Mattea 2018, Malonza and Veith 2012).

In this study, only few species were recorded from higher elevations and there is no any species recorded above 2050 m of elevation however survey was done up to 4200 m elevation. However similar type of study in eastern Himalaya showed that amphibian species are present up to 3400 m asl in Nepal. There are lots of reasons for declining amphibian species globally, because of climate change and other anthropogenic activities (Baillie et al. 2004). Moreover; a number of ecosystems are being fragmented and degraded by climate change (Araujo et al. 2006) and environmental pollution (Davidson 2004). Similarly, other researchers have shown that without any known reason amphibian species are going to extinct from higher elevations globally (Pliasu et al. 2010). One additional information was obtained during field survey from field guide and other local persons around study sites; there was overexploitation of *Nanorana liebigii* in higher elevation. This species is being consumed by tourists and Yarsagumba (*Ophiocordyceps sinensis*) collectors during pre-monsoon season. These peoples come from outside of study area and local peoples are not involved in consumption of frogs. Information obtained from local guides; overcrowding on habitat, overconsumption of frogs, pollution and disturbing their nests before breeding season is one of the strong reasons behind absence of species in higher elevations in this study area. This is supported by many other researchers, their finding is that only high elevation is not the cause of declining species but human disturbances have negative impact on species composition and assemblages. (Cayuela et al. 2006, Bell and Donnelly 2006). Some studies have shown that mid peak elevation occurs in amphibian research (*Hu* et al. 2011, Fu et al. 2006). There is less biodiversity in higher elevations due to less surface area (Korner 2007, Brown 2001) and therefore monotonic decline of species richness occurs in amphibian species in the higher elevations.

Canonical correspondence analysis showed that elevation, Humidity, and substrate temperature cause strong effects on amphibian species distribution. This means that abiotic factors are more responsible for species distribution than biotic factors, such as canopy cover. In this study almost individuals were captured from open area. This is supported by the study of Khatiwada and Haugaasen (2015). There was not significant effect of canopy cover on amphibian's distribution elevation was major factor for species distribution. But in some studies, canopy cover and leaf litter level were the major factors for amphibian species distribution (Mattea 2018). Similarly, Keller et al. (2009) found not only environmental variables are responsible for amphibian distribution, cross- product of both environmental parameters and spatial parameters are associated with composition of anurans.

Diversity and mean abundance of amphibians in different habitat types, forest, cropland and stream was calculated. Highest species diversity was observed in forest habitats i.e. 2.374. Diversity value of cropland and stream were 1.983 and 1.973 respectively. This result indicated that forest habitat is commonly used by both frogs which are found in cropland and tree frogs, other leaf litter frogs, *Polypeadates maculatus, Polypedates taeniatus, Uperodon globulossus* *and Uperodon systomus*. Other studies are supportive to this result their finding is amphibian species diversity in forest habitat is higher than other habitats (Syamili and Nameer 2018, Ray 1999). Their findings also support present result. *Polypedatus maculatus* and *Uperodon taprobanicus* were only present in the forests. In this study, abundance of amphibians was observed higher in cropland habitat than other habitats due to the higher probability of more number of prey in croplands.

5.2 Body size variation along elevation gradient

In this study, effect of elevation on body size; snout-vent length (SVL) of three families; Bufonidae, Dicroglossidae and Microhylidae were analyzed by linear regression model. SVL of all individuals from six families were measured however only these three families were used in analysis; because of individuals of other three families were not recorded from more than one elevation bands. *Amolops marmoratus* belonging to Ranidae family has only one species and was recorded from one elevation points i.e. 980 m asl. Family Megophridae has only two individuals and Family Rhacophoridae has also three individuals of two species from lower elevations.

It is still controversial whether all amphibian species follow Bergmann's rule or not (Adams and Church 2008). Present study result showed that body size of amphibian species belonging to Bufonidae and dicroglossidae families was increased with increasing elevation. Body size of both male and female frogs in lower elevations was smaller than those from higher elevations. Family Microhylidae did not followed Bergmann's rule in present study. Body size of individuals belonging to this family was declined with increasing elevation. Similar type of study was conducted by (Khatiwada et al. 2019) and their result was also similar to present result as all individuals from all families followed the Bergmann's rule except Microhylidae family. Some other researchers have also shown that some species of amphibians follow Bergmann's rule. Similar type of study was conducted in altitudinal gradient in China and their result indicated that variation in body size of three *Rana limnocharis* populations inhibiting different elevations showed that individuals from higher elevations were larger than those from lower elevations. And same results are reported in earlier studies for *Rana limnocharis* from two relatively larger altitudinal gradient populations (Liao et al. 2011, Lu et al. 2006, Ma et al. 2009). Snout-vent length of frog species inhabiting cold habitat has larger than those which were from warmer habitat (Manjano and Bautista 2017). Some other studies have shown that amphibian species follow Bergmann's rule but Urodeles have shown opposite of this (Olalla-Tarraga and Rodriguez 2007).

In present study, family Microhylidae did not followed the Bergmann's rule; similar results have shown by other researchers, their finding is that Bergmann's rule is not valid for all taxa. Their study found that there was negative correlation between species of Microhylidae and elevation because of their narrow distribution range (Hu et al. 2011, Khatiwada et al. 2019). Some amphibian species has followed the Bergmann's rule but it is not valid for anuran species that mean species specific trend is seen in case of body size variation with altitudinal gradient (Meiri and Dayan 2003, Mousseau 1997).

6. CONCLUSIONSION AND RECOMMENDATIONS

6.1 Conclusion

This study indicated that Mahakali River basin is moderately rich in amphibian diversity, which decreases along the elevation gradients. This is because lower elevation areas provides suitable habitats for amphibian species (i.e., more temperature, humidity and food).

Diversity of amphibians and influence of environmental variables on their distribution pattern in Mahakali River basin was determined. Species richness along elevation gradient showed that declining of species richness with increasing elevation. Effect of environmental variables on species distribution was analysed by canonical correspondence analysis (CCA). Result of CCA showed that effect of elevation, substrate temperature, and humidity has strong effect on species distribution. Species turn-over was observed in the study area. Most of the species were recorded from lower elevation and only few species are restricted to the higher elevations. There was no record of amphibians above 2050 m asl. The reason behind this is predicted that overexploitation of *Nanorana liebigii* species by peoples before their breeding season. Disturbing the habitat and exploitation of nests before breeding may have created eradication of amphibian species from study area. And other unknown causes are there for loss of amphibian species from higher elevations this information was obtained from other literatures.

Body size variation along elevation gradient was analysed by linear regression. Three families; Bufonidae, Dicroglossidae and Microhylidae were used for this analysis. Among them individuals from two families; Bufonidae and Dicroglossidae followed the Bergmann’s rule. Body size was increased with increasing elevation except family Microhylidae.

6.2 Recommendations

Based on this research, following recommendation were made which will be useful for conservation and further study of amphibian species in Mahakali River basin.

- There may be more than 17 species of amphibians in Mahakali River basin therefore further studies recommended.
- Possible other reasons of no any species in higher elevation i.e. above 2050 m elevation need to be identified.

7. REFERENCES

Adams, D.C. and Church, J.O. 2008. Amphibians do not follow Bergmann's rule. Evolution **62**: 413-420.

Araujo, M.B., Thuiller, W. and Pearson, R.G. 2006. Climate warming and the decline of amphibians and reptiles in Europe. Journal of Biogeography **33**: 1712-1728.

Ashton K.G. and Feldman C.R. 2003. Bergmann's rule in non-avian reptiles: Turtles follow it, lizards and snakes reverse it. Evolution **57**: 1151-1163.

Ashton, K.G. 2002. Do amphibians follow Bergmann's rule? Canadian Journal of Zoology **80**: 708-716.

Avise J.C. 2000. Phylogeography: the history and formation of species. Cambridge, MA: Harvard University Press.

Baillie, J., Hilton-Taylor, C. and Staurt, S.N. 2004. IUCN red list of threatened species: a global species assessment. IUCN, Gland, Switgerland and Cambdrige, UK.

Bell, K.E. and M.A. Donnelly. 2006. Influence of forest fragmentation on community structure of frogs and lizards in north-eastern Costa Rica. Conservation Biology **20**(6):1750-1760.

Bhattarai, S., Pokheral, C.P., Lamichhane, B. and Subedi, N. 2017. Herpetofauna of a Ramsar Site: Beeshazar and Associated Lakes, Chitwan National Park, Nepal. IRCF Reptiles & Amphibians **24**(1): 17-29.

Bhattarai, S., Pokheral, C.P., Lamichhane, B.R., Regmi, U.R., Ram, A.K., and Subedi N. 2018. Amphibians and reptiles of Parsa National Park, Nepal. Amphibian & Reptile Conservation **12**(1): 35-48.

Brehm G, Colwell, R.K, and Kluge J. 2007. The role of environment and mid-domain effect on moth species richness along a tropical elevational gradient. Global Ecology and Biogeography **16**: 205-219.

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

Butchart, S.H.M., Walpole, M., Collen, B., Van Strien, A., Scharlemann, J.P.W., and Almond, R.E.A. et al. 2010. Global biodiversity: indicators of recent declines. Science **328**: 1164-1168.

Campbell, H.W. and Christman, S.P. 1982. Field techniques for herpetofaunal community analysis. In: Scott, N.J. (Ed.), Herpetological Communities. US Fish and Wildlife Service, pp. 193-200 (Wildlife Research Report 13).

Cayuela, L., Golicher, D.J., Benayas, J.M.R, Gonzalez-Espinosa, M. and Ramirez-Marcial, N. 2006. Fragmentation, disturbance and tree diversity conservation in tropical montane forests. Journal of Applied Ecology **43**: 1172-1181.

Chen, B.Y., Lio, W.B. and Mi, Z.P. 2011. Body size and age of the China Wood Frog (*Rana chensinensis*) in northeastern China. North-Western Journal of Zoology **7** (2): 236-242.

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

Colwell, R.K., Rahbek, C. and Gotelli, N.J. 2004. The mid-domain effect and species richness patterns: what have we learned so far? American Naturalist **163**: 1-23.

Cooper N., Bielby, J., Thomas G.H. and Purvis A. 2008. Macroecology and extinction risk correlates of frogs. Global Ecology and Biogeography **17**: 211-21.

Cunningham, H.R., Rissler, L.J., Buckley, L.B. and Urban, M.C. 2015. Abiotic and biotic constraints across reptile and amphibian ranges. Ecography **38**: 001-008.

Cvetkovic, D., Tomasevic, N., Ficetola G.F., Crnobrnja-Isailovic, J. and Miaud, C. 2009. Bergmann's rule in amphibians: combining demographic and ecological parameters to explain body size variation among populations in the common toad Bufo bufo. Journal of Zoological Systematics and Evolutionary Research **47**:171-180.

Davidson, C. 2004. Declining downwind: amphibian population declines in California and historical pesticide use. Ecological Applications **14**:1892-1902.

Dobremez, J.F. 1976. Exploitation and Prospects of Medicinal Plants in Eastern Nepal. Mountain Environment and Development. Kathmandu. SATA (Swiss Association for Technical Assistance), pp. 97-110.

Dubois, A. and Matsui, M. 1983. A new species of frog (genus *Rana*, subgenus *Paa*) from western Nepal (Amphibia: Anoura). Copeia (4): 895-901.

Dubois, A. 1987. Living amphibians of the world: a first step towards a comprehensive checklist. Alytes **5** (3): 99-149.

Dubois, A. 1999. Miscellanea nomenclatorica batrachologica. 19. Notes on the nomenclature of Ranidae and related groups. Alytes **17** (1–2): 81-100.

Duellman, W.E., and Trueb, L. 1994. Biology of amphibians. JHU press, Baltimore, USA.

Ernst, R., Linsenmair, K.E. and Rodel, M.O. 2006. Diversity erosion beyond the species level: dramatic loss of functional diversity after selective logging in two tropical amphibian communities. Biological Conservation **133**(2):143-155.

Foerster, N.E. and Conte, C.E. 2018. Anuran diversity in an Araucaria Forest fragment and associated grassland area in a sub-tropical region in Brazil. Herpetology Notes, **11**: 421-428.

Freckleton, R.P. and Watkinson, A.R. 2003. Are all plant Populations metapopulations? Journal of ecology **91**: 321-324.

Frost, D.R. 2017. Amphibian Species of the World: An Online Reference. Version 6.0. American Museum of Natural History, New York, USA.

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

Gaeta J.W., Ahrenstorf T.D., Diana J.S. Fetzer W.W., Jones T.S., Lawson Z.J. et al. 2018. Go big or… don't? A field-based diet evaluation of freshwater piscivore and prey fish size relationships. PloS ONE **13**(3):0194092. https://doi.org/10.1371/journal.pone.0194092.

Gemi, J., Morgadol, L.N., Semenova-Nrlsen, T.A. and Schilthuizen, M. 2017. Changes in richness and community composition of ectomycorrhizal fungi among altitudinal vegetation types on Mount Kinabalu in Borneo New Phytologist **215**: 454-468.

Gouveia S.F. and Correia I. 2016. Geographical clines of body size in terrestrial amphibians: water conservation hypothesis revisited. Journal of Biogeography **43**: 2075-84.

Greenbum, E. and komar, O. 2005. Threat assessment and conservation prioritization of the amphibians of El Salvador. Biodiversity and Conservation **14**:2377-2395

Hammer, O., Harper, D.A.T. and Ryan, P.O. 2001. PAST. Paleontological statistics software package for education & data analysis, Paelentologia Electrica **4**(1): 9pp.

Hasan, M.K. and Feeroz, M.M. 2014. Species diversity and habitat preferences of amphibian fauna in six protected areas of Bangladesh. Bangladesh Journal of Zoology **42**(1): 105-116.

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

Hsu, F.H., Hsieh, Y.S., Wu, S.H., and Kam, Y.C. 2014. Altitudinal variation in body size and age structure of the Sauter's frog Rana sauteri in Taiwan. Zoological Studies. 53:62.

Hu, J., Xie F., Li, C. and Jiang, J. 2011. Elevational Patterns of Species Richness, Range and Body Size for Spiny Frogs. PLoS ONE **6** (5): e19817. https://doi.org/10.1371/journal.pone.0019817

Hunter, M.L., and Yonzon, P. 1993. Altitudinal distributions of birds, mammals, people, forests, and parks in Nepal. Conservation Biology 7: 420-423.

Iturra-Cid, M., Ortiz, J.C., and Ibarguengoytia, R. 2010. Age, size, and growth of the Chilean frog Pleurodema thaul (Anura: Leiuperidae): latitudinal and altitudinal effects. Copeia 609-617.

Jinadasa, J., Herath, H.M.B.K. and Samarasinghe, J.M.S.H.K. 2003. Species Composition and Altitudinal Distribution of Fish in Upper Walawe River Basin in Sri Lanka Journal of Aquatic Sciences **8**: 67-83.

Kastle, W., K. Rai, and H.H. Schleich. 2002. Field Guide to Amphibians and Reptiles of Nepal. ARCO-Nepal e.V., Munchen, Germany.

Keller, A., Rodel, M.O., Linsenmair, K.E. and Grafe, T.U. 2009. The importance of environmental heterogeneity for species diversity and assemblage structure in Bornean stream frogs. Journal of Animal Ecology **78**: 305-314.

Khatiwada, J.R. and Haugaasen, T. 2015. Amphibian species richness and composition along an elevational gradient in Chitwan, Nepal. M.Sc. dissertation, Norwegian University of Life Sciences, Norway.

Khatiwada, J.R., Zhao, T., Chen, Y., Wang, B., Xie, F., Cannatella, D.C. and Jiang, J. 2019. Amphibian community structure along elevation gradients in eastern Nepal Himalaya. BMC Ecology **19**:19.

Koirala, B.K., Cheda, K. and Penjor, T. 2019. Species diversity and spatal distributon of amphibian fauna along the alttudinal gradients in Jigme Dorji Natonal Park, western Bhutan. Journal of Threatened Taxa **11**(10): 14249-14258.

Korner, C. 2007. The use of 'altitude' in ecological research. Trends in Ecology & Evolution **22**: 569-574.

Lai Y.C., Lee, T.H. and Kam, Y.C. 2005. A skeletochronological study on a subtropical, riparian ranid (*Rana swinhoana*) from different elevations in Taiwan. Zoological Sciences **22**: 653-658.

Laugen, A.T., Laurila A., Jonsson K.I., Soderman F., Merila J. 2005. Do common frogs (*Rana temporaria*) follow Bergmann's rule? Evolutionary Ecology Research **7**:717-31.

Leviton, A., Myers, G. and Swan, L. 1956. Zoological results of the California Himalayan expedition to Makalu, Eastern Nepal. Amphibians and reptiles. Natural History of Museum Stanford University, Stanford, USA.

Liao, W. and Lu, X. 2010. Age structure and body size of the Chuanxi Tree Frog *Hyla annectans chuanxiensis* from two different elevations in Sichuan (China) Zoologischer Anzeiger **248**: 255- 263.

Liao, W.B., Lu, X., Shen, Y.W., Hu, J.C. 2011. Age structure and body size of two populations of the rice frog *Rana limnocharis* from different altitudes. Italian Journal of Zoology **78**: 215-221.

Lips, K. 1999. Mass mortality and population decline of anurans at an upland site in western Panama. Conservation Biology **13**: 117-125.

Liu, Y.H., Zeng, Y., Liao, W.B., Zhou, C.D., Mi, Z.P., Mao, M. et al. 2012. Altitudinal variation in body size in the rice frog (*Rana limnocharis*) in Southwestern China. Acta Herpetologica **7**(1): 57-68.

Lou, S.L., Jin, L., Liu,Y. H., Mi, Z.P., Tao, G., Tang et al. 2012. Altitudinal variation in age and body size in Yunnan Pond Frog (*Pelophylax pleuraden*). Zoological Science **29**: 493-498.

Lu, X., Li, B., Liang, J.J. 2006. Comparative demography of a temperate anuran, *Rana chensinensis*, along a relatively fine altitudinal gradient. Canadian Journal of Zoology **84**: 1789-1795.

Ma, X., Tong, L. and Lu, X. 2009. Variation of body size, age structure and growth of a temperate frog, *Rana chensinensis*, over an elevational gradient in Northern China. Amphibia-Reptilia **30**: 111- 117.

Malonza, P.K. and Veith, M. 2012. Amphibian community along elevational and habitat disturbance gradients in the taita hills, Kenya. Herpetotropicos **7** (1-2): 07-16.

Manzano, R.L. and Bautista, A.M. 2017. Diet comparison between rainforest and cave populations of *Craugastor alfredi* (Anura: Craugastoridae): does diet vary in contrasting habitats? Journal of Natural History 51: 2345-2354.

Mattea, R.C. 2018. Environmental Factors Influencing Amphibian Species Richness and Distribution in Chocoan Rainforest, NW Ecuador. M. Sc. Dissertation. University of Sussex.

Matthews, K.R. and Miaud, C. 2007. A skeletochronological study of the age structure, growth, and longevity of the mountain yellow-legged Frog, *Rana muscosa*, in the Sierra. Copeia 986-993.

Mazerolle, M.J. 2001. Amphibian activity, movement patterns, and body size in fragmented peat bogs. Journal of Herpetology **35**:13- 20.

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

Meiri, S. and Dayan, T. 2003. On the validity of Bergmann's rule. Journal of Biogeography **30**: 331-351.

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

Mousseau T.A. 1997. Ectotherms follow the converse to Bergmann's Rule. Evolution **51**: 630-632.

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

Naniwadekar, R. and Vasudevan, K. 2007. Patterns in diversity of anurans along an elevational gradient in the Western Ghats, South India Journal of Biogeography **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**: 82-91.

Ollala- Tarraga, M.A. and Rodriguez, M.A. 2007. Energy and interspecific body size patterns of amphibian faunas in Europe and North America: anurans follow Bergmann's rule, urodeles its converse. Global Ecology and Biogeography **16**: 606-617.

Ortiz-YustY, C.E. 2013. Temperature and Precipitation as predictors of species richness in Northern Andean amphibians from Colombia **35**(1): 65-80.

Parris, K.M. 2004. Environmental and spatial variables influence the composition of frog assemblages in sub-tropical eastern Australia. Ecography **27**: 392-400.

Pawar, S., Koo, M.S., Kelley, C., Ahmed, M.F., Chaudhuri, S. and Sarkar, S. 2007. Conservation assessment and prioritization of areas in Northeast India: Priorities for amphibians and reptiles. Biological Conservation **136**: 346-361.

Pliasu, R., Bancila, R. and Cogalniceanu, D. 2010. Body size variation in *Rana temporaria* populations inhabiting extreme environments. Ovidius University Annals of Natural Sciences, Biology - Ecology Series **14:** 121-126.

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

Ray, P. 1999. Systematic studies on the amphibian fauna of the district Dehradun, Uttarpradesh, India. Memoirs of the Zoological Survey of India, Kolkata **18**(3): 90.

Ribeiro, J.W., Lima, A.P. and Magnusson, W.E. 2012. The Effect of Riparian Zones on Species Diversity of Frogs in Amazonian Forests. Copeia **3**: 375-381.

Schleich, H.H., and Kastle, W. 2002a. Amphibians and reptiles of Nepal. ARG. Ganther Verlag KG. Ruggell.

Schleich, H.H., and Kastle, W. 2002b. Amphibians and reptiles of Nepal: Biology, Systematics, Field Guide. ARG. Ganther Verlag KG, Ruggell, Germany.

Schuble, C.S. 2004. Variation in body size and sexual dimorphism across geographical and environmental space in the frogs *Limnodynastes tasmaniensis* and *L. peronei*. Biological Journal of the Linnean Society, **82**: 39-56.

Shah, K.B. and Tiwari, S. 2004. Amphibians of Nepal: A Conservation Companion. IUCN the World Conservation Union, Kathmandu, Nepal.

Sizling, A.L. and Keil, D.P. 2009. Rapport's rule, species tolerances, and the latitudinal diversity gradient: geometric considerations. Ecology **90** (12): 3575-86.

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

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

Subba, B., Arvind, N.A. and Ravikanth, G. 2016. Amphibians of the Sikkim Himalaya, India: an annotated checklist. Journal of biodiversity data.

Syamili, M.S. and Nameer, P.O. 2018. The amphibian diversity of selected agroecosystems in the Southern Western Ghats, India. Journal of Threatened Taxa, **10**(8): 12027-12034.

terBraak, C.J.F. 2009. Biometris- quantitative methods in the life and earth sciences: Plant Research International, Wageningen University and Research Centre Box 100, 6700 AC Wageningen, the Netherlands.

Tocher, M.D., Gascon, C. and Zimmerman, B.L. 1997. Fragmentation effects on a central Amazonian frog community: a ten-year study. Tropical forest remnants: ecology, management, and conservation of fragmented communities. University of Chicago Press, Chicago 124-137.

Vetaas, O.R. 2000. Comparing species temperature response curves: population density versus second-hand data. Journal of Vegetation Science **11**:659-666.

Vetaas, O.R., and 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**:291-301.

Vitt, L.J., Pianka, E.R., Cooper, W.E. Jr., and Schwenk, K. 2003. History and the global ecology of squamate reptiles. American Naturalist 162: 44-60.

Wagh, G.A., Rawankar, A.S., Sharma, V. and Wadatkar, J.S. 2017. A preliminary study on the amphibian diversity in different habitats of Amravati district, Maharashtra. Journal of Entomology and Zoology Studies **5**(1): 158-162.

Werner, E.E., Skelly, D.K., Relyea, R.A. and Yurewicz, K.L. 2007. Amphibian species richness across environmental gradients. Oikos **116**: 1697-1712.

Whiles, M.R., Lips, K.R., Pringle, C.M., Kilham, S.S., Bixby R.J., Brenes, R. et al. 2006. The effects of amphibian population declines on the structure and function of Neotropical stream ecosystems. Frontiers in Ecology and the Environment **4**:27-34.

Wiafe, E.D. and Agyei, D. 2013. Species richness, diversity and distribution of amphibians along elevational gradient on mountain Afadjato, Ghana. Eurasian Journal of Forest Science **1**(2): 68-76.

Zancolli, G., Dewenter, I.S. and Rodel, M.O. 2014. Amphibian diversity on the roof of Africa: unveiling the effects of habitat degradation, altitude and biogeography. Journal of Conservation Biology. Diversity and Distributions **20:** 297-308.

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

Zhang, L.X., Ma, X., Jiang, J. and Lu, X. 2012. Stronger condition dependence in female size explains altitudinal variation in sexual size dimorphism of a Tibetan frog. Biological Journal of Limnological Society **107**:558-565.

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

PHOTOGRAPHS

Photo 1: Polypedates maculatus

Photo 2: ***Uperodon globulossus***

Photo 3: ***Duttaphrynus stomasticus***

Photo 4: ***Amolops marmoratus***

Photo 5: ***Hoplobatracus tigerinus***

Photo 6: ***Minervarya teraiensis***

Photo 7: ***Duttaphrynus stomasticus***

Photo 8: ***Microhyla teraiensis***

APPENDIX

DATA SHEET

S.N	Date	Line Transect Number	GPS location	Name of Species	Number of Individuals	Habitat type	Canopy Cover	SVL	Sex	Remarks