HERPETOFAUNAL ASSEMBLAGE OF RAMAROSHAN WETLAND COMPLEX, ACHHAM, NEPAL

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Submitted to:

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

September, 2022

DECLARATION

I hereby declare that the work presented in this thesis **"Herpetofaunal Assemblage of Ramaroshan Wetland Complex, Achham, Nepal"** 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 the author(s) or institution(s).

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Date: 2022-08-22

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This is to recommend that the thesis entitled **"Herpetofaunal Assemblage of Ramaroshan Wetland Complex, Achham, Nepal"** has been carried out by Janaki Paudel for the partial fulfillment of Master's Degree of Science in Zoology with special paper Ecology and Environment. This is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

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LETTER OF APPROVAL

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Janaki Paudel entitled **"Herpetofaunal Assemblage of Ramaroshan Wetland Complex, Achham, Nepal"** has been accepted as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Ecology and Environment.

EXAMINATION COMMITTEE

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LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviations
asl	Above Sea Level
GLM	Generalized Linear Model
IUCN	International Union for Nature Conservation
Km^2	Square kilometer
LC	Least Concern
LULC	Land Use and Land Cover
LULCC	Land Use and Land Cover Change
RWC	Ramaroshan Wetland Complex

ABSTRACT

Herpetofauna are key bio-indicators of environmental health and habitat quality and are useful to assess habitat conditions of threatened ecosystems. Herpetofaunal diversity along elevational gradient in Nepal has rarely been studied. This study was carried out in Ramaroshan Wetland Complex (RWC) of Achham District, Sudurpaschim Province, Nepal which is one of the threatened wetlands due to anthropogenic activities. It aimed to explore the land use and land cover (LULC) changes in the RWC quantifying the area of wetlands and document the diversity and distribution pattern of herpetofauna. The LULC in the area (13.94 Km^2) was analyzed for 1989, 2000, 2010 and 2021 using supervised classification of remote sensing images from United States Geological Survey (USGS) geo-portal. Herpetofaunal survey were conducted by line-transect and opportunistic survey. Environmental variables such as nearest distance to the settlement, nearest distance to water, elevation, habitat type, etc. were measured along each transect. LULC analysis showed a considerable change in the land use pattern of the RWC from 1989 to 2021. Among major five LULC classes, vegetation (39%) was the dominant. The results demonstrated that there was a decrease in barren land, grassland and water body by 2% each from 1989 to 2021. Agricultural land and vegetation were increased by 3% and 2%, respectively. A total of 179 individuals of herpetofauna belonging to 11 species under five families and two orders i.e., Anura and Squamata, were recorded. Dicroglossidae family was the most dominant among them. The herpetofaunal community had Shannon-Wiener diversity index of H=1.88312 and evenness E=0.3642. Results showed that herpetofauna are not uniformly distributed along the elevational gradient (1401m to 2540m asl) of the RWC. The highest abundance of herpetofauna was found in agricultural land at an elevation of 2300m asl. Amphibian abundance increased with increasing distance to nearest water sources, whereas reptile abundance decreased with an increase in distance to water sources and increased with increase in distance to settlement. The study concluded that the wetland in the RWC is declining and herpetofaunal community in the area has low diversity.

1. INTRODUCTION

1.1 Background

Herpetofauna, which includes amphibians and reptiles, can be found in a wide range of environments, including deserts, grasslands, woods, and open water, as well as isolated places and human settlements (Johari et al. 2021). Their fundamental function is to sustain the cycling of nutrients and energy between trophic levels, and they play a key role in many ecosystems and greatly contribute to the diversity of vertebrates worldwide (Folt & Guyer 2021). Numerous factors, including as habitat loss and deforestation, land conversion, forest fires, illicit logging, climate change, and rapid water level drops, have contributed to the large population losses of the herpetofaunal species (Disi et al. 2014, Folt & Guyer 2021, Lindsay 2021). As a result, it's crucial to register and monitor amphibian and reptile diversity in order to evaluate the status of the species' populations and give relevant data for managing ecosystems.

Wetlands, as proposed by the Ramsar Convention (1971) are “areas of marsh, fen, peat land, or water, whether natural or artificial, permanent or temporary, with water that is static of flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six meters”. Wetlands provide important ecosystem services that include flood protection, enhanced water quality, support for the food chain, and carbon sequestration (Barbier 2011). Despite their great ecological values, wetlands are among the highly threatened ecosystems. Dewatering and infill activities, hydrologic alterations, contaminant flow, algal blooms, embankment, and subdivision by highways and drainage systems are a few examples of the stressing modifications that have been made to wetlands (Klemas 2011). Potential threats to the wetland ecosystems have been brought on by industrial production, agricultural use, pesticide residues, wetlands contaminated by chemical outflows, changes in natural habitats, and overuse of natural resources (Chen et al. 2011, Yang & Mao 2011, Kotze et al. 2012, Herbert et al. 2015). Hence, assessment of the status of the wetland using scientific tools and techniques and formulation of proper management plans is of utmost importance.

Geographic information technology could be one of the major tools for the assessment of the wetland spatial distribution and quality (Mathiyalagan et al. 2005). It enhances the inclusion of geographical information of both land and water sources, to promote information sharing (Goodchild et al. 1992). Geographic information in depth can serve as a framework for the techniques, policies, and personnel required to promote the sharing of geographical information across all levels of government, the commercial non-profit sphere, and the scientific researchers, and can be used successfully to wetland ecosystems (Mathiyalagan et al. 2005). Due to significant shifts brought on by anthropogenic and natural forces, as well as their effects on the local, national, and regional environment and climate, land use and land cover (LULC) have been changing continuously in Nepal over the past few decades (Paudel et al. 2016). Land cover change science continues to place a high priority on identifying and tracking the patterns of land use and land cover transitions, including their magnitude, extent, determinants, and implications (Napton 2002, Lu et al. 2004). In order to better comprehend the spatial and temporal as well as multidimensional aspects of changes in land use and land cover at both regional and global levels, remote sensing and geographic information systems (GIS) have gained popularity (Weng 2001, Rogan & Chen 2004).

Human activities, like industrial activity and habitat alteration to create tourist destinations, are typically to blame for the decline in water quality and quantity (Effendi & Wardiatno 2015), these actions disturb, harm, and have an impact on all living species that depend on water supplies. Biological indicators can be employed to offer qualitative data to assess the ecological resource (Barrett & Guyer 2008). The presence of herpetofauna (reptiles and amphibians) in an ecosystem can be used to assess the environmental conditions (Wilson & McCranie 2003). Herpetofauna play a significant role in ecology as both primary consumers and predators (Iskandar & Erdelen 2006). Since amphibians in particular are extremely sensitive to environmental changes, it can be used to detect the harm done to the ecosystem (Wilson & McCranie 2003). These taxa are excellent candidates for indicator species due to the dependence of amphibians on water and the habitat specialization of many reptiles (Barrett & Guyer 2008). Herpetofaunal diversity is severely impacted by significant changes to forests, such as clear-cutting and conversion to agroforestry (Gillespie et al. 2005, Gardner et al. 2007, Burivalova et al. 2014).

There are 56 amphibian species recorded till date in Nepal, belonging to three orders: Anura (frogs and toads), Caecilians (limbless amphibians), and Caudata (salamanders and newts) (Shah & Tiwari 2004). The habitat of organism plays an important role in the study of its diversity, distribution and abundance (Barnagaud et al. 2021). There are extraordinary global losses in the populations of reptiles and amphibians. Therefore, a comprehensive study on herpetofaunal diversity and status is of urgent need for their conservation and identifying risk associated with them.

1.2 Rationale of the study

Herpetofaunal species are one of the major groups that are highly threatened by anthropogenic activities and global climate change because they are sensitive to changing environments. The indiscriminate exploitation of amphibians and reptiles has threatened the survival of many species. Wetlands are biodiversity rich areas. Wetlands of the western Nepal including the Ramaroshan Wetland Complex in Achham District had never been explored systematically in order to document herpetofaunal diversity and abundance. The inclusion of small vertebrates in management plans for overall conservation of biodiversity at the local as well as landscape level is crucial. This research aimed to document the herpetofaunal species of conservation importance that the Ramaroshan areas hold and the change pattern in the land use and land cover. Ramaroshan Wetland Complex is one of the potential Ramsar sites from the far-western Nepal. Therefore, the findings of this study give insight on the distribution of the wetlands and herpetofaunal community therein.

1.3 Research objectives

1.3.1 General objective

The general objective of the study was to assess the herpetofaunal assemblage in the Ramaroshan Wetland Complex, Sudurpaschim Province, Nepal.

1.3.2 Specific objectives

The specific objectives of the study were as below:

i. To assess the LULC change of Ramaroshan Wetland Complex (RWC) using remote sensing data.
ii. To identify the herpetofaunal diversity of RWC.
iii. To establish the relationship between the herpetofaunal abundance and environmental variables in the RWC.
iv. To understand people's perception towards herpetofaunal conservation in the RWC.

1.4 Limitations of the study

- The survey was only carried out for one season, which limited the possibilities of discovering new species.

2. LITERATURE REVIEW

2.1 Mapping geographically isolated wetlands and its significance

Multiple methods (such as semi-automated methods, remote sensing techniques, etc.) have been developed for mapping the widespread wetlands with reduced and efficient efforts (Baker et al. 2006). When considering land values, the biological benefits of riparian zones and wetlands lead to the conclusion that these ecosystems are more crucial in terms of both the economy and the environment than the majority of other types of land use (Mitsch & Gosselink 2000). On-site assessments, aerial photo interpretation, and digital image processing are the three main inventory methods used today to map wetland habitats (Baker et al. 2006). Among them, remote sensing has been considered as a valuable method for factual information about the dimensions, fluctuations, and situations of wetlands (Gallant 2015). The delineation and analysis of watershed characteristics clearly shows promising results employing remote sensing tools. For example, the extensively utilized Landsat collection (such as Landsat-5 TM, Landsat-7 ETM+, and Landsat-8 OLI) using geo-referenced images acquired by optical imagery have abilities to perform efficacy in studying wetland habitats and flood catastrophe (Baker et al. 2006).

The use of different wavelengths in remote sensing imaging has made it possible to map a large number of land cover classifications (Maleki et al. 2019). Remote sensing images of fine spatial and hyperspectral resolution have been widely used in mapping the wetlands (Bansal et al. 2017, Maleki et al. 2019). For identifying and forecasting changes in land use, geospatial analysis employs a variety of statistical and rule-based modeling techniques (Overmars et al. 2003). Models that are frequently used in land use and land cover change (LULC) investigations include analytical approaches (Hyandye et al. 2015), phylogenetic approaches (Aitkenhead & Aalders 2009), cellular approaches (Singh et al. 2015), Markov approaches (Yang et al. 2012), composite approaches (Subedi et al. 2013), expert modeling methods (Stefanov et al. 2001), and multi-agent approaches (Ralha et al. 2013). Wetland function may be understood and wetland response to natural and human-caused activities can be monitored with the use of accurate wetland mapping (Mitsch & Gosselink 2000). Wetland mapping is employed to assess land-use choices and track the efficacy of mitigating measures (Muller et al. 1993).

2.2 Diversity of herpetofauna

Amphibian and reptile research are less prioritized by conservationists globally than bird and mammalian research (Fazey et al. 2005). Amphibian and reptile research instances were also discovered in Nepal. Between 1826 and 1854, Hodgson collected herpetofauna in Nepal, which has been the subject of baseline study for herpetofauna (Günther 1860). After that, Nepalese herpetology study advanced to a new level by contributing to amphibian and reptile zoogeographic data, (Dubois 2004), (Kramer 1977), all contributed to Nepal herpetology study. Mitchell & Zug (1995), investigated the variety and distribution of amphibians and reptiles in Chitwan National Park, together with habitat descriptions. In Nepal, the first documentation of a total number of amphibian and reptile species was given, and 54 species of amphibians were described (Shah & Tiwari 2004). This documentation marks a watershed moment in Nepalese herpetology study.

Some researchers in Nepal investigated amphibians and reptiles on a regional scale. According to them, a total of 16 species of herpetofauna were identified in the Manaslu Conservation Area (Pokhrel & Thakuri 2017), 22 species of herpetofauna belonging to eight species of amphibian representing three families and 14 species of reptiles representing six families were recorded from Manaslu Conservation Area, Gorkha District (Shrestha & Shah 2017). Forty species of snakes belonging to five families were recorded from Kaski district (Baral et al. 2020), 51 species of herpetofauna belonging to 12 species of amphibians and 39 species of reptiles were documented from Parsa National Park (Bhattarai et al. 2018). Various climatic factors like temperature and rainfall plays a major role in the diversity, distribution and richness of herpetofaunal species (Pyron 2014). However, the presence of water resources nearby terrestrial habitat also influences the diversity and abundance of herpetofauna (Wells 2010) as amphibians need water sources along with terrestrial habitats to complete their life cycle.

2.3 Herpetofaunal assemblage in wetlands and their determinants

The variety of the amphibians and reptiles, particularly reproducing amphibians, can be utilized to predict the general health of the ecosystem (Welsh & Droege 2001). Multiple amphibian species' breeding phenology frequently overlaps, resulting in a sophisticated aquatic ecosystem and a abundant supply of food for different organisms, predators of amphibians include many species of reptiles (Preston & Johnson 2012). Poikilothermic herpetofaunal species have experienced large-scale population loss in recent decades due to a variety of factors such as habitat modification, global warming, contamination, and disease (Erwin et al. 2016). For the purpose of tracking population patterns through time and defining the communities that make use of these wetlands, long-term surveillance of herpetofaunal assemblages in aquatic habitats is crucial (Erwin et al. 2016). Wetlands have historically been defined and delineated using hydrologic measurements, hydric soils, and botanical standards, which has led to a concentration on the "wet" aspect of the wetland as the essential attribute (Christen 2022). Because the integrity of many animal populations across the landscape depends on terrestrial corridors connecting wetlands, the interaction between terrestrial habitat and faunal communities of isolated wetlands is unique (Gibbons 2003). However, wetlands of the Nepal Himalaya are underexplored and the wetlands of the Ramaroshan area are not properly identified, not physiochemically characterized and neither the faunal and floral diversity are explored.

2.4 Conservation challenges of herpetofauna

Some amphibian and reptile species have experienced fluctuations in the length of their reproductive cycles as a result of habitat changes, threats in combination with other factors such as, the loss of breeding grounds, genetic diversity, changes to their home ranges, population isolation as a result of being unable to cross anthropogenically created habitats, changes to individual growth rates and activity patterns, and changes in how they use microhabitats (Crump 2003, Gibbons 2003, Gardner et al. 2007). Greater habitat specialization makes species more vulnerable to extinction in fragmented ecosystems because it makes it impossible for them to travel between native forest patches or withstand rapid microclimatic changes (Schlaepfer & Gavin 2001, Urbina-Cardona et al. 2006).

Over few decades, the discipline of conservation biology has grown tremendously (Lawler et al. 2006). Even though they face a high risk of extinction, amphibians and reptiles are the terrestrial vertebrate groups that have received the least amount of research globally, with only 5 and 2.5% of all studies on vertebrates and the impacts of habitat loss, respectively (Lawler et al. 2006, Gardner et al. 2007). Conservation with an academic focus Study in biology and the social sciences rarely collaborate, which limits the applicability of ecological scientific research to "on-the-ground" conservation (Fazey et al. 2005). There is a significant gap between management and implementation of theoretical conservation biology research (Fazey et al. 2005). It's possible that the lack of interest in reptile-focused study is caused by the fact that these creatures have cryptic habits, small populations, and are more elusive than other vertebrate groups in the field (Gibbons et al. 2000, Tolson & Henderson 2011). The conservation of amphibians and reptiles requires multidisciplinary studies that complement amphibian and reptile survey with several subject areas (such as social science, education, ethnography, medical sciences, biological sciences, business, and legislation), at the global scale (to clarify common trends), national level (to define policy objectives), regional level (to focus solely on primary concern areas for study and protection), and community level (to recognize local issues) to finally implement conservation plans (Schlaepfer & Gavin 2001).

3. MATERIALS AND METHODS

3.1 Study area

The present study was conducted in Ramaroshan Wetland Complex (Fig.1). Ramaroshan is a mid-hill rural wetland complex extending from 1401m to 3792m asl in Achham District of Sudurpashchim Province. Ramaroshan wetland area is popularly known as Barha Banda Athhara Khanda (12 lakes and 18 meadows) which consists of twelve lakes interconnecting with each other forming a complex. The only source for the outlet of lake water is Kailash River. Ramaroshan lies 42 km away from the district headquarter of Achham District, Mangalsen.

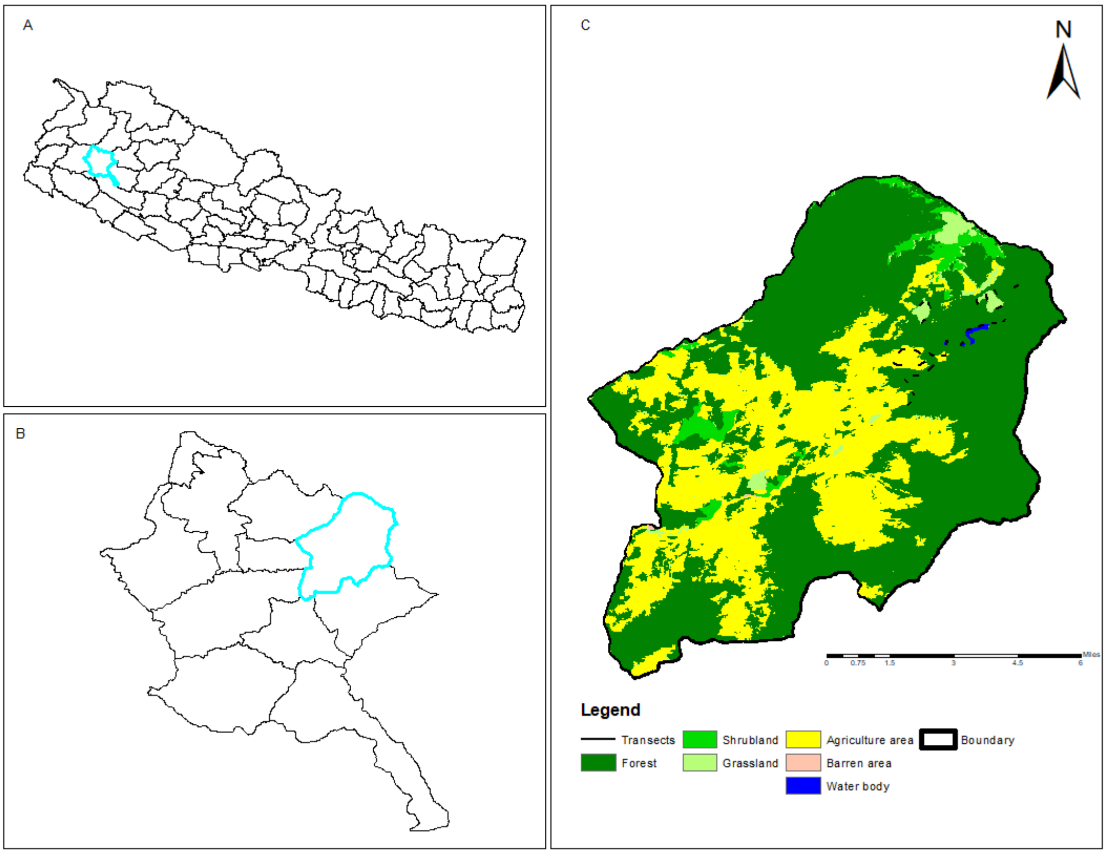

Figure 1. Map of the study area. A- Map of Nepal showing Achham District, B- Map of Achham District showing Ramaroshan Rural Municipality, and C- Map of Ramaroshan Rural Municipality showing transects

3.1.1 Flora and fauna

Major surrounding vegetation comprises of Gurans (*Rhododendron*), Katush (*Castanopsis indica*), Chilaune (*Schima walichii*), Utis (*Alnus nepalensis*), Salla

(*Pinus roxburghii*), Loth salla (*Taxus wallichiana*), Moso bamboo (*Phyllostachys pubescens*) etc. Sixteen species of rhododendron are found in the area. Various shrubs include Bahramase (*Lantena camera*), Titepati (*Astemisia vulgaris*), Amriso (*Rubus ellipicus*), etc. Danfe (*Lophophorous)*, Kalij (*Lophura leucomelanos*), Mallard (*Anas platyrhynchos*), Black-headed Jay (*Garrulus lanceolatus*) (Bird that is found only in far-western region of Nepal) etc. are the main birds found and Bear (*Ursidae*), Musk-deer (*Moschus chrysogaster*), Leopard (*Panthera pardus*) are the animals of this area. Ramaroshan is also storage of many medicinal plants.

3.1.2 Climatic condition

The climatic condition of Ramaroshan is sub-tropical, mild-temperate, cool-temperate, where temperature ranges between average minimum 1 °C to maximum 30 °C and annual rainfall amount 1800 ml.

3.2 Materials used

- GPS: Garmin Etrex 10
- Camera: Nikon P900
- Ethanol
- Collecting jars
- Tracing papers
- Torch light

3.3 Methods

3.3.1 Herpetofaunal survey

Preliminary field survey was conducted during the third week of May 2021 in order to locate the wetlands and herpetofaunal survey areas. Line transects method and opportunistic survey was carried out for the collection of data in and around the major wetlands of Ramaroshan area from 02 October to 07 October 2021. A total of 25 transects each of 200m lengths were established along the walking trail and every species that were encountered two meters on either side of transects were captured and counted. The species were recorded along the trail from 4PM to 10PM.

Opportunistic records and captures were carried throughout the day. Transects on each elevation band were surveyed over lakes, streams, forests, settlement areas and croplands whenever possible. While walking along transect, every encountered species was photographed on the spot and collected in the ventilated jars and then further identification was carried out. After identification the species were released back to avoid repetition and unidentified species were supplied with ethanol for paralyzing and were tagged on the hind leg with tracing paper and pencil. All the tagged species were kept in absolute ethanol in a closed bottle and brought to the laboratory of the Central Department of Zoology, Tribhuvan University for further identification. Captured individuals were identified in the field by using the field guidebook "Herpetofauna of Nepal" (Shah and Tiwari, 2004). Unidentified species were later identified by experts with the aid of samples and photographs. Key informant interview and focus group discussion were made in order to gather information on uses of herpetofauna by the local people, risks they perceive and their perception towards the conservation.

3.3.2 Environmental variables

The relation of environmental variables distance to water, distance to human habitat and elevation on the richness of amphibian species were tested for selected habitat types; agricultural land, forest, human habitat and marshy land. The distance to nearest water sources and distance to human settlement were measured with the help of Geographic Information System and elevation was measured by using GPS.

Table 1. Environmental variables and their description

Parameter	Variables	Description	Codes used
Habitat variables	Habitat type	Forest, marshy land, agricultural land, grassland, human habitat	F, ML, AL, G, HH
	Nearest distance to water resource	Euclidean distance measured from sampling location to the closest waterhole	DW
Topographic variables	Elevation	Elevation (meter) above sea level	Elev
Disturbance variables	Nearest distance to human settlement	Euclidean distance measured from sampling points to nearest human settlements	DH

3.3.3 Remote sensing data acquisition and land-use classification

Landsat images from 1989, 2000, 2010 and 2021 were used to detect LULCC within 10-year time interval. The Landsat 5-TM (Thematic mapper) for 1989, Landsat 7-ETM (Enhanced Thematic Mapper) for 2000, Landsat 5-TM for 2010 and Landsat 8-OLI (Operational Land Images) for 2021 with same spatial resolution (30m) were downloaded from United States Geological Survey (USGS) geoportal (Adhikari et al. 2022). Cloud cover and undesired shade-free images were defined as criteria during image selection because their existence could affect the categorization work's accuracy significantly. The entire Landsat image consists of around 0-10% of cloud cover. In addition, Google Earth was used as reference for verification. The area of latitude 29.250899˚, 29.251255˚, 29.223386˚, 29.222702˚ and longitude 81.445734˚, 81.489519˚, 81.489888˚ and 81.445793˚ was selected for the classification. Data sets were digitized by the ArcGIS tools then land cover were categorized into five land use type i.e. vegetation, grassland, water bodies, barren land and agricultural land. The supervised classification was performed. The signature classes or training samples were prepared from Google Earth map.

Accuracy assessment is an important part of any classification project. It compares the classified image to another data source that is considered to be accurate or ground truth data. Ground truth was collected in the field. Accuracy assessment was done on the resulting classified imagery using error matrix and Kappa index, to test the precision and accuracy of imagery and comparing them with actual points from the field. Ground truthing points (n=30) were used as reference for the accuracy assessment of classified image 2021. For Landsat images of 1989, 2000 and 2010, 90 stratified random points were generated and compared them with reference Google Earth. The user's accuracy, producer accuracy and overall accuracy was obtained from error matrix. Kappa coefficient is generally used to determine how precisely the agreements between model prediction and reality match. It is the statistical evaluation of the classified map's accuracy. The Kappa coefficient ranges from 0 to 1. If the Kappa coefficient is 0, it means there are no agreements. Values between 0-0.2 denote mild agreements, 0.21–0.40 indicate fair agreements, 0.41–0.60 indicate moderate agreements, 0.61–0.80 indicate satisfactory or good agreements, and 0.81–1 indicate almost perfect accords. Accuracy was calculated using following formulas:

$$\text{User's Accuracy} = \frac{\text{Number of correctly classified pixels}}{\text{Total number of classified pixels (Row Total)}} \times 100$$

$$\text{Producer Accuracy} = \frac{\text{Number of correctly classified pixels}}{\text{Total number of reference pixels (Column Total)}} \times 100$$

$$\text{Overall Accuracy} = \frac{\text{Total number of correctly classified pixels(Diagonal)}}{\text{Total Number of Reference Pixels}} \times 100$$

$$\text{Kappa Coefficient} = \frac{(\text{TS} \times \text{TCS}) - \Sigma(\text{Column Total} \times \text{Row Total})}{\text{TS}^2 - \Sigma(\text{Column Total} \times \text{Row Total})} \times 100$$

Where, TS= Total Sample

TCS= Total Corrected Sample

Table 2. Major land use and land cover type in RWC

SN	Land cover types	Description
1	Water body	Lakes, marshy land, river
2	Barren land	Dry places, flood plains without vegetation, landslide and no vegetation areas
3	Grassland	Meadows and irregular bushes
4	Vegetation	Forested area, mixed forest type
5	Agricultural land	Crop (e.g. paddy, maize, millet etc.) cultivated land

3.3.4 Data analysis

Shannon-Wiener diversity index

Biodiversity index was calculate by using Shannon and Wiener function (H'). It assumes that individuals are drawn at chance from a sizable, autonomous community, with members of every species present. It is calculated as,

$H' = -\Sigma (n_i/N) \log_e (n_i/N)$

Or, if $P_i = n_i/N$

$H' = -\Sigma P_i \log_e P_i$

Where,

$H' = -\Sigma P_i \ln P_i$

Where, Σ represents sum of $P_i (\ln P_i)$

H' = Index of species diversity

P_i = the proportion of individuals in the i^{th} species, $P_i = n_i/N$

n_i= number of individuals of each species

N= total number of individuals

Shannon’s evenness index (E):

When the complexity of the habitat increases, species diversity also increases. This species diversity considers both the species richness and species evenness. Species richness simply gives the presence of total number of species at a particular area and is calculated as,

S= total number of species recorded. Where, S= Species richness

The richness of an area is measured by its evenness, which is a measure of the relative abundance of various species. This evenness, which expresses the distribution of individuals among the many species equally, is a crucial part of diversity indices. Thus, to calculate whether species are distributed evenly across landscape, evenness index was determined by the following equation

$E = H'/\log S$

Where,

H' = Shannon-Weiner diversity index

S = Total numbers of species in the sample.

Abundance and diversity of the species were determined by using PAST version 3.5. The generalized linear model (GLM) was performed by using R-studio. Based on the eigen values and axes length redundancy analysis (RDA) was done by using CANOCO version 4.5 to examine influence of environmental variables on herpetofauna distribution (Ter Braak 2009). Final results were 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.

4. RESULTS

4.1 Land use and land cover pattern of Ramaroshan Wetland Complex

The land use and land cover classification of the RWC from year 1989 to 2021 showed variation in the classes with the time. The results of analysis of 1989 image (Fig.2) demonstrated that vegetation, barren land and grassland dominated the landscape with 37%, 31% and 27%, respectively. Compared to 1989, findings from the year 2000 the vegetation and barren land remained constant whereas the water bodies decreased and the agricultural land increased by 1%. Similarly in comparison to that of 2000, in the year 2010, the vegetation and agricultural land increased, grassland remained almost constant; and barren land and water body decreased by 1% (Table 3). Similarly, vegetation and agricultural land increased and barren land, grassland and water body decreased from 2010 to 2021.

Table 3. Spatial extent of land cover classes in RWC

Land use and land cover classes	**Year**								**Remarks**
	1989		2000		2010		2021		
	Area (Km^2)	Area (%)	Area (Km^2)	Area (%)	Area (Km^2)	Area (%)	Area (Km^2)	Area (%)	
Agricultural land	0.46	3	0.53	4	0.76	6	0.78	6	Increased
Barren land	4.40	31	4.39	31	4.08	29	4.07	29	Decreased
Grassland	3.71	27	3.68	26	3.61	26	3.49	25	Decreased
Vegetation	5.12	37	5.10	37	5.30	38	5.39	39	Increased
Water	0.25	3	0.24	2	0.19	1	0.21	1	Decreased
Total	**13.94**	**100**	**13.94**	**100**	**13.94**	**100**	**13.94**	**100**	

The overall changes showed that there was a decrease in barren land, grassland and water body by 0.33, 0.22 and 0.04 Km^2, respectively (Fig. 3). Agricultural land and vegetation were increased by 0.32 and 0.27 Km^2 respectively.

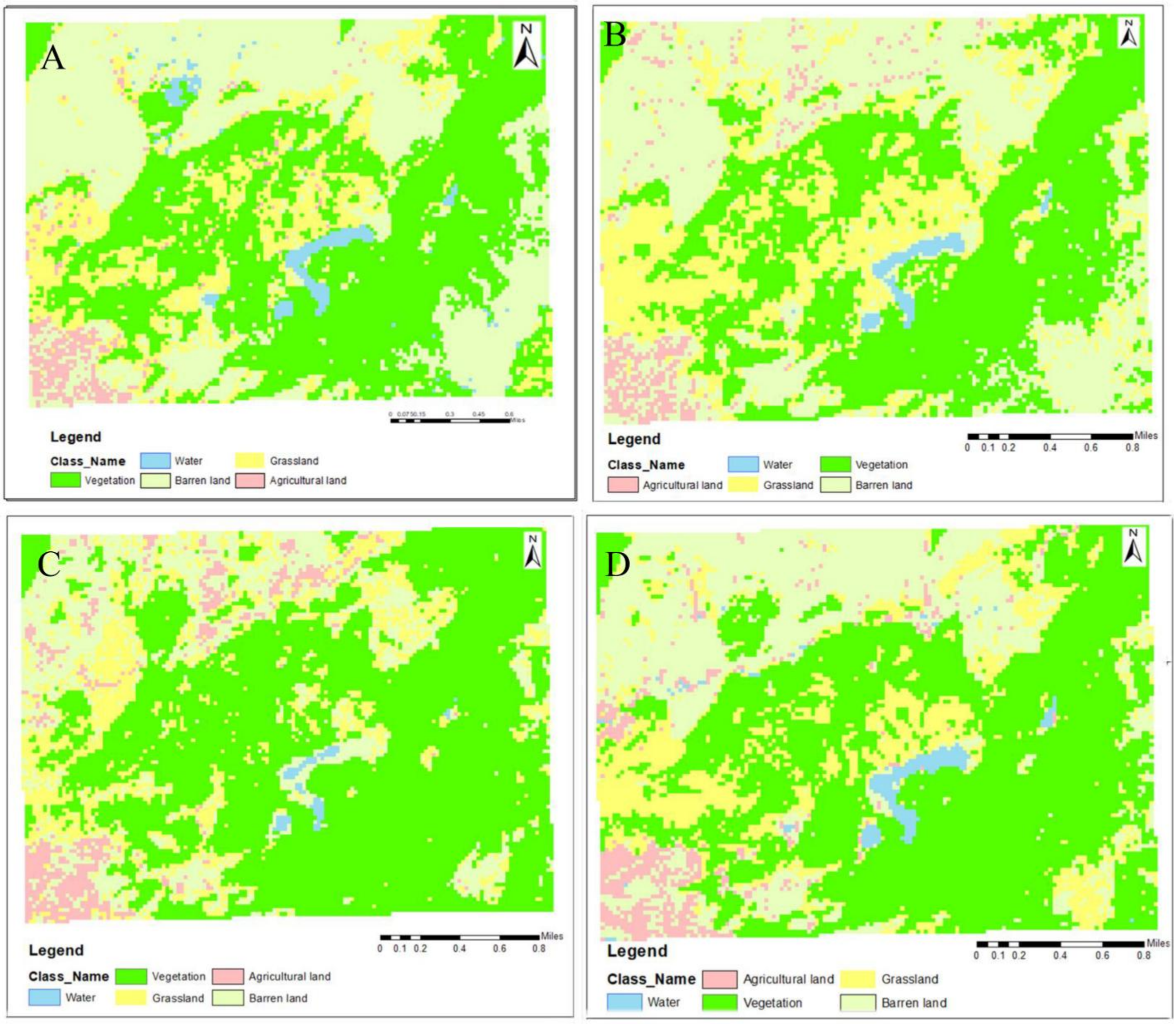

Figure 2. Patterns of land use and land cover from 1989-2021. A- LULC of the RWC for 1989; B- LULC of RWC for 2000; C- LULC of RWC for 2010; and D- LULC of RWC for 2020

The results from the LULCC showed that although the RWC holds 12 lakes and 18 meadows only six among them were with visible amount of water and other lakes are degrading. The total water area in the wetland was about 25 ha. There was consistent decrease in the water area from 1989 through 2000 and 2010 to 2021. The results from the image analysis of year 1989 (Fig.2) showed that water area was of total 0.25 Km^2. Compared to 1989 results, findings from the year 2000, the water area decreased by 0.01 Km^2. Similarly compared to results from year 2000, findings of year 2010 showed decrease in water area by 0.05 Km^2. Furthermore, compared to findings from year 2010, the water area increases by 0.02 Km^2. The net decrease in wetland area from 1989 to 2021 was 0.59 Km^2 (6% of the wetland area in 1989).

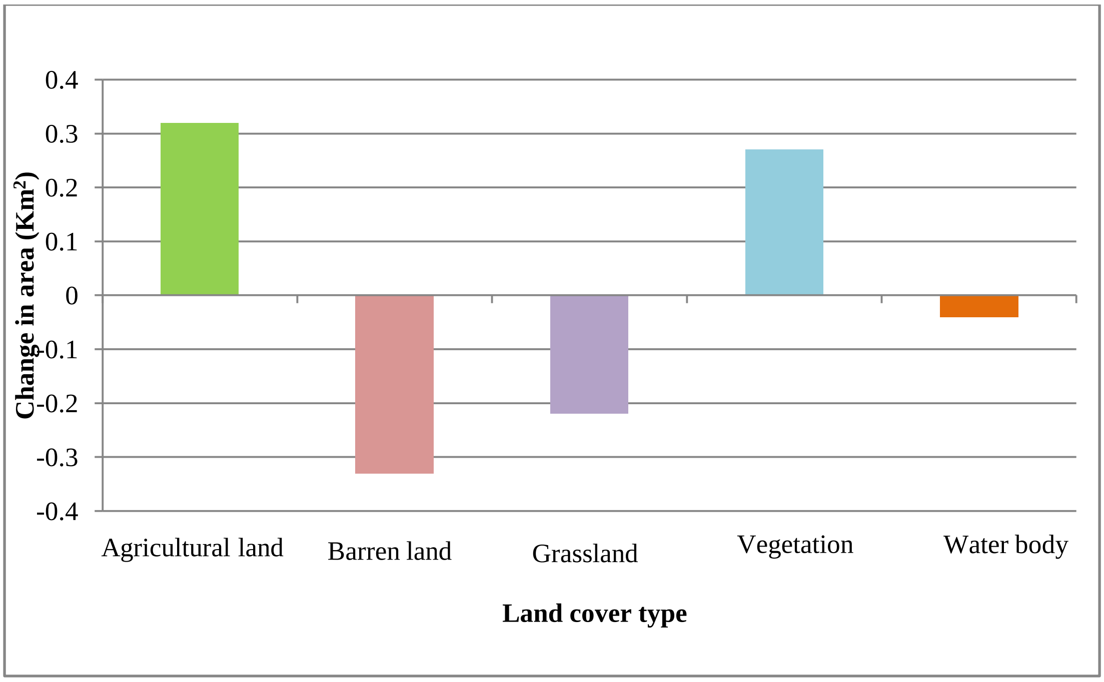

Figure 3. Land use and land cover change in RWC

The overall accuracy of classified images of 1989, 2000, 2010 and 2021 was 70%, 80%, 83.33% and 86.66% respectively. The user's accuracy ranged from 50% to 83.33% in 1989, 60% to 85.71% in 2000, 66.66% to 89.97% in 2010 and 71.42% to 91% in 2021. The kappa coefficient for the years 1989, 2000, 2010 and 2021 were 0.62, 0.74, 0.79 and 0.83 respectively.

Table 4. Accuracy assessment of the classified images

Land cover	**1989**		**2000**		**2010**		**2021**	
	UA	PA	UA	PA	UA	PA	UA	PA
Agricultural land	80	50	60	75	89.97	62.5	87.33	62.5
Barren land	71.42	71.42	85.71	75	85.71	100	71.42	100
Grassland	66.66	66.66	83.33	71.42	66.66	80	83.33	83.33
Vegetation	50	96.23	83.33	100	83.33	91.34	91	94
Water body	83.33	83.33	83.33	83.33	83.33	83.33	83.33	92.56
Overall accuracy	70		80		83.33		86.66	
Kappa coefficient	0.62		0.74		0.79		0.83	

Notes: UA- User Accuracy, PA- Producer's accuracy

4.2 Diversity and abundance of herpetofauna in the RWC

A total 179 individuals of 11 species of herpetofauna were recorded from the study area (Table 4). Among them five species were of amphibians and six species were reptiles. All five species of amphibians belonged to order Anura. Dicroglossidae was the dominant family consisting of three species followed by Bufonidae consisting of two species. The relative abundance of *Duttaphrynus melanostictus* was the highest (0.284) and that of *Elaphe hodgsonii* and *Nanorana rostandi* was the least (0.005).

Table 5. List of herpetofaunal species recorded from the RWC with their relative abundance and codes used

S.N.	Species	Family	Order	Relative abundance	Code used
1	*Duttaphrynus himalayanus*	Bufonidae	Anura	0.201	H1
2	*Duttaphrynus melanostictus*	Bufonidae	Anura	0.284	H2
3	*Nanorana minica*	Dicroglossidae	Anura	0.067	H3
4	*Nanorana polunini*	Dicroglossidae	Anura	0.106	H4
5	*Nanorana rostandi*	Dicroglossidae	Anura	0.005	H5
6	*Calotes versicolar*	Agamidae	Squamata	0.072	H9
7	*Laudakia tuberculata*	Agamidae	Squamata	0.178	H8
8	*Amphiesma platyceps*	Colubridae	Squamata	0.011	H7
9	*Elaphe hodgsonii*	Colubridae	Squamata	0.005	H6
10	*Asymblepharus himalayanus*	Scincidae	Squamata	0.016	H10
11	*Asymblepharus ladacensis*	Scincidae	Squamata	0.050	H11

All six species of reptiles belonged to order Squamata. Three families Colubridae, Agamidae and Scincidae were recorded with each consisting of two species (Fig. 4).

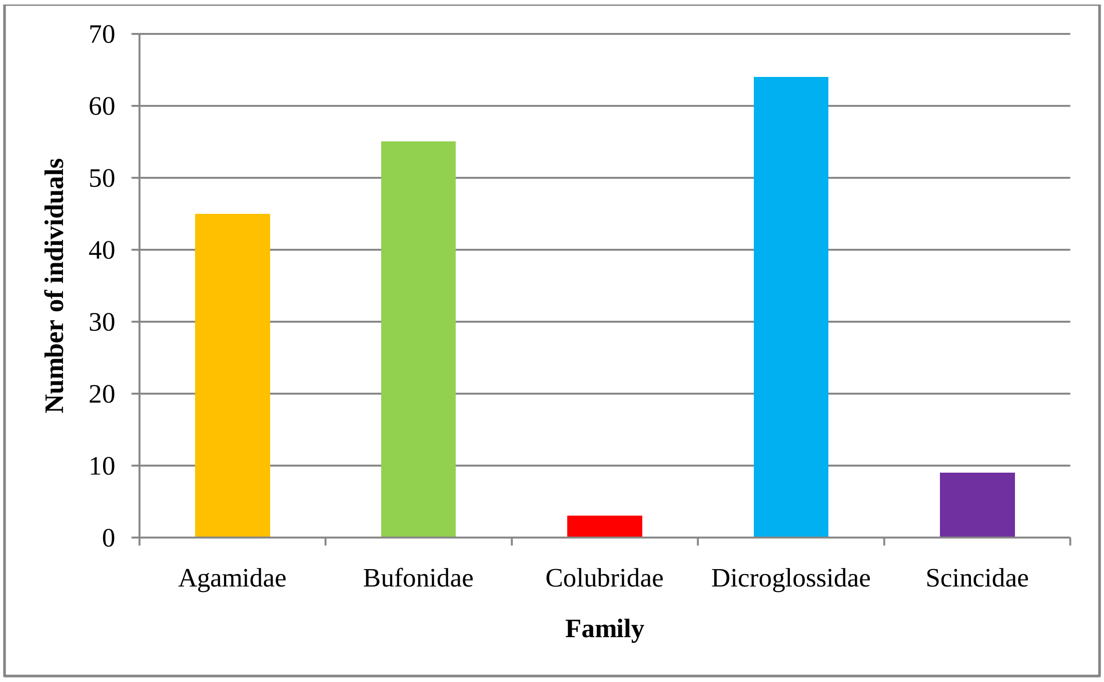

Figure 4. Family wise abundance of herpetofauna in theRWC

The biodiversity index (H') and evenness index for amphibian were 1.2892 and 0.6211, respectively. Similarly, biodiversity index (H') and evenness index (E) for reptiles were 1.2825 and 0.7213, respectively. The overall biodiversity index (H') was 1.88312 and the evenness index (E) was 0.3642.

The species accumulation curve (Fig. 5) showed a stable condition, indicating that there are no prospects of finding new species even with increased sampling efforts.

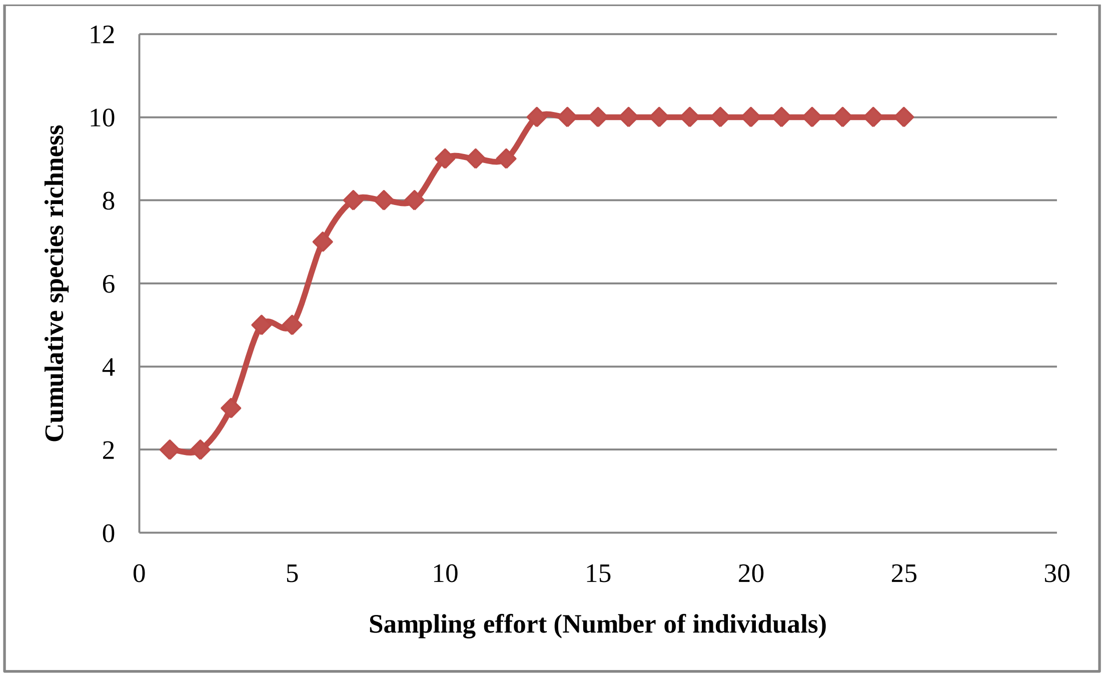

Figure 5. Species accumulation curve of herpetofauna recorded in the RWC

The highest total abundance of herpetofauna was found in agricultural land (42%) followed by marshy land (24%), human habitat (15%), grassland (10%), and forest (9%).

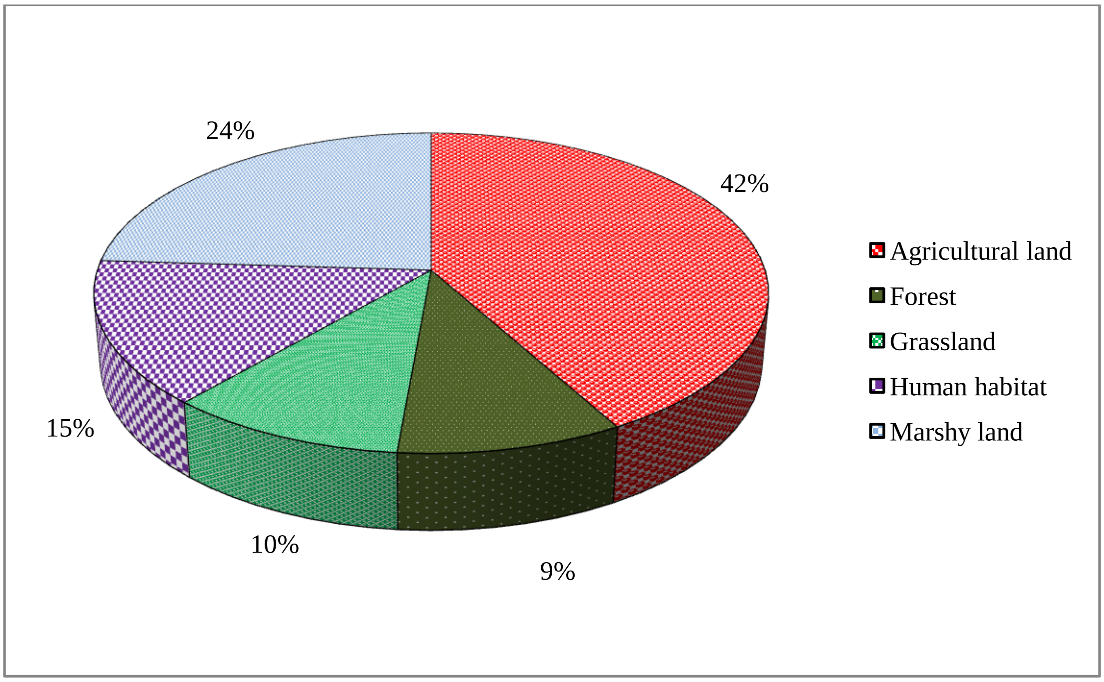

Figure 6. The abundance of herpetofauna along different habitats

4.3 Effects of environmental variables on abundance of herpetofauna

Herpetofaunal abundance increased from the elevation 1600m to 2400m asl. It peaked at the elevation of 2400m and then gradually began to decline with increasing elevation.

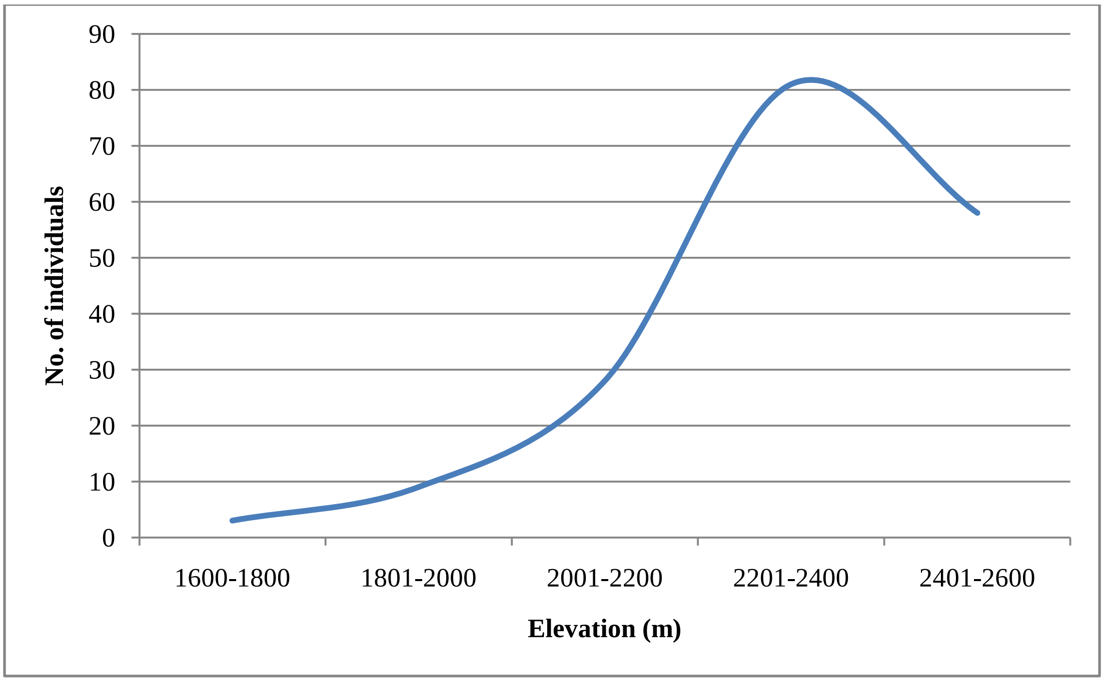

Figure 7. Herpetofauna along altitudinal gradients in RWC

Amphibians revealed a statistically significant negative relationship with distance to water, implying that as distance to water increases, amphibian abundance tends to decrease. Amphibian richness did not have a significant relation with elevation and distance to the human settlement.

Table 6. Summary of GLM showing the effects of variables on the abundance of amphibian

Variables	Estimate	SE	Z	P
Intercept	1.1223	1.9323	0.581	0.561
Elevation	0.0005	0.0008	0.673	0.501
Nearest distance to water	-0.0097	0.0023	-4.231	2.32e-05 ***
Nearest distance to settlement	0.0001	0.0001	1.407	0.160

Reptile abundance showed strong positive association with distance to water and negative association with elevation (Table 5). The probability of herpetofaunal abundance was less in transects with increasing elevation however, the mid elevation had highest abundance. Amphibian abundance increases with increasing distance to nearest water sources, whereas reptile abundance decrease with an increase in distance to water sources and increases with increase in distance to settlement (Table 6).

Table 7. Summary of GLM showing the effects of variables on the abundance of reptiles

Variables	Estimate	SE	Z	P
Intercept	4.0049	1.4716	2.752	0.0059 **
Elevation	-0.0015	0.0006	-2.430	0.0150 *
Nearest distance to water	0.0005	0.0001	3.466	0.0005***
Nearest distance to settlement	-0.0005	0.0004	-1.278	0.2013

Table 8. Summary of GLM showing the effects of variables on the abundance of herpetofauna

Variables	Estimate	SE	Z	P
Intercept	-1.049e-01	1.239e+00	-0.085	0.9325
Elevation	9.458e-04	5.210e-04	1.815	0.0695
Nearest distance to water	-2.775e-04	1.634e-04	-1.699	0.0893
Nearest distance to human settlement	1.427e-04	9.629e-05	1.482	0.1385

The Monte-Carlo permutation test of significance of all the reduced model axes revealed significance preference of the amphibian species (Trace=0.384, F-ratio=3.118, P=0.0120) to different habitat types and environmental variables. The amphibian species were more associated with marshy land (Fig. 8). Similarly, reptilian species also showed significant preference (Trace=0.448, F-ratio=1.967, P=0.05). Reptiles were more associated with forest, human habitat and grassland (Fig. 9). The Monte-Carlo permutation test of significance for overall species (Fig. 10), showed significance preference (Trace=0.394, F-ratio=1.300, P-value=0.2320).

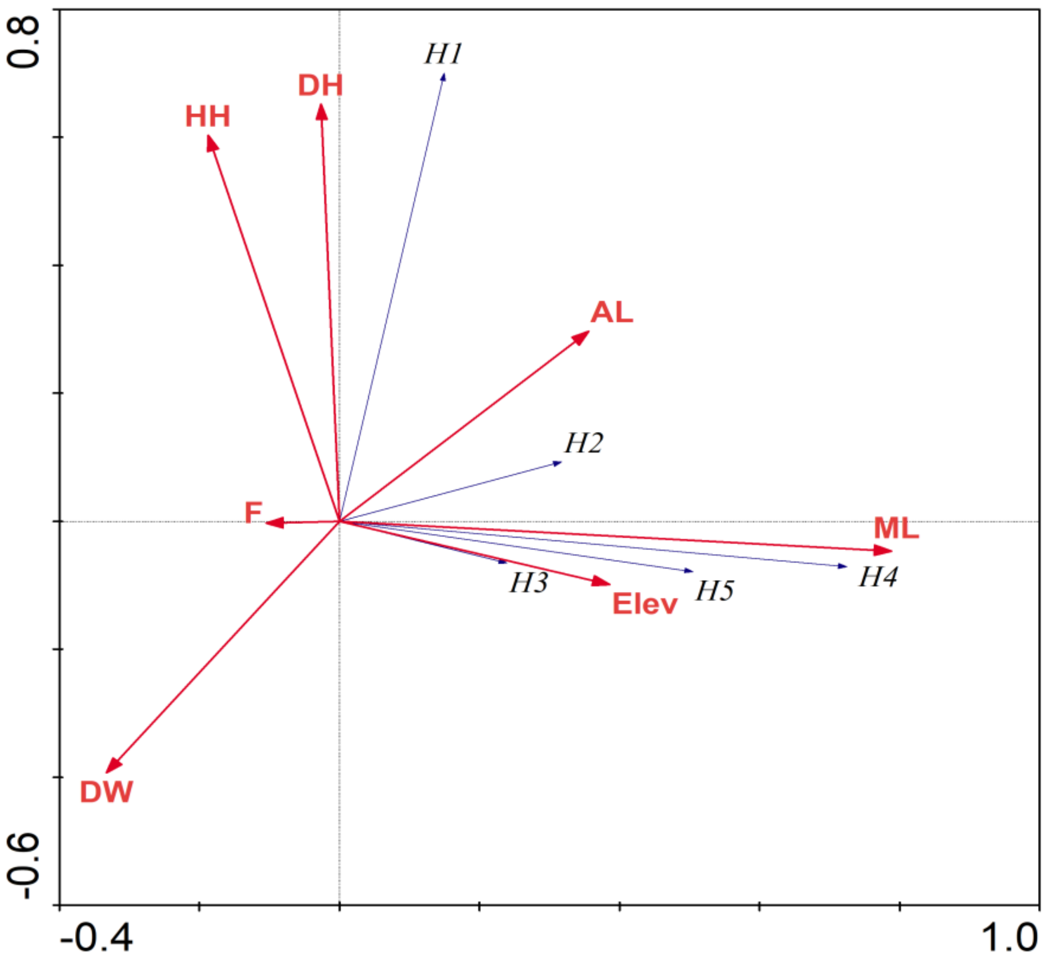

Figure 8. RDA ordination diagram (biplot) showing the effect of environmental variables and habitat types on amphibian species in RWC

The RDA ordination diagram showed that *Duttaphrynus himalayanus* is more associated with human habitat. *D. melanostictus* is associated to agricultural land. *Polunini paa*, *Nanorana minica* and *Nanorana rostandi* are more associated with marshy land. Among the variables, the association of maximum abundance of the species was higher with elevation.

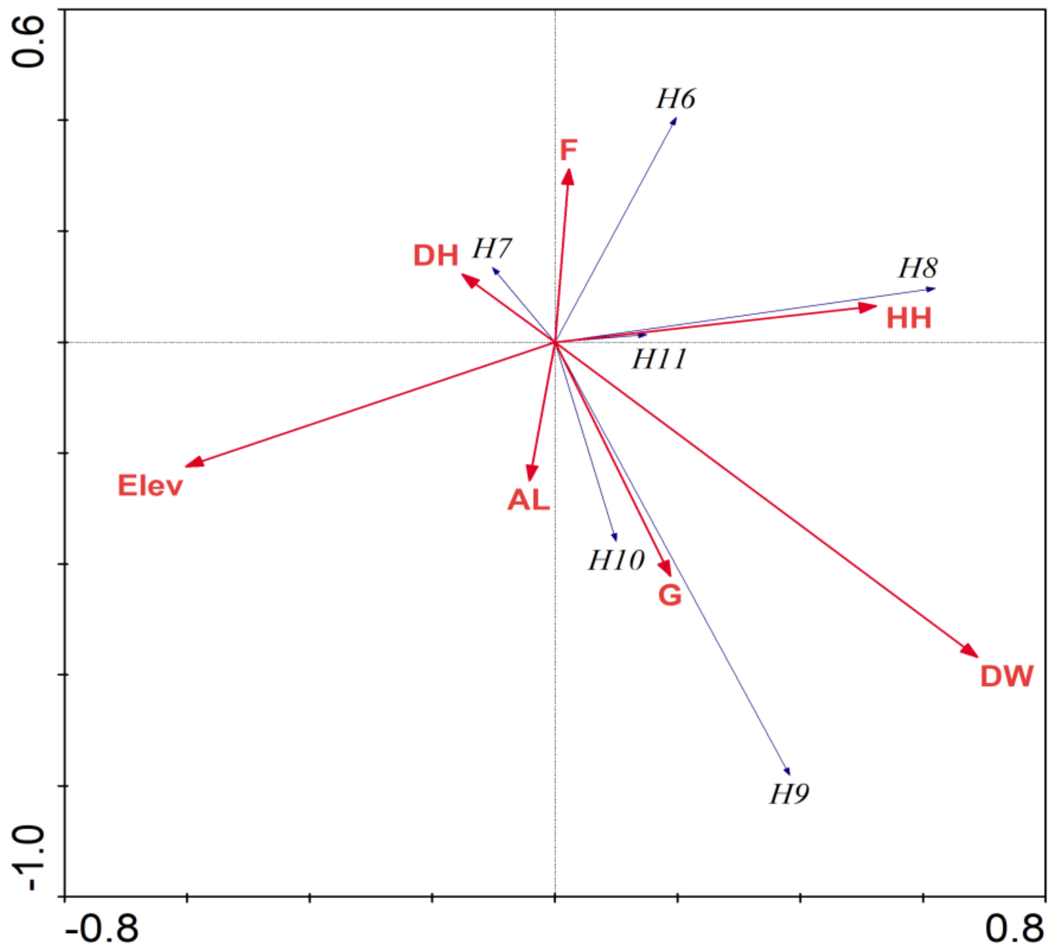

Figure 9. RDA ordination diagram (biplot) showing the effect of environmental variables and habitat types on reptile species in RWC

The RDA ordination diagram showed that *Elaphe hodgsonii* was more associated to the forest area. *Laudakia tuberculata* and *Asymblepharus ladacensis* are more associated to human habitat area. *Calotes versicolar* and *Asymblepharus himalayanus* are more associated to grassland. Among the variables, the association of maximum abundance of the species was higher with distance to human habitat.

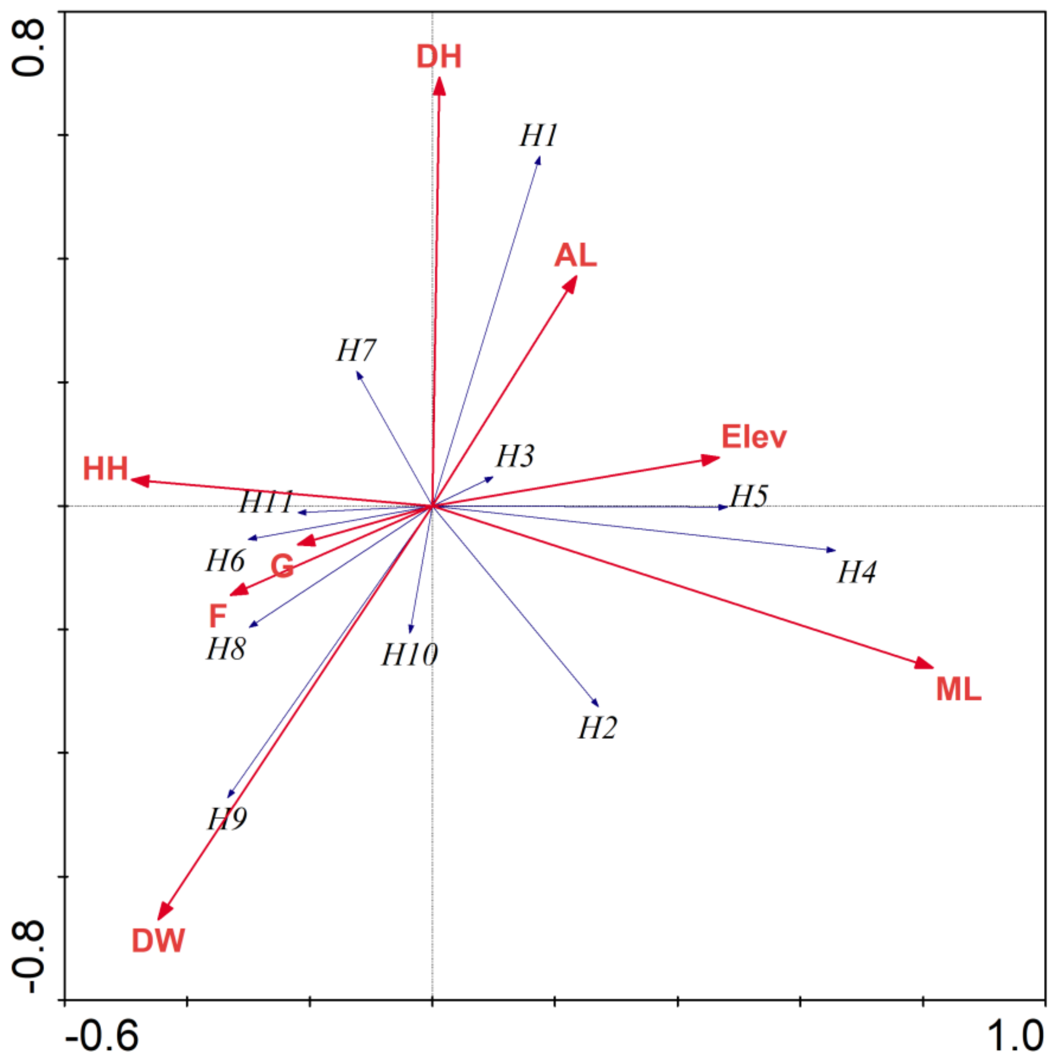

Figure 10. RDA ordination diagram (biplot) showing the effect of environmental variables and habitat type on overall herpetofauna in the RWC

4.4 Perception of local people towards herpetofauna

In the present study, the majority of respondents believed that the number of herpetofauna is decreasing (66%) with time, whereas the minority believed that the population is rising (8%) (Fig. 11).

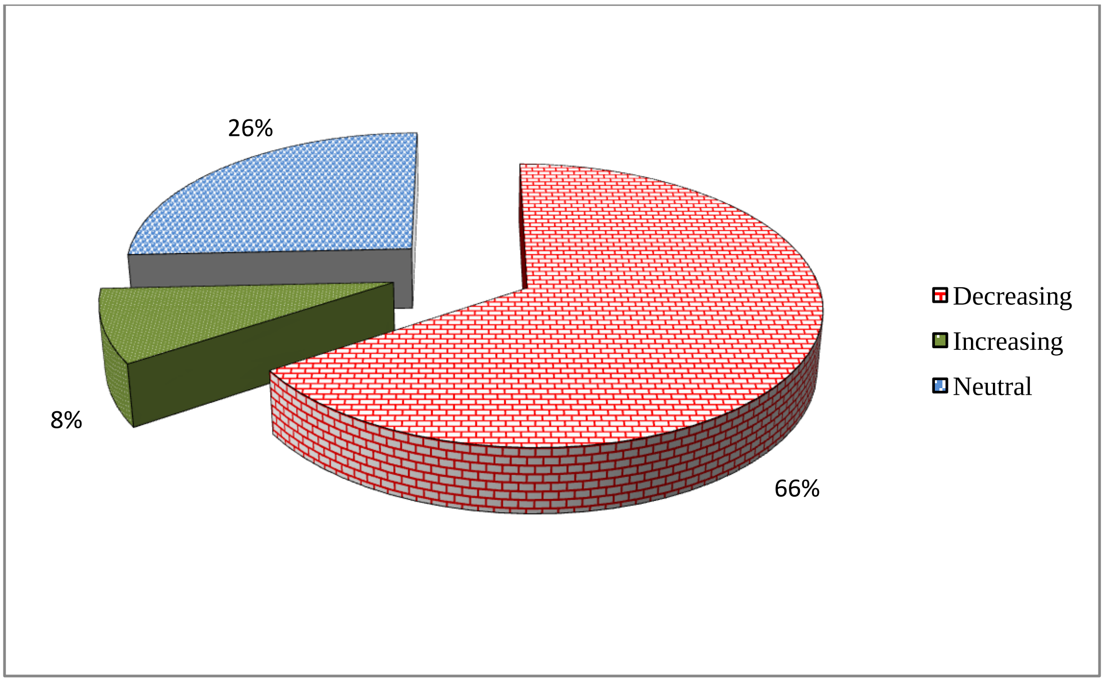

Figure 11. Perception of local people towards population trend of herpetofauna

In this study, most of the respondents believed that the population is decreasing due to climate change which poses the greatest threat to herpetofauna. Other threats that were responsible for the declining number of herpetofauna were habitat loss, disease, and pesticides respectively (Fig. 12).

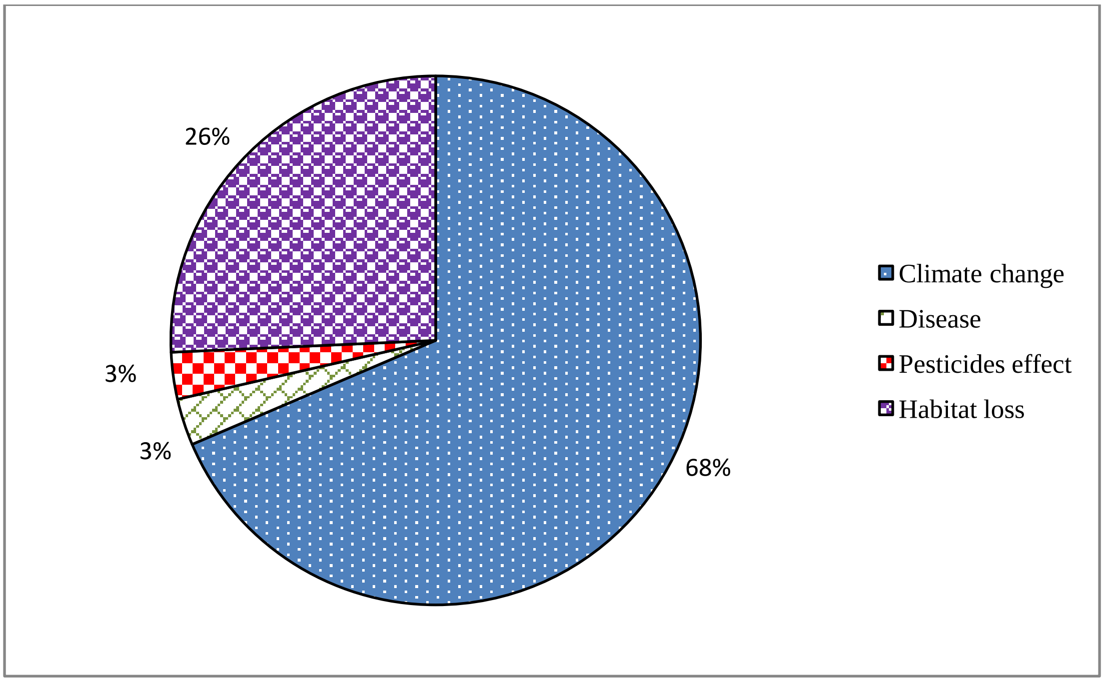

Figure 12. Perception of local people towards potential threats to herpetofauna population

5. DISCUSSION

5.1 Land use and land cover change in RWC

Changes in LULC around RWC indicate an increase in population and a rise in the need for land for habitation and cultivation. The results of LULCC from 1989 to 2021 indicated that there was a decrease in barren land, grassland and water body by 0.33, 0.22 and 0.04 Km^2, respectively. These reductions serve as a warning that unsustainable resource use, the conversion of land to habitation centers, and the effects of climate change could lead to a decline in ecosystem services and an increase in food poverty (Wang et al. 2020). Agricultural land and vegetation were increased by 0.32 and 0.27 Km^2 respectively. Present study categorized five land cover classes; vegetation was the most common in RWC. However, this region is very dispersed because of the agriculture and scattered human populations. The lake system (Jingale, Batuta, Mathillo Dhaunne, Tallo Dhaunne, Gagre, Lisedali, Lamidaha, Dauthe Daha and associates) and meadows (Kinniminni, Rame, Roshan and associates) play significant role in maintaining different ecosystems.

Using the initial land cover (land cover 1989) as a baseline, the patterns of LULCC analysis demonstrated the direction of land cover changes. Classified images of RWC clearly showed a decrease in water might be due to overexploitation by the local people. The lake named Dallena has been made a farmland by the people of RWC and the lake was dried during Maoist insurgency. The biggest decrease by proportion to 1989 is the grassland coverage probably due to overgrazing, which has undesirable environmental impacts on the soil's preservation, diversity, and water holding capacity (Hyandye et al. 2015, Singh et al. 2015, Lamchin et al. 2018). The results also demonstrated decrease in grassland and barren land and increase in vegetation and agricultural land. The increase in agricultural land might be due to returning of people to the place after a huge loss from flood caused due to Kailash River in the year 2020. The degradation of a water body necessitates immediate management actions, especially given the ongoing global warming-related drying up of water supplies worldwide (Wang et al. 2020). On account of people moving from rural to urban areas for higher quality of life and more economic prospects, the cropland they left behind gradually turned into a forest (Garrard et al. 2016).

The barren area is decreased in the RWC that might be because most of area might have been replaced by forest. The grassland also showed a progressive reduction, which may be because locals use the grassland in the mountains as pasture land (Rai et al. 2018). Interestingly, the coverage of vegetation in RWC area while coming to 2021 increased which can be attributed to the commendable support from the Ramaroshan Tourism Board and local level government.

5.2 Diversity and abundance of herpetofauna

A checklist of eleven species of herpetofauna provides an update on herpetofaunal richness in the Ramaroshan Wetland Complex, Achham, Nepal. The presence of 179 individuals of 11 species (five amphibians and six reptiles) in the field study implies that herpetofaunal diversity is likely impacted by the time of visit (Post monsoon). Because of the minimal rainfall during the post-monsoon season, species discovery may be limited during this field observation. Similar type of result was found that was herpetofaunal diversity less in pre-monsoon season (Shrestha & Shah 2017). The species diversity in RWC was found to be less (1.88321) that might be due to the time of data collection which was during post-monsoon and less favorable condition for the survival of the herpetofaunal species. According to Shah & Pandit (2013), anytime the diversity index rises in value, the evenness index rises in the opposite manner. The current research also shows that the diversity index (H'=1.88321) and evenness index (E=0.3642) have a negative relationship.

Among 11 species of herpetofauna, *Duttaphrynus melanostictus* was the most abundant species in the area followed by *Nanorana polunini, Laudakia tuberkulata, Duttaphrynus himalayanus, Calotes versicolar, Nanorana minica, Asymblepharus himalayanus, Asymblepharus ladacensis, Elaphe hodgsonii, Nanorana rostandi* and *Amphiesma platyceps*. However, according to local people the area also holds tree frogs which this study was unable to record. The family Dicroglossidae was largest in amphibians and family Scincidae was largest in reptiles. *Calotes versicolar* has been recorded from an altitude of 2540m in this study. *C. versicolar* can be found in a variety of terrestrial environments below 2000 meters (Shah & Tiwari 2004). The highest total abundance of herpetofauna was found in agricultural land that may be because amphibians congregate around or at water sources this could be attributed to the abundance of more number of preys in agricultural fields. However, multiple studies have demonstrated that agricultural contamination (e.g.,using pesticides and insecticides too frequently) and habitat loss owing to large road systems are key drivers in global amphibian decrease (Christin et al. 2004, Cothran et al. 2013). The grassland had lowest abundance probably due to monotonous kind of food options and less diverse habitat range. The research area's most common species was *D. melanostictus*. A similar result was reported by Fu et al. (2006) where *D. melanostictus* was the most abundant species in the study area.

5.3 Effects of environmental variables on herpetofaunal abundance

In this study, a wide range of elevational levels revealed a hump-shaped distribution in the abundance and diversity of the herpetofauna. Present study showed the species richness gradually increasing with increasing elevation, at 2200-2400m the richness peaked and again the richness falls with increasing elevation. The humped relationship between species richness and elevation in this study might be because the RWC's major wetlands are situated at 2100–2500m providing both wet and dry condition for herpetofaunal assemblage which is fundamental for their various life stages. Many amphibians have relocated their ranges towards higher elevations due to the growing trends of global warming and climate change (Pounds et al. 1997). Similar altitudinal richness patterns were documented by other studies on the diversity of plants in the Nepal Himalaya, the Indian Western Himalaya, and the Gaoligong Mountains, as well as the diversity of frogs, lizards, and snakes in the Hengduan Mountains and small mammals on Mount Qilian (Vetaas & Grytnes 2002, Li et al. 2003, Bhattarai et al. 2004, Oommen & Shanker 2005, Fu et al. 2006, Fu et al. 2007).

Rahbek (2005) identified three fundamental forms of elevational diversity patterns: a reduction in species richness with elevation, a peak at low elevation plateaus, and a peak at mid height. Four broad patterns of reptile richness on mountains can be seen: declining, low plateau, low plateau with a mid-elevation peak, and unimodal with a mid-elevation peak (McCain 2010). Amphibians and reptiles, on the other hand, show a consistent reduction in species richness as altitude rises (Nathan & Werner 1999). With regard to distance to water, amphibians showed a negative but statistically significant connection, suggesting that amphibian abundance tends to decline with increasing distance to water might be because the species are associated with moist

(Aryal et al. 2020), as a proxy for water availability, the soil moisture is a crucial predictor of amphibian occurrences (Blaustein et al. 2010).

The GLM between species richness and environmental variables shows reptile abundance is positively linked with distance to water as the distance to water increases the richness also increases but is negatively associated with elevation as the elevation increases the richness tends to decrease contrastly species richness is higher for lizards at high elevations in southern latitudes because high elevations in southern latitudes have wildly varying seasonal temperatures and dry soils, which seem to benefit the physiological ecology of lizards (Navas 2002). Distance to human settlement has significant effect on herpetofaunal species in RWC where both amphibian and reptile species were associated with distance from the nearest human settlement. Of all 11 species of herpetofauna *L. Tuberculata* was found mostly in the habitats associated to human settlement like on the roof of houses, holes made in houses might be because of the high number of preys in and around the human habitat.

5.4 Conservation challenges of herpetofauna

According to local people, the number of herpetofauna in RWC compared to past has decreased and most of the respondent believed that the major threat for their declining number is due to climate change that might be because compared to other vertebrate, they are more vulnerable to climate change because of their ectothermic nature (Acharya & Chhetri 2012), similarly habitat loss and fragmentation, infectious diseases, pesticides and other contamination, road kill can be the some other factors causing the declination of herpetofaunal communities (Lesbarrères et al. 2014). All the species recorded were of common status in Nepal. However, no any consumption record of herpetofauna was found through questionnaire survey that might be due to the unfavorable attitudes people have toward this animal group, the herpetofauna is barely understood and valued by society (Sousa et al. 2016). In contrast, because of excessive exploitation for human consumption, the herpetofauna has become less numerous, less diverse, and even endangered (Natusch & Lyons 2012, Shaney et al. 2016). In this study, the locations that combined a variety of wet habitat types were the most crucial for maintaining semi-aquatic herpetofauna (i.e. sites in agricultural zone, marshy sites), on a broader scale, amphibians that breed in ponds are known to depend on such habitat diversity as one of their key breeding determinants (Brodman et al. 2003).

6. CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This study revealed that the LULC change around RWC is the sign of human encroachment and rise in the need for the settlement and agriculture. The dominant LULC class in the study area was vegetation. Barren land, water body, grassland showed gradual decrease from 1989 to 2021 whereas agricultural land and vegetation increased. A total of 11 species of herpetofauna (five amphibians and six reptiles) were recorded from the RWC. Amphibian abundance has significant negative relationship with distance to nearest water source and reptile abundance has strong positive association with distance to nearest water source and negative association with distance to settlement area and elevation has strong effect on the both species distribution. The herpetofauna showed hump shaped distribution where the abundance increased up to mid elevation (2400m asl) and gradually began to fall as elevation increased. Additionally, the research was limited by its short duration and by the off-season. However, the data collected are crucial and will serve as the standard for any further herpetofaunal research in the region. Due to its varied habitat and anthropogenic activities altering the land use pattern and threatening the ecosystem, biodiversity in the RWC demands strong conservation initiatives.

6.2 Recommendations

1. LULCC showed that the water body in the RWC is decreasing. To mitigate the trend, effective management and restoration programs should be employed.
2. Long-term sampling may be required to properly characterize patterns and variety of herpetofaunal richness, abundance, and diversity at isolated wetlands since amphibian and reptile captures fluctuate with temporal fluctuations in environmental conditions.
3. Species richness and diversity values are valuable indicators of community composition and structure, but they have limitations that must be recognized.
4. The study's method for documenting the herpetofauna was strictly limited, which accounts for the study's lesser representation of species. Additional sampling efforts might document unreported species.

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PHOTOGRAPHS

Photo 1: ***Duttaphrynus himalayanus***

Photo 2: ***D. melanostictus***

Photo 3: ***Nanorana minica***

Photo 4: ***Polunin paa***

Photo 5: ***Nanorana rostandi***

Photo 6: ***Calotes versicolor***

Photo 7: ***Laudakia tuberculata***

Photo 8: ***Elaphe hodgsonii***

Photo 9: ***Amphiesma platyceps***

Photo 10: ***Asymblepharus ladacensis***

Photo 11: ***A. himalayanus***

Photo 12: Questionnaire survey

APPENDICES

Appendix 1. Data sheet used for herpetofaunal survey

SN	Date	Line Transect Number	Name of Species	Number of Individuals	GPS Location	Elevation	Distance to Water Source	Distance to Settlement	Habitat	Remarks

Appendix 2. Questionnaire set

Date: ……………….

Name of respondent: ……………….

Age: ……………….

Sex: ………………..

Q. N 1 How many herpetofauna have you seen?

( ) Only amphibian ( ) Only reptiles ( ) Both ( ) None

Q. N 2 What is the Population trend of herpetofauna in your area?

( ) Increasing ( ) Decreasing ( ) Neutral

Q. N 3 Have you ever consumed herpetofauna for any reason?

( ) Yes ( ) No

If yes, why?

( ) Food ( ) Medicine ( ) Other

Q. N 4 What are the potential threats to herpetofauna in your area?

( ) Habitat loss ( ) Pollution and disease ( ) Pesticides effect ( ) Climate change