DIET COMPOSITION AND NICHE OVERLAP OF SYMPATRIC ANURANS IN CROPLAND AND FOREST OF WESTERN SIDE OF BARANDABHAR CORRIDOR FOREST, NEPAL

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DECLARATION

I hereby declare that the work presented in this thesis 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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This is to recommend that the thesis entitled "**DIET COMPOSITION AND NICHE OVERLAP OF SYMPATRIC ANURANS IN CROPLAND AND FOREST OF WESTERN SIDE OF BARANDABHAR CORRIDOR FOREST, NEPAL**" has been carried out by Sovit Sapkota for the partial fulfillment of Master's Degree of Science in Zoology with special paper Ecology and Environment. This is his 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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LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviations
ANOVA	Analysis of Variance
BA	Standardized Niche Breadth
BCF	Barandabhar Corridor Forest
CNP	Chitwan National Park
DFO	Division Forest Office
GPS	Global Positioning System
IRI	Index of Relative Importance
masl	Meters above sea level
NMDS	Non-Metric Multidimensional Scaling
SVL	Snout Vent Length
TAL	Terai Arc Landscape

ABSTRACT

Dietary variation in sympatric anurans is important for understanding ecological niche of a species which have allowed it to live in various habitats and ecosystems. Croplands are crucial for amphibians that are useful for farmers by controlling crop pest and frogs also play vital role in forest ecosystem as well. Hence, this study aimed to focus on dietary habits and niche overlap of anurans in cropland and forest of western side of Barandabhar Corridor Forest, Central Nepal. Nocturnal time-constrained visual encounter transect survey using 100 m long transect at least 250 m apart from 19.00 to 21.00 hr was used for the anuran survey during monsoon (June-August). Non-lethal stomach flushing technique was applied for the extraction of diet and analyzed microscopically. Twenty nine (22.83%) individuals out of 127 stomachs flushed were found with empty stomach. The diet contained 442 prey items which were further categorized into 16 taxonomic groups. Hymenoptera (62.89%) was highly abundant prey, followed by Coleoptera (10.40%) and Anurophagy (6.78%). The relation between weight of prey uptake was significantly correlated with the Snout-Vent Length (SVL) (R^2=0.399, $p<0.0001$). Similarly, dietary preferences was no significant with habitat types (t =0.08, p=0.931). The result of Non-metric Multidimensional Scaling revealed that there was high degree of dietary niche overlap between the anurans species in both cropland and forest habitat. Common Asian Toad had the highest niche breadth (BA = 0.42) and high dietary niche overlap (0.985) followed by *Minervarya teraiensis* (average SVL= 46.13) and *Duttaphrynus melanostictus* (average SVL= 63.68 mm). This study evaluated the dietary preferences in different habitats and indicated importance of anurans. Hence, this study will be baseline for the conservation of amphibians.

1. INTRODUCTION

1.1. Background

Amphibians are one of the diverse vertebrates in animal kingdom, comprised of more than 8461 species worldwide (Frost 2022) and 57 species in Nepal (Shah and Tiwari 2004; Rai et al. 2022). The class Amphibia includes the orders Anura (frogs and toads), Caudata (Salamanders and Newts) and Gymnophiona (Caecilians) where Anura alone comprises 55 species (Shah and Tiwari 2004; Rai et al. 2022). Amphibians are paired limbs (except caecilians) and cold blooded vertebrates having soft, scale less, moist and naked skin. The larvae of frogs and toads (Order Anura) are grossly different from adults and have many developmental (Altig and Johnston 1989) and morphological (Altig and McDiarmid 1999) features not seen in other larvae of amphibians. The main habitat of amphibians includes terrestrial areas like agricultural fields, forest, cliffs, rocks, burrowing, grasslands, alpine meadows, trees, tunnels and so on. whereas the aquatic habitat include rivers, lakes, ponds, wetlands, and even houses as macro-habitat and micro-habitat (Schneider et al. 2001).

In the context of Nepal, anurans can be found in a variety of habitats and at elevations gradients, although overall anurans species richness and abundance are decreasing as elevation increases (Khatiwada and Haugaasen 2015). Barandabhar Corridor Forest (BCF) connects Chitwan National Park (CNP) in the South and the Mahabharat Mountains to the Annapurna Himalayan range in the Northern part. BCF, part of the Terai Arc Landscape (TAL), is considered to be biologically important for various flora and fauna (Aryal et al. 2012). However, the study on anuran is still in outset, their distribution and current status of population is poorly known in Nepal (CEPF 2005). Very little anuran species have been described from disturbed habitats, indicating a diminished species composition when compared with the original habitat (Molur 2008). From a conservation point of view also, anurans conservation efforts have been limited (Shah and Tiwari 2004). Temperature and vegetation cover are the key environmental variables affecting the distribution and abundance of frogs while seasonality also influences the distribution of certain anuran (Contreras 2018). Study of amphibians is an important part of biodiversity and conservation research because nearly one-third of the global amphibian species are threatened with extinction (Baillie et al. 2004). Currently, amphibians are facing serious threats due to habitat loss and degradation (Marsh and Trenham 2001), invasion, environmental pollution, excessive use of pesticides, disease and global climate change (Gibbons et al. 2000).

Amphibians are extensively distributed on every continent except Antarctica (Duellman and Trueb 1994) due to their generalist nature, as they consume a wide range of prey including arthropods, annelids, mollusks, and sometimes small vertebrates such as frogs and snakes (Freed 1982). Anurophagy has been reported in 228 species (Measey et al. 2015), in general larger species of frogs eating much smaller ones. Some small sized frog may also prey on the other frogs which even secret bufotoxin, the term called Batracophagy (Ceron et al. 2018). Major Anuran diet consists of insects of order Hymenoptera, Coleoptera, Lepidoptera, Orthoptera, Homoptera and Hemiptera (Mahan and Johnson 2007) but Hymenoptera was abundant followed by Coleoptera and Lepidoptera in case of rice field in Nepal (Khatiwada et al. 2016). The ecosystem services provided by frogs are not limited to control crop pest but also include consumption of large number insects known to be vital vectors of different zoonotic diseases (Khatiwada et al. 2016). Hence act as potential biological control agents of pest.

Niche overlap describes the situation of different species in which co-occurring species share parts of their niche space with each other (Pianka 1974). Availability of food and the degree of diet overlap assist to define the ecological function and tropic niche of a population within a community (Marques-Pinto et al. 2019). Diet preferences of various species play a substantial role in competition of resources (Lawlor 1980) and prey-predator interactions (Richter-Boix et al. 2007), which in turn shapes the community structure (Duellman and Trueb 1994). As a result, diets are most important indicators of the functioning and structuring of an ecosystem (Duffy et al. 2007). All the frogs have more or less similar feeding habitat and clear niche overlap can be observed which suggest that competition for food resource is not only the key driver to find out the frog distribution and community structure (Piatti and Souza 2011). Diet contained in different types of frogs is affected by various factors like distance to foraging ground, hunting approach, feeding behavior, duration and time of foraging.

The relationship between several species competing in a one-dimensional continuum for resources like food may be predicted by using niche overlap (May and Mac Arthur

1972). However, the degree of difference in use of food resources between similar species could reduce competition and would therefore allow their coexistence (Pianka 1973). A community with more resource sharing or greater niche overlap may hold up more species than that with less niche overlap (Rusterholz 1981). In case of dense population of closely related species of frogs, inter-specific competition for various resources is predicted to be higher (Crawford et al. 2009) which leads in consumption of similar prey items by multiple species of frog.

1.2. Rationale of the study

Information about variation of diet is important for understanding the ecological niche of a species, habitat exploration and ecology of organisms. Diet composition also gives knowledge on potential attributes that have allowed it to live in different habitats and ecosystems and the degree of dietary specialization (Darst et al. 2005). Frog plays a crucial role on ecosystem services as they generally uptake variety of small invertebrates including vector of the diseases and crop damaging pests as food. Despite a number of studies describing dietary habits of anurans (Hirai and Matsui 1999; Santos et al. 2004; Sabagh and Carvalho-e-Silva 2008; Piatti and Souza 2011; Khatiwada et al. 2016; Sapkota et al. 2022), there is a insufficiency of studies about frog diets in cropland and forest and very less information is available from Nepal. The feeding habit of anurans helps to evaluate the benefits of frog in ecosystem as well as ecosystem services provided by frogs. Moreover, its feeding ecology helps in understanding of the foraging habitat, prey selection and the feeding behavior of the frog species. The information on diet can also be applied for the conservation of anurans populations but even scare from Central Nepal. Hence, this study is designed to explore the dietary composition between different species of frog which would contribute to better understanding of distribution, feeding ecology and ecological functions of anurans in cropland and forest habitats. This will also helpful for formulating the strategies and plans in conservation and exploration of roles of different frog species as important biological pest controller agent during the development of pest management and strategies in agricultural landscapes.

1.3. Objectives

1.3.1 General objective

The general objective of the study was to assess diet composition and niche overlap of sympatric anurans in cropland and forest areas of western side of Barandabhar Corridor Forest, Nepal.

1.3.2 Specific objectives

- ❖ To assess the dietary composition of different anuran species in cropland and forest areas of western side of BCF, Nepal.
- ❖ To identify the dietary niche overlap between different anuran species in cropland and forest areas of western side of BCF, Nepal.

2. LITERATURE REVIEW

In the IUCN's list of threatened species, amphibians are declining to the great extent globally so the declines in amphibian populations have raised concerns. The increasing urbanization (Aryal et al. 2020), intensive agriculture (Luría-Manzano and Ramírez-Bautista 2017), change in water quality (Burraco and Gomez-Mestre 2016) and climate change (Davis et al. 2019) are emerging threats to anurans populations. To monitor the current data and potential risks, we require reliable demographic information. These studies address the inadequacy of anuran research, lack of information so the prominent need for anuran study.

2.1 Dietary composition

Limited studies on the diets of anurans have been recorded despite the fact that Nepal is home to a varied range of anurans. Norval et al. (2014) carried out study on the diets of five amphibian species in southwestern Taiwan. Three thousand four hundred and six prey items were reported from 21 orders and 6 classes, with ants (Formicidae) being the most abundant prey item in the diets of all five anuran species.Castro et al. (2016) studied the diet of *Dendropsophus branneri* at a cocoa plantation in Brazil. *D. branneri* has a small volume of prey per stomach, suggesting that it adopts a "sit and wait" foraging strategy. One surprising discovery from this study was that stomach flushing can be successfully implemented to frogs not less than 14.4 mm in size. The dietary niche breadth of *Fejervarya limnocharis* was the greatest, followed by *Duttaphrynus melanostictus, Microhyla fissipes, Microhyla astejnegeri, and Microhyla heymonsi*. There were also significant dietary overlaps among the anurans investigated. Dietary content varies amongst comparable species of anurans that thrive in different habitat types. Although Orthopterans were dominating in both the rainforest and cave populations of *Craugastor alfredi*, the dietary variety and feeding intensity were found to be greater in the rainforest population. This might be due to the species' smaller body size, direct development, and semi-arboreal habit inside the cave (Luría-Manzano and Ramírez-Bautista 2017). Apart from other aspects, distinct anuran foraging approaches may help in various feeding nature and habits. Sapkota et al. (2022) conducted study on diet composition of sympatric Amphibians in paddy fields of Western Nepal and the study found Hymenoptera (mostly ants) was the most abundant food group in the stomach contents, comprising 35.77% of the total number of prey items, followed by Coleoptera and Lepidopteran larva. In addition to this diet composition did not differ significantly among amphibian species and there was a high degree of dietary overlap among amphibian species in the paddy fields.

The diet of *Fejervarya limnocharis* showed significant difference in the prey composition rate between the habitat i.e farmland and natural habitat (Chang et al. 2016). The index of IRI showed Orthoptera and Coleoptera as dominant prey species in between habitat reflects the change in species composition due to anthropogenic disturbances. In the diet composition of Japanese tree frog, 71% of the callings male were found with empty stomach and most abundant prey categories was Arthropoda (Park et al. 2018). The most common prey categories observed were beetles, flies, bugs, and moths. In the diet assessment of tadpole of five anuran's species Asrafuzzaman et al. (2018) reported detritus and phytoplankton. This study reported the first ever Batracophagy in the diet of small sized frog *Leptodactylus policipnus,* which might due to its sit and wait foraging strategy. In the study of *Leptodactylus spixii*in Brazil, Sole et al. (2019) recorded 169 prey items. The most common prey category was Orthoptera followed by Acarina and Formicidae. They reported *L. spixi*as generalist feeder, with sit and wait predation habits.

Khatiwada et al. (2016) investigated the feeding patterns of anuran species in croplands of Chitwan, Nepal and discovered that frogs consume a substantial amount of crop pest. The research was carried out approximately 3-4 weeks after rice plantation. Furthermore, diets changed between species and even between individuals of the same species throughout wet and dry seasons. The findings found that frogs eat insects, which are known to be key carriers of zoonotic diseases. Chowdhary et al. (2018) also concluded that *Sphaerotheca breviceps* also acts as an important biological agent for controlling harmful pest and helps in ecosystem management. Their result revealed that this frog was primary predator of nocturnal terrestrial arthropods feeding mainly on insects and variety of other invertebrates.

The diet also differs in the sense that either the anurans feed primarily upon terrestrial or aquatic prey items. Vignoli et al. (2009) conducted research in dietary patterns of amphibians in pond of central Italy where they found two types of prey category: terrestrial 6 prey and aquatic prey in diet of frog. Micro-habitat, resource partitioning and body size in terrestrial species plays important role to influence dietary pattern while in case of aquatic species high dietary niche overlap was seen due to generalist feeding habits.

2.2 Dietary niche overlap

Since the majority of anurans are generalist predators that prey mostly on invertebrates and small vertebrates, dietary niche overlap across various species and habitat types can be greater. The feeding and tropic impacts of the invasive *Hoplobatrachus tigerinus* on the Andaman were studied by Mohanty and Measey (2018). They discovered that although most frogs eat tiny invertebrates, *H. tigerinus* primarily eats small vertebrates. While *Fejervarya sp*. and *Limnonectes sp*. were discovered in the same area, they did not share the same dietary niche.In Southern Brazil, a research on the diet and trophic niche overlap of the *Boana bischoffi* and *Boana marginata* was undertaken by Moser et al. (2018). These two frogs shared a high degree of tropic niche overlap (0.90), since they both prey on dominant Araeneda and Coleopteran species. As the niche breadth ranged from 0.35 to 0.42, the result also indicates to these two species generalist eating habits. This could change when comparing the sizes of large and tiny anurans. Generally, larger anurans can eat larger prey than smaller anurans, which leads to dietary portioning.

The diet composition of two sympatric frog species *Pseudis minuta* and *Scinax squalirostris* were determine by stomach content analysis and estimation of prey availability (Huckembeck et al. 2018). Body size and microhabitat use by the species also influences the dietary niche between anurans. Positive relationship can be seen between predator-prey body sizes which results in preference to different prey categories thus minimizing the niche overlap. During the survey, *Pseudis minuta* displayed a bigger body size and mouth width and shown a wider utilization of microhabitats, mostly in and around major water bodies, but *S. squalirostris* had a lower body size and mouth width and was only discovered in or close to *Phytotelmata*. Compared to *S. squalirostris*, *P. minuta* had a wider range of diet specialization. Likewise the study on two sympatric species *Ischnocnema henselii* and *Adenomera marmorata* in the forest of Southern Brazil raveled the effect of anuran body size on prey preferences (Santos-Pereira et al. 2015). The anuran species *Ischnocnema henselii* with larger SVL and jaw size considerably affected the volume of prey ingested. The niche overlap between the species was 0.52 as the larger body size of *I. henselii* enables it to prey on larger species and *A. marmorata* mostly depend on smaller species, which determines the difference in diet composition. Oliveira et al. (2015) recorded less overlap (O_{jk}= 0.28) between two phylogenetically and sympatric species *Physalaemus graciluis* and *Physalaemus biligonogerus*. Le et al. (2018) examined the diet composition and dietary overlap among Montane frog community in Vietnam by using stomach flushing technique, the result revealed interesting facts among the diet selection by frogs. *Leptobrachium pullum* was found to be specialist, only feeding on Orthoptera.

Cajade et al. (2010) conducted study on the trophic ecology of two anuran species *Dendrobates auratus* and *Oophaga pumilio* in La Selva Biological Station, Costa Rica. The result was interesting that in spite of feeding of similar prey categories dietary overlap was not significant, suggesting the absence of negative feeding interactions. Microhabitat use, body size and gape size makes differentiation on the use of spatial resource. The estimated tropic niche overlap between 8 the species was moderate and probably there was no significant competition for food resources between different species in the places with sympatric distribution (Mollov and Stojanova 2010). Anurans might prefer to similar prey categories but the species consumed by them might be different which separates the dietary preferences among species. Sabagh et al. (2012) carried out the study from Central Amazonia on food niche overlap between two sympatric leaf litter anuran species. Ants were main food item in the diets of both frog species. The coexistence between these frog species might be facilitated by the significant differences in the size of their mouths. This difference made them able to consume prey items of different sizes.

2.3 Research gap

The researchers are still in the phase of identification of species and very less research can be found in anuran diet and about its habitat. Hence this study aimed to fulfill this research gap in some instant. Especially, Central Nepal is still unexplored area and comparative analysis of diet assemblage in cropland and forest areas will provide the baseline information for further research on frog ecology and conservation. There is inadequate research conducted in the dietary composition with anurans body size and niche overlap in Nepal. This suggested that there is a research gap and demand the research on anurans in Nepal.

3. MATERIALS AND METHODS

3.1. Study area

The study was carried out in the different cropland and forest area of western side of Barandabhar Corridor Forest (BCF) of Chitwan District, Bagmati Province, Nepal. The BCF is divided into two parts by an east-west highway; the southern part is in the buffer zone and maintained by Chitwan National Park (CNP), while the northern part is protected forest and controlled by Division Forest Office (DFO). The climate is humid and subtropical monsoon climate. The mean monthly temperature varies from 15 °C in January to 29 °C in June. High monsoon rainfall occurs between June to September and annual rainfall ranges from 1,800 to 2,200 mm/annum in BCF (DHM 2019).

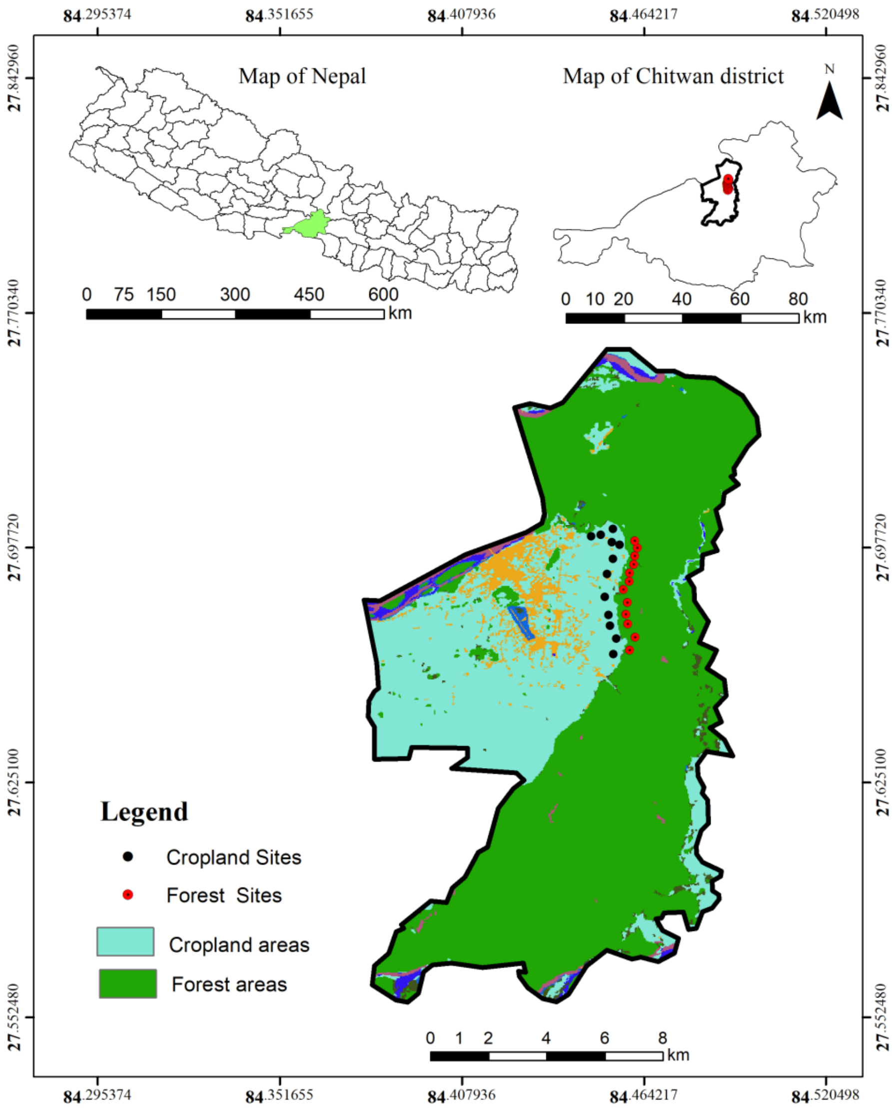

Figure 1: Map of study area showing sampling sites

Two different categories of habitats were surveyed: cropland and forest.

Cropland habitat: Agricultural paddy field area was taken as cropland. Anurans were surveyed in microhabitats like trail between fields, grassy banks of the rice fields, inside the field and associated terrestrial habitats of cropland.

Forest habitat: Forest around the study area was chosen for anuran survey. The vegetation of the BCF is dominated primarily by Sal forest following riverine forest, mixed forest, and grasslands. Anurans were surveyed in microhabitats including leaf litter, tree leaves, tree branches, and grasslands inside forest.

Altogether 199 species of vegetation were found in the BCF area, sal (*Shorea robusta*) and asna (*Terminalia alata*) are the most prominent species, whereas simal (*Bombax ceiba*), bot dhayiro (*Lagerstromia parviflora*) etc are the associated floral species (Lamichhane et al. 2016).

A total of 31 species of Herpetofauna including 12 species of anurans, 3 species of lizards, 9 species of snakes, 3 species of gecko and single species of crocodile were recorded in BCF (Lamichhane et al. 2016). In addition to this, 329 species of birds were recorded from this area. This diverse ecosystem of BCF is home to 32 animal species, including wild ungulates such as chital (*Axis axis*), sambar deer (*Rusa unicolor*), northern red muntjac (*Muntiacus veginalis*), wild boar (*Sus scrofa*), hog deer (*Axis porcinus*) greater one-horned rhino (*Rhinoceros unicornis*), elephants (*Elephas maximus*) and so on (Lamichhane et al. 2016; Adhikari et al. 2021).

3.2 Materials

- Camera: Nikon D5600
- Diet Extraction Set
- Digital Caliper
- Field Guide Book (Shah and Tiwari 2004)
- GPS: Garmin eTrex® 10
- Measuring Tape
- Microscope
- Three-digit Weighing Machine
- Torch Light

3.3 Methods

3.3.1 Field sampling design

The preliminary survey was conducted during last week of June, 2021 to gather the necessary information about the study area and the feasibility of the research. Habitats were divided into two categories as cropland and forest, and sampling design were made accordingly. A total of 24 transects of 100 m length were made with twelve transect representing each habitat. Equal sampling effort was applied in terms of time and manpower in both habitat types.

3.3.2 Anuran survey

Anuran surveys were carried out during the months of April to September, 2021. This coincides with the monsoonal rainfall and breeding season of frogs (Schleich and Kästle 2002). Time-constrained, nocturnal visual encounter survey (Campbell and Christman 1982) was employed for the anuran survey. Two people systematically walked at a slow pace for 30 min along transect of 100 m length and cover 2 m on either side using torch light from 19.00 to 23.00 hr. Transects were placed at least 250 m apart from each other. The number of anuran species and individuals encountered in each transect were recorded. Anurans encountered in all transects were captured and kept in small cotton bag to avoid the recapture. All anurans species were identified in the field with the help of field guide book (Shah and Tiwari 2004).

Each captured anuran individual in transect was taken to nearby dry area and measured Snout-Vent Length (SVL) and body weight along with sex identification. Individuals with SVL greater than 15 mm were only selected for the extraction of diets. Males were identified based on secondary sexual characteristics such as the presence of blue pigment vocal sac on either side of throat and nuptial pads, and females based on enlargement of the coelomic cavity.

Figure 2: Anuran survey (A) cotton bag to collect frogs (B) male frog having dual blue-colored vocal sacs on either side of the throat (C) measuring SVL of frog (D) measuring weight of frog by using analog weighing machine.

3.3.3 Diet extraction

For the extraction of diet, frog diet was collected by using a non-lethal stomach flushing technique described by Sole et al. (2005). The stomach contents of each frog were flushed by using 50 mL syringe with attached soft surgical plastic tube (15 cm long and 2 mm in diameter). Thumb and spatula were used to open the mouth and the soft surgical plastic tube was inserted carefully through the esophagus into the stomach of the frogs. Once the tube was introduced, 50 mL of water was squeezed slowly from the attached syringe into the stomach and any content ejected from the stomach was then collected in plastic sieve. The stomach-flushing procedure was repeated frequently up to three times until no further stomach content was ejected. Food items present in the oral cavity after flushing were carefully collected using entomological forceps. The retrieved stomach contents were stored in vile containing 70% ethanol for further identification and measurements. At last, frogs were released at its original habitat approximately 30 minutes after flushing, once normal activity had been regained.

Figure 3: Process of diet extraction (A) capturing frog from cropland- nocturnal VES (B) using spatula to open the frog's mouth (C) introducing soft surgical plastic tube into the stomach of frog (D) stomach contents of *H. tigrinus* (E) stomach contents in vile containing 70% ethanol (F) releasing frog after 30 min after flushing.

3.3.4 Diet analysis

Stomach contents of individual frog were kept on filter paper from vile to remove the moisture from the stomach contents and collected prey were weighted by using 3-digits weighing machine. Prey items were placed carefully in a Petri dish and examined under a stereoscopic microscope. Reference slides of antenna, wings and legs were used to recognize the unidentified prey items so antenna and legs were used to prepare the reference slides from collected prey items. All the prey items collected from stomach of frogs were identified to lowest achievable taxonomic level (Order or Family) at the lab of Central Department of Zoology, Tribhuvan University. In the laboratory, those prey items which were fully digested could not be identified and natural objects such as grass, leaves, mud, pebbles and stones were excluded from the stomach contents as they might have been accidentally consumed during feeding.

Those prey items found as whole specimen in stomach were used for analysis. All recorded prey items were categorized up to orders in case of Insecta and other were categorized as their type such as larva, snail, earthworm, crab, spider, snake and frogs.

3.4 Data analysis

The gathered information was organized, structured, and entered into Microsoft Excel for additional analysis. The sum of the numbers for each prey item and prey category produced the percentage contribution.

The correlation between anuran body weight and stomach content was examined using a linear regression model. The model was analyzed for each species, and the results only included those species that showed a significant association. Regression in the Data Analysis Tool set was used to construct a linear regression model in Microsoft Excel and create a graph. To investigate the dietary habits of anurans in two distinct habitat types, a paired t-test was employed.

The dietary niche overlap between various species was determined using Non-Metric Multidimensional Scaling (NMDS). To identify the similarities between prey categories, the Bray-Curtis Similarity Index was used. PAST 4.0 was used to prepare the final graph (Hammer et al. 2001).

Levin's niche breadth

Niche breadth of different anurans was calculated by using Levin's Niche breadth (Levins 1968) formula,

$$B = \frac{1}{\sum Pi2}$$

Where, B is the Levin's measure of Niche breadth and P_i is the proportion of individuals found using resources.

Standard niche breadth

Levin's Niche breadth is further standardized to range of 0-1 by using formula,

$$B_A = \frac{B-1}{n-1}$$

Where, BA is the standardized niche breadth, and n is the total number of prey categories for the species.

Pianka's niche overlap

The niche overlap between two species of anuran was calculated by using the formula given by (Pianka 1973). The value of Pianka's Index varies from 0-1.

$$O_{jk} = \frac{\sum PijPik}{\sqrt{\sum P^2ijP^2ik}}$$

Where, O_{jk} is Pianka's measure of overlap between species j and species k, P_{ij} is the proportion by number that resource i is of the total resources used by species j, and P_{ik} is the proportion by number that resource i is of the total resources used by species k.

4. RESULTS

4.1 Dietary composition

A total of 127 stomachs of frogs were flushed to extract stomach contents. Of these, 29 (22.83%) had empty stomach or completely digested foods. From the remaining 98 (60 males and 38 females) anurans of eight species belonging to four families identifiable prey items were extracted (Table 1). A total of 54 individuals with stomach content were recorded from croplands and remaining 44 anuran individuals were captured from forest habitat.

Table 1: Anurans species with individual records in cropland and forest

S. N	Family	Name of species	Individuals with stomach content		Individuals with Empty stomach	
			Cropland	Forest	Cropland	Forest
1	Ranidae	*Hoplobatrachus tigerinus* (Daudin, 1802)	26	17	5	4
		Euphlyctis cyanophlyctis (Schneider, 1799)	17	11	4	0
		Hoplobatrachus crassus (Jerdon, 1853)	2	7	2	0
		Minervariya teraiensis (Dubois, 1984)	4	3	1	0
2	Rhacopphoridae	*Polypedates maculatus* (Gray, 1830)	2	0	5	1
		Polypedates taeniatus (Boulenger, 1906)	0	1	4	1
3	Bufonidae	*Duttaphrynus melanostictus* (Schneider, 1799)	3	3	0	0
4	Microhylidae	*Uperodon taprobanicus* (Parker, 1934)	0	2	0	1
		Microhyla ornata (Duméril and Bibron, 1841)	0	0	1	0
	Total		**54**	**44**	**22**	**07**

The prey items after diet analysis were classified into 16 categories (Table 2). Prey items from the class Insecta were additional classified up to the order level, whereas other prey items were classified based on their type. A total of 442 identifiable prey items were identified. Algae and different plant parts were removed from dietary habit descriptions and comparisons, assuming they were consumed coincidently by anurans.

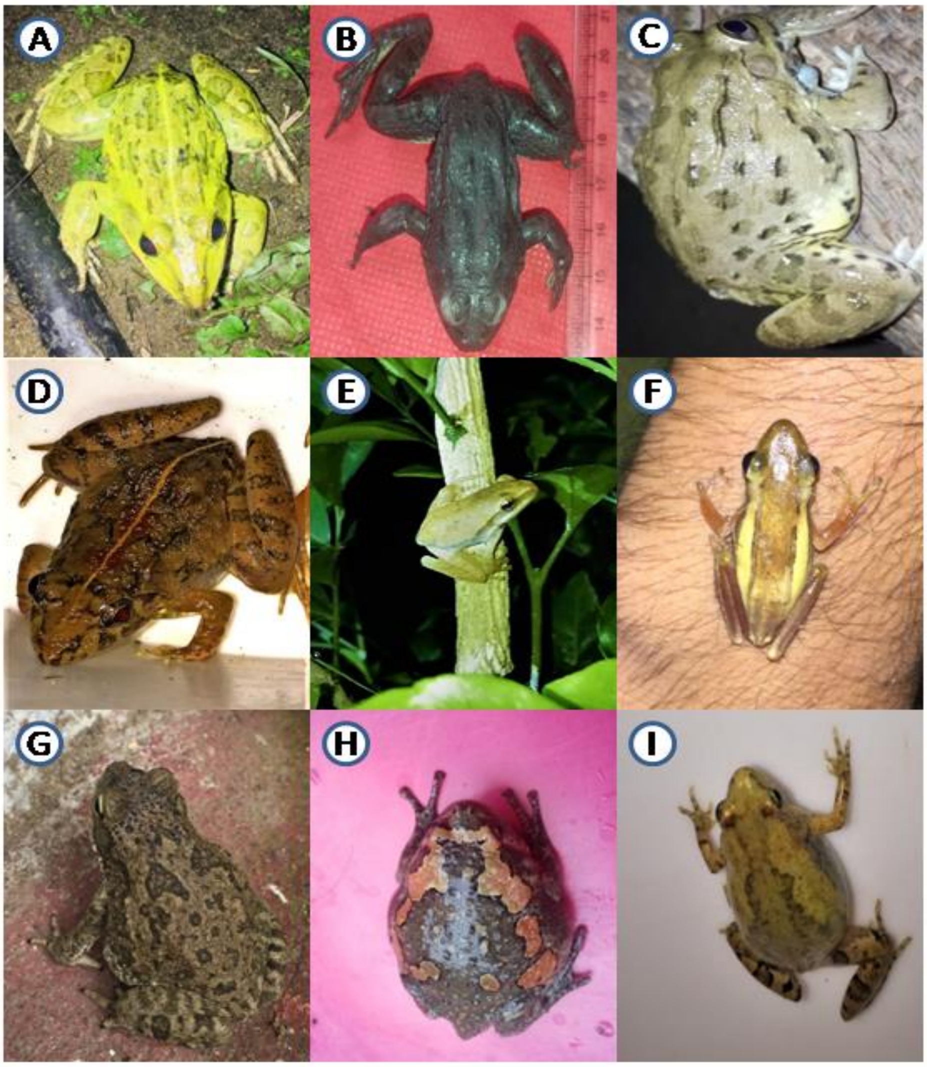

Figure 4: Anurans of cropland and forest studied for diet analysis. (A) Asian Bullfrog (*Hoplobatrachus tigerinus*), (B) Common Skittering Frog (*Euphlyctis cyanophlyctis*), (C) Jerdon's Bullfrog (*Hoplobatrachus crassus*) (Photographed by Suman Sapkota), (D) Terai Cricket Frog (*Minervarya teraiensis*), (E) Indian tree frog (*Polypedates maculatus*), (F) Six lined tree frog (*Polypedates taeniatus*), (G) Common Asian Toad (*Duttaphrynus melanostictus*), (H) Sri Lankan painted frog (*Uperodon taprobanicus*), (I) ornate narrow-mouthed frog (*Microhyla ornata*).

Table 2: Prey categories and prey items obtained after stomach flushing

S.N	Prey categories	Individual (s) of prey	Relative abundance (%)	Species recorded
1	Hymenoptera	278	62.89	Cropland and forest
2	Coleoptera	46	10.40	Cropland and forest
3	Anurophagy	30	6.78	Cropland and forest
4	Larva	25	5.65	Cropland and forest
5	Hemiptera	14	3.16	Cropland and forest
6	Spider	12	2.71	Cropland and forest
7	Orthroptera	12	2.71	Cropland and forest
8	Diptera	11	2.48	Cropland and forest
9	Earthworm	3	0.67	Cropland only
10	Snail	3	0.67	Cropland and forest
11	Tricoptera	2	0.45	Cropland only
12	Blattodea	2	0.45	Cropland and forest
13	Isopoda	1	0.23	Cropland only
14	Lepidoptera	1	0.23	Cropland only
15	Mariopoda	1	0.23	Forest only
16	Snake	1	0.23	Cropland only
	Total	**442**	**100.00**	

Hymenoptera was the most dominating prey category which was consumed by all of the captured species of anurans. Coleoptera was the second most preferred prey category by the anurans which is followed by rest of the prey categories. Anurophagy was found in thirty individuals of *Hoplobatrachus tigerinus*, *Hoplobatrachus crassus* and *Euphlyctis cyanophlyctis*.

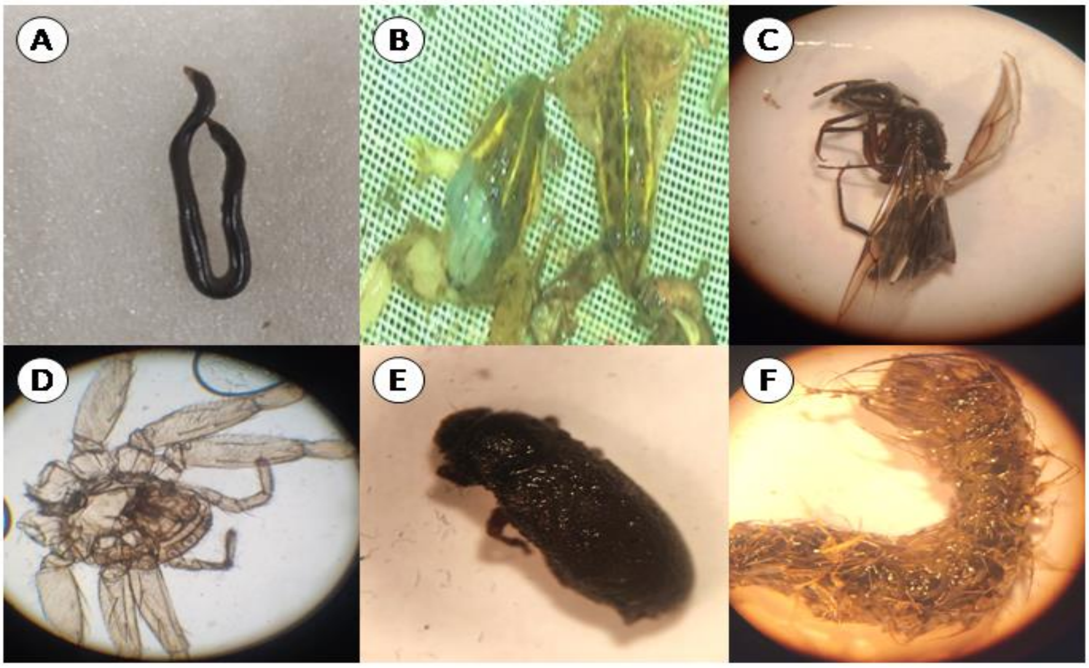

Figure 5: Prey items obtained after stomach flushing of *Hoplobatrachus tigrinus* (A) Blind Snake in diet (B) Anurophagy in diet (C) Hymenoptera in diet (D) Spider in diet (E) Coleoptera in diet (F) Larva in diet

Pest and non-pest categories

Hymenoptera (Ant), Blattodae (Cockroach), Spider, Anurans, Earthworm, Reptiles (Snakes), and Mariopoda are among the prey types listed as non-pest in cropland and forest (Figure 6).

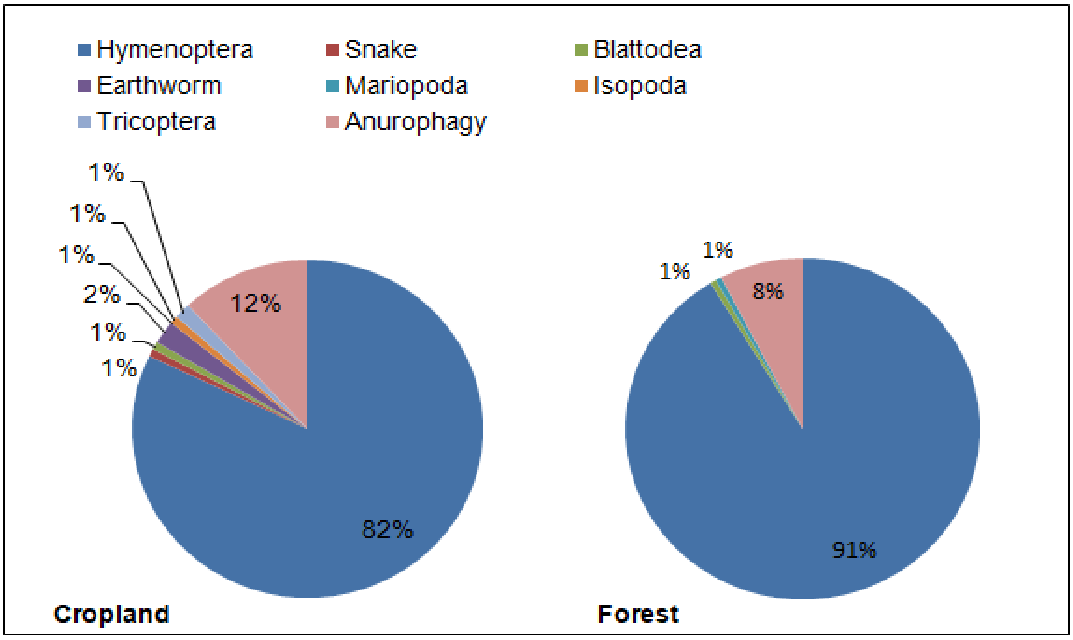

Figure 6: Non-pest prey categories in cropland and forest habitat

Prey pest categorizes which were characterized as pest consists of Coleoptera (Blue Beetles), Larva (moth larvae, moth nymphs, larvae of any worm or other species), Hemiptera (Leaf hopper), Lepidoptera (Caseworm), Diptera (Housefly) and Snail cropland and forest (Figure 7).

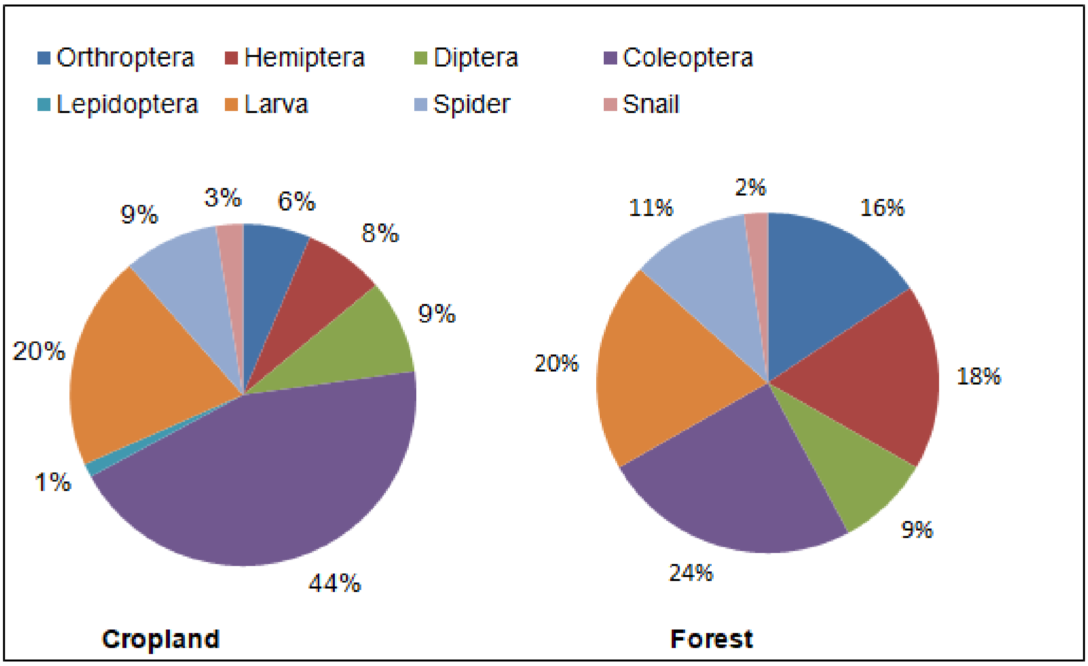

Figure 7: Pest prey categories in cropland and forest habitat

A total of 7 prey categories with 134 individuals of non-pest were recorded from cropland whereas 4 prey categories with 184 individuals of prey recorded from forest. The non-pest categories in anuran diet are higher in forest. However the abundance of prey pest items was comparatively higher in cropland than forest. A total of 8 prey categories with 79 individuals of pest were recorded in cropland whereas only 7 prey categories with 45 individuals of prey recorded in forest.

4.2 Dietary profile of individual species

Hoplobatracus tigerinus

Hoplobatracus tigerinus was the most abundant frog in the study area consisting of 52 individuals. Out of 52 individuals, 43 individuals were found with stomach content while 9 individuals (16.98%) with empty stomach. The stomach content of this species contained all 16 prey categories including 278 prey items. This species showed maximum proportion of anurophagy among other species. Hymenoptera was the most dominating prey category consisting of 188 items which was followed by 24 items of Coleoptera, 17 individuals with anurophagy and other prey categories.

Euphlyctis cyanophlyctis

Euphlyctis cyanophlyctis was the second most abundant frog with 32 individuals. Out of 32 individuals, 28 individuals were found with at least one prey item while 4 individuals (12.5%) were found with empty stomach. Out of 16 prey categories, this species contained 10 prey categories including 70 prey items. The most dominating prey category was Hymenoptera including 27 items which was followed by 12 individuals of larva.

Hoplobatrachus crassus

A total of 11 individuals of *Hoplobatrachus crassus* were stomach flushed out of which 9 individuals were found with stomach content while a 2 individuals (18.18%) was found with empty stomach. The diet of this species consisted of 27 prey items belonging to 6 prey categories. Hymenoptera was the most dominating prey category consisting of 18 items which was followed by 4 items of anurophagy.

Minervarya teraiensis

Eight individuals of *Minervarya teraiensis* was stomach flushed out of which diet was extracted only from seven individuals and remaining one individual (11.11%) was found with empty stomach. A total of 37 prey items belonging to 4 prey categories were found. Hymenoptera was the most dominating prey category consisting of 27 items which was followed by 5 items of Coleoptera.

Polypedates maculatus

Eight individuals of *Polypedates maculatus* was stomach flushed out of which diet was extracted only from two individuals and remaining 6 individuals (75%) were found with empty stomach. A total of 9 prey items belonging to 3 prey categories were found. Hymenoptera and Coleoptera was abundant consisting 4 individual of each.

Polypedates taeniatus

A total of 6 individuals of *Polypedates taeniatus* were stomach flushed out of which only one individual was found with stomach content while a 5 individuals (83.33%) was found with empty stomach.A total of 1 prey item belonging to Hymenoptera was found.

Duttaphrynus melanostictus

Total number of stomach flushed individuals of *Duttaphrynus melanostictus* was six. *Duttaphrynus melanostictus* was only species found all individuals with stomach contents, no individuals were found with empty stomach. The diet of *Duttaphrynus* consisted of 17 prey items belonging to 3 categories. The most dominating prey category was Hymenoptera including 12 items which was followed by 3 items of Coleoptera.

Uperodon taprobanicus

Out of 3 individuals of *Uperodon taprobanicus*, 2 individuals were found with at least one prey item while single individual (33.33%) was found with empty stomach. Out of 16 prey categories, this species contained 3 categories containing snail, Hymenoptera and Coleoptera each of single item.

Microhyla ornata

Microhyla ornata was the least abundant among all of the above. Only single individual was found and stomach flushed. It was found with empty stomach and this species was not kept in further data analysis.

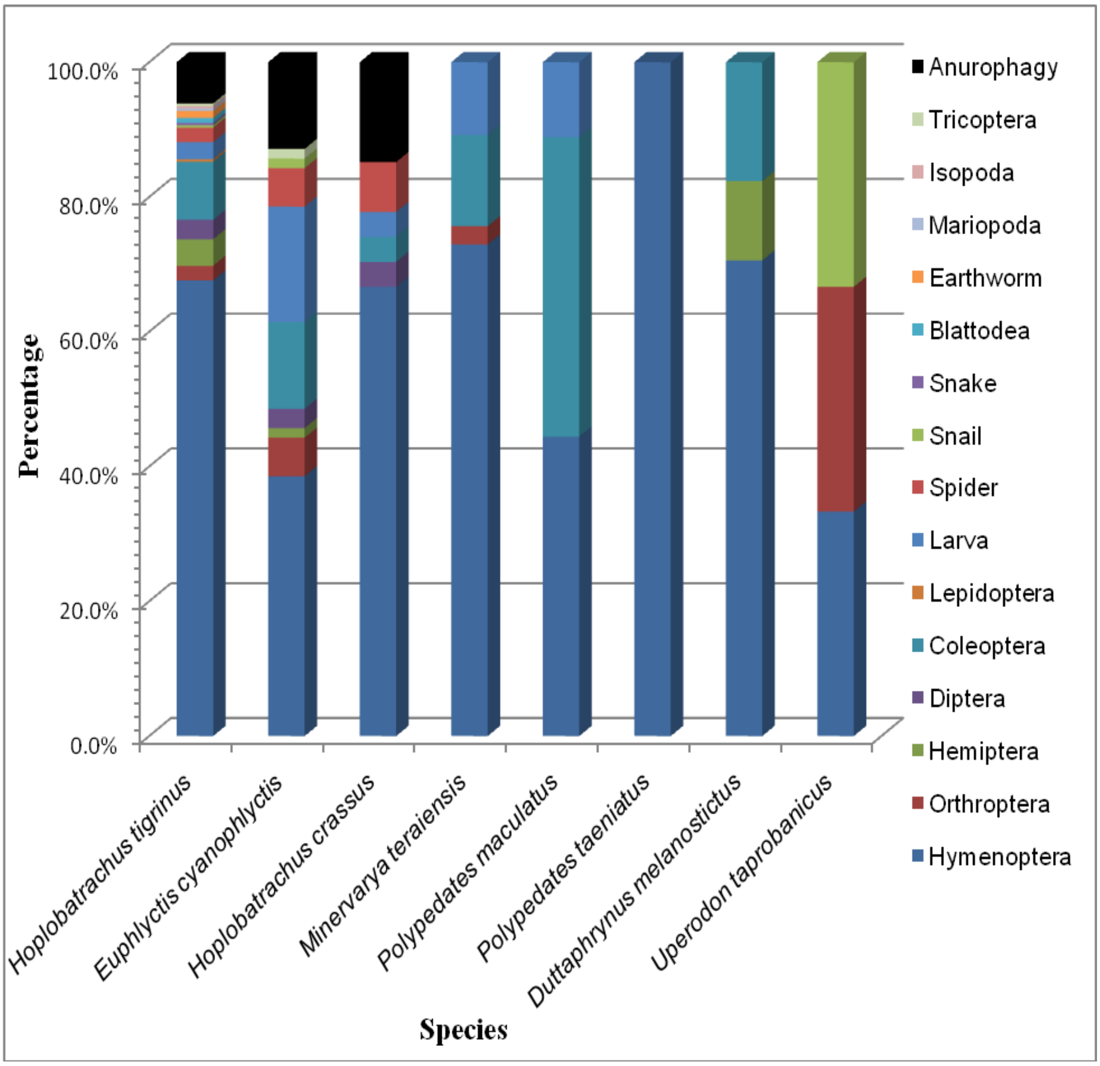

Figure 8: Dietary composition of different anurans species

4.3 Effect of body size on prey consumption

The average Snout-Vent Length (SVL) of all the stomach flushed anurans was 69.21 mm and the average body weight was 46.33 gm. The average weight of stomach content after being dried was 0.312 gm. There was a positive relation between SVL and body weight of captured frogs ($R^2 = 0.88$, $p < 0.001$).

The largest frog in the study area was *H. tigerinus* with average SVL of 89.07±13.8 mm which was followed by *Duttaphrynus*, 63.68±3.95 mm. The average body size of *Uperodon taprobanicus, H. crassus, E. cyanophlyctis, M. teraiensis and Polypedates maculates* was 60.20±7.35 mm, 57.48±8.96 mm, 53.51±8.49 mm, 52.42±10.81 mm and 46.97±9.04 mm respectively.

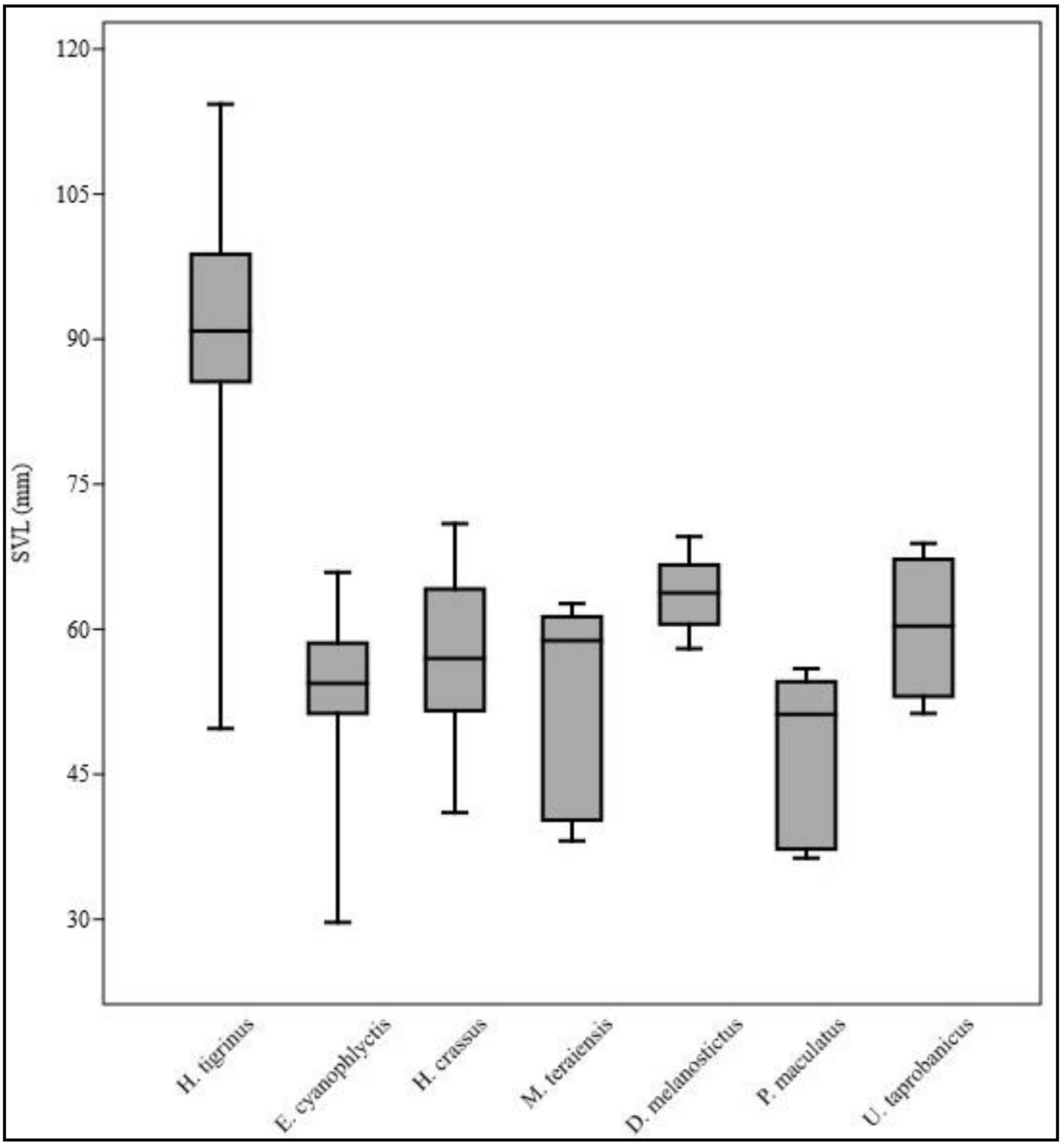

Figure 9: Snout-Vent Length (Mean ± SD) of seven species in 95% interval.

The linear regression analysis showed the positive relationship (R^2= 0.399) between the total weights of prey consumed with the Snout-Vent Length (SVL) of frog ($p<0.0001$). It was seen that large sized frogs generally consumed more prey (Figure 10).

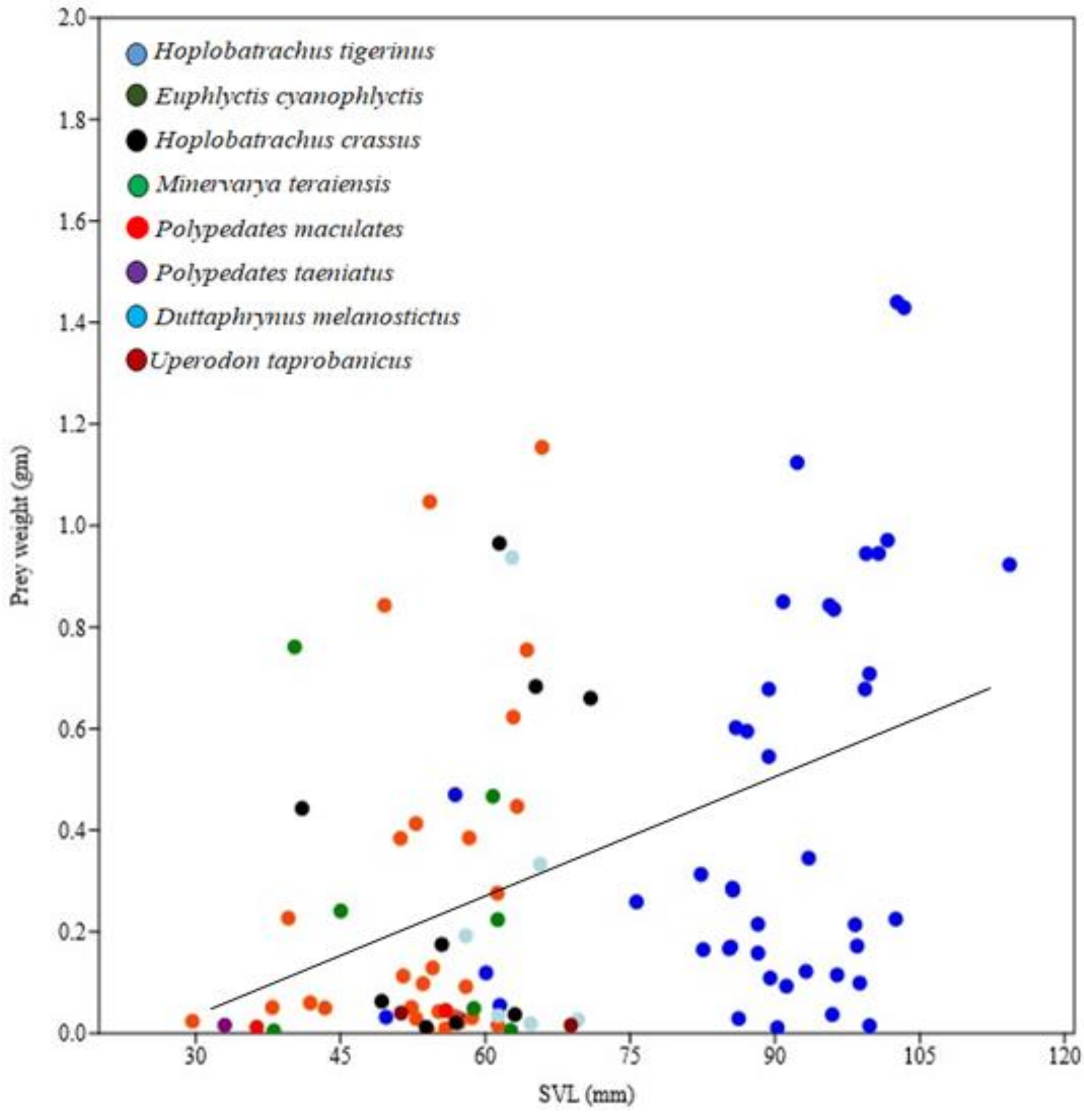

Figure 10: The relationship of snout vent length of captured frog with total weight of prey consumed.

4.4 Effect of habitat type on dietary habit

A paired t-test results showed that there were no significant effect of habitat on the dietary habits of anurans (t =0.08, p=0.931). The variation in the mean among the treatment groups was not great enough to exclude the possibility that the differences are due to random sampling variability.

4.5 Dietary niche overlap

The highest Standarized niche breadth (0.42) was observed in Common Asian Toad (*Duttaphrynus melanostictus*) followed by 0.40 in Tik-tike paha (*Euphlyctis cyanophlyctis*), 0.37 in Rukh Byakuta (*Polypedates maculates*). The lowest niche breadth was 0.07 which was observed in *Hoplobatracus tigerinus*, reaveled that it depends on greater in hymenoptera order in diet among seven different species in the study area (Table 3).

Table 3: Levin's niche breadth and Standard niche breadth of the seven anurans species

SN	Name of Species	Levin's Niche Breadth (B)	Standardized Niche Breadth (BA)
1.	*Hoplobatracus tigerinus*	2.16	0.07
2.	*Euphlyctis cyanophlyctis*	4.56	0.40
3.	*Hoplobatrachus crassus*	2.11	0.22
4.	*Minervarya teraiensis*	1.78	0.26
5.	*Polypedates maculates*	2.45	0.37
6.	*Duttaphrynus melanostictus*	1.84	0.42
7.	*Uperodon taprobanicus*	1.50	0.25

The high dietary niche overlap was 0.985 which observed between two medium sizes species *Minervarya teraiensis* (average SVL= 46.13) and *Duttaphrynus melanostictus* (average SVL= 63.68 mm). The dietary niche overlap between *Hoplobatracus tigerinus* and *Duttaphrynus melanostictus* was also found to be high (i.e 0.981) followed by *Euphlyctis cyanophlyctis* (average SVL= 53.51 mm) *and Hoplobatracus tigerinus* (i.e 0.97), *Minervarya teraiensis* (average SVL= 46.13 mm) and *Duttaphrynus melanostictus* (0.95). Where as least dietary niche overlap was recorded between medium sizes tree frog *Polypedates maculates* (average SVL= 46.13 mm) frogs and large sizes species *Hoplobatracus tigerinus* (i.e 0.77).

The feeding habit and diet preferences of all the species was found to be comparatively similar to each other and the NMDS plot demonstrated the high degreeof niche overlap between all the anurans. All the anurans species were generalist predators and fed on almost similar type of prey categories.

Hoplobatrachus tigrinus was observed with larger dietary niche followed by *Euphlyctis cyanophlyctis* in both cropland and forest habitat. NMDS plot for cropland and forest area shows high niche overlap among the anurans species, as most of the prey items are clumped in one definite region and each symbol represents single individual. The final plot figure 11(A) was obtained in 3-D to reduce the stress configuration from 0.31 to 0.23. In plot 11(B) Final stress configuration in 2-D was 0.25 hence graph was prepared in 3-D to reduce the final stress configuration to 0.21. Bray-Curtis Similarity Index was used to get graph from NMDS.

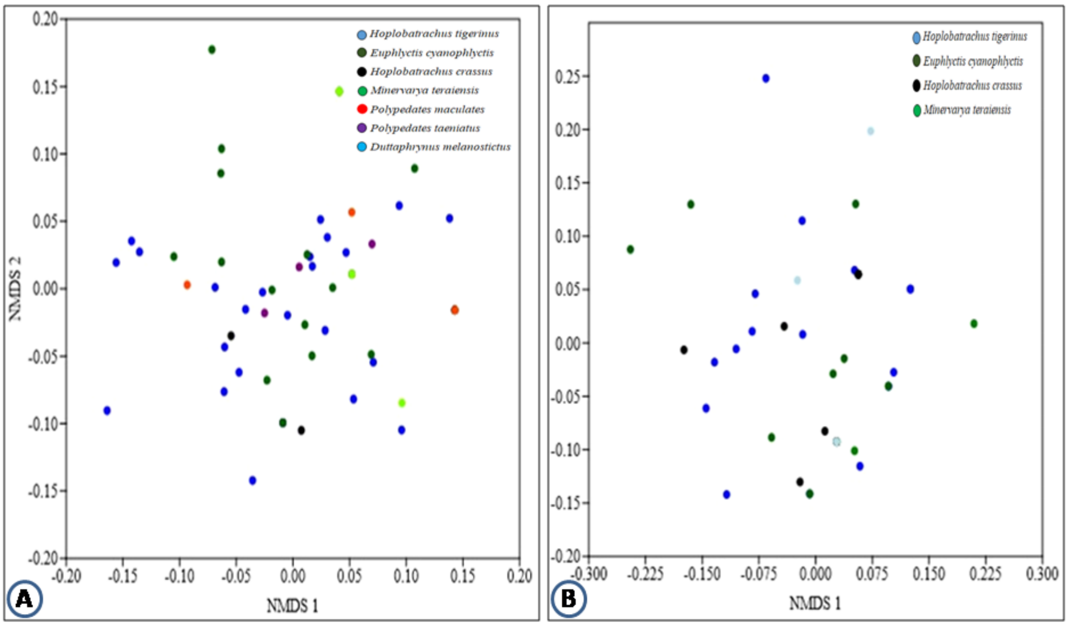

Figure 11: Non-metric multidimensional scaling (NMDS) ordination of diet overlap of anurans (A) Dietary niche overlap between seven different species in cropland area (B) Dietary niche overlap between four different species in forest area.

5. DISCUSSION

5.1 Dietary composition

Hymenoptera was the most dominant category in diets of all frog species, followed by Coleoptera, Anurophagy and larva. The diet composition result of this study was similar to the outcome by (Khatiwada et al. 2016) in rice fields of Chitwan, Nepal. They found Hymenoptera (35.5%) was the most abundant prey species in the rice field followed by Coleoptera (22%). However similar types of patterns were not seen in forest areas as Hymenoptera (73.36%) was the most dominant prey of anuran species followed by Anurophagy (6.11%). All amphibian species' diets were dominated by Hymenoptera (mostly ants). Eating ants has major ecological and evolutionary significance in amphibians, such as the usage of ants as alkaloid precursors (Daly et al. 1994), and this dietary activity can also help to understand prey capture technique (example, active vs. sit and wait).The distinct preference for Hymenopterans and Coleopterans may be due to their chitinous exoskeletons, which may take more time to digest compared soft-bodied prey like insect larvae (Mahan and Johnson 2007).

A total of 29 individuals were detected with empty stomachs, which might be due to the time of capture. The presence of stones, silt, and grass in a frog's stomach might be due to accidental intake along with its prey. During the research period, a small number of tree frogs were captured and observed with an empty stomach. Tree frogs have a particular feeding habit, consuming only selected prey items among the available ones (Araujo-Vieira et al. 2018).The frequency of empty stomachs of tree frogs and *Microhyla* was very high, this could be linked to previous findings that frogs stop eating, or reduce prey intake during the breeding season (Hirai and Matsui 2000; Tiberti et al. 2016). In addition to this, the empty stomach of *Polypedates* species may be caused by the limited number of species ability to capture tree frogs and its specialized diet.

One of the most interesting findings of this study was small blind snake consumed by frog (*H. tigrinus*). Frogs are amazing creatures that are sometimes excellent hunters (Jancowski and Orchard 2013). Frogs of various species have a diverse prey range and can prey on some unlikely animals. Frogs can kill snakes by striking them and consuming them whole and alive. The snake becomes caught in the frog's sticky tongue. It is immobile and suffocates in the stomach of the frogs (Goetz et al. 2018).

5.2 Dietary profile of individual species

Frogs in cropland and forest relied heavily on Hymenopterans, Coleopterans, and Orthopterans for their diet. These findings are consistent with the few previous anuran dietary studies (Hirai and Matsui 2000; Piatti and Souza 2011). Pest categories dominated the diet of anurans in the cropland areas than forest areas. The dominating prey items found in the diet of *H. tigrinus* were pests (except Hymenoptera) such as Coleoptera, Larva and Orthoptera and so on. *Euphlyctis cyanophlyctis* also consumed greater amount of Larva, Coleoptera and Orthroptera in their diet in cropland and significant amount of Hymnoptera in forested area. All 16 categories of pest items were found in the diet of *H. tigrinus* in both cropland and forest habitat. This result revealed that all of the anuran species consist significant amount of pest in their diet as similar to study of Khatiwada et al. (2016). Their research showed that during dry season, the large aquatic species (*Euphlyctis cyanophlyctis*) consume a lot of crop pest.

Anurans also ingested insects such as mosquitoes, house flies, and cockroaches in the research area, which are physically detrimental to human health. This result was partially similar to one reported by Khatiwada et al. (2016), who discovered culex mosquitoes, sand flies, and a housefly that transmit vector-borne disease. Moreover, in cropland, anurans consumed a significant proportion of harmful insects and clearly represent a vital ecological service by destroying pests.

5.3 Effect of body size on prey consumption

The weight of prey consumed and frog body size (SVL) were shown to be positively correlated (0.39). Prey weight is frequently used as an appropriate parameter for prey size analysis, and a few species revealed a positive relationship between body size and prey weight (Hirai and Matsui 2000). Large frogs were observed feeding large prey items such as other frogs. However, there was no relationship between frog weight and prey weight ingested. The total frog research revealed a positive correlation between body size and prey weight.

Only one species (*Hoplobatrachus tigerinus*) was shown to have a positive relationship between body weight and body size and with prey weight. This might be due to the species' size. With an average SVL of 89.07 mm and a maximum of 114.3 mm, *H. tigerinus* was the biggest frog in the study area. This frog's increased size may allow it to ingest larger prey items and more food by weight. A similar pattern was discovered in a research done by Mohanty and Measey (2018). *H. tigerinus* had a significant positive relationship between prey size and volume and body size, although *Limnonectes* and *Fejervarya sp*. did not follow the same trend. Because of its generalized dietary habits, the frog may consume fewer large prey items but consume a large number of little prey items (Vignoli and Luiselli 2012). As a result, it is very difficult to forecast the anurans feeding habits and preferred prey.

5.4 Effect of habitat on feeding habit

Two different habitat types (cropland and forest) were studied, and there was no significant difference in prey selection among these habitat types. This might be due to the feeding habit and generalist predation by all the anurans. Generally, anurans forage at night and the insects might also follow the same pattern so they are active during the night in cropland and forest. In addition to this, the whole cropland area was a paddy field and the tree composition was also relatively uniform. This could be the cause of similar insect types becoming prevalent, leading to similarities in feeding preferences. In contrast, the feeding habit of frogs and floristic composition, forest type, time, temperature, and habitat heterogeneity have an impact on insect diversity (Chung et al. 2000). In short, the generalist feeding habit might be the reason that anurans feed on every possible available prey type showing no significant difference in dietary preferences among habitat types (Menin et al. 2015).

5.5 Dietary niche overlap

The seven species of anurans present in the study area had a high degree of dietary niche overlap in cropland. Diets of all frogs comprised mostly similar types of prey, with Hymenoptera and Coleoptera dominating prey categories. Their feeding habits and consumption of similar types of prey might be due to their foraging approach. The application of an active search approach in combination with a sit and wait strategy resulted in the eating of similar types of prey (Mohanty and Measey 2018). In addition to this the anuran species recorded in the study area were common and found in both habitat types which might result in foraging upon similar prey items. The high dietary niche overlap among sympatric anuran species might be explained by high prey availability and similar foraging habits (Piatti and Souza 2011).

Hoplobatrachus tigerinus was the only species which consumed all 16 prey categories found in study area which suggest it can consume every possible prey found in the environment. It was followed by *Euphlyctis cyanophlyctis* with 10 prey categories. The higher niche overlap (O_{jk}=0.985) was recorded between two medium sizes species *Duttaphrynus melanostictus* and *Minervarya teraiensis*. This might be due to larger individuals consume larger prey as well as smaller ones, whereas similar-sized individuals or species feed on almost similar prey categories, suggesting greater dietary overlap (Maragno and Souza 2011). *Euphlyctis cyanophlyctis, Minervarya teraiensis,* and *Duttaphrynus* are medium-sized frogs that exclusively eat smaller prey items. As large sized frogs consume a huge number of smaller sized prey on which small sized frogs rely, there is a high degree of dietary niche overlap. Large frogs seek larger prey but due to opportunistic and sit and wait foraging technique, similar prey content in different species. Although anurans rely on similar types of food, prey constraint, varying mouth breadth, and microhabitat use help to coexistence in similar habitat (Santos-Pereira et al. 2015). *H. crassus* and *H. tigerinus* could ingest larger prey items (Small frogs, Beetles etc.) in the study area, whereas *E. cyanophlyctis, Duttaphrynus,* and *M. teraiensis* relied on smaller prey items. The dietary niche overlap between two medium sized frogs was more than two large sized frogs. This might be due to the small head width and gap size of medium sized frog which enables them to consume the limited size and shape of prey.

The highest Standard niche breadth was observed in *Duttaphrynus melanostictus* followed by *Euphlyctis cyanophlyctis.* This result suggested that *Duttaphrynus melanostictus* is the most habitat generalist among seven different species of frogs. The different in body size, body weight and dietary preference might be the reason for their coexistence in similar environment. Different feeding behavior and prey preferences reduce the competition for the food between species which facilitate co-existence (Pianka 1973). Additionally, differences in niche width and the absence of particular prey categories in some species may facilitate in coexistence in the similar environment (Santos-Pereira et al. 2015). Very less value of standard niche breadth was observed in *H. tigerinus.* This might be due to the cropland area was with similar rice field and other faunal composition.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The most dominating prey category was Hymenoptera which was preferred by all of the captured species of anurans, followed by Coleoptera, Anurophagy, Larva, Hemiptera, Spider, Orthroptera, Diptera, Earthworm, Snail, Tricopoda, Blattodea, Isopoda, Lepidoptera, Mariopoda and Snake. However, Coleoptera was the most dominant pest prey category recorded in cropland and forest trailed by Larva and Orthoptera. Anurophagy was also reported from the study which supports that large anurans (*H. crassus* and *H. tigerinus)* can also consume smaller frogs found in the same habitat. Anurans body size (SVL) and the weight of consumed prey was found to have a positive relationship. While comparing effect of habitat type on dietary preferences or habits, no significant difference was seen in cropland and forest due to availability of similar prey items in both habitats. Dietary niche overlap was significant across the all species. All of the anurans in the study area were dependent on the same type of prey and had similar dietary preferences. All the species has almost similar niche breadth; *Duttaphrynus melanostictus* possessing the higher niche breadth. Medium sized anurans had very high dietary niche overlap as compared to large sized anurans. The high dietary niche overlap among species of anurans suggests that there is a strong competition for the food resources. Higher abundance of pest prey items in cropland concluded that frogs as important biological pest controllers during the development of pest management and strategies.

6.2 Recommendations

Some of the recommendations based on the current study are as follows:

- ❖ Overall this study was only based on habitat specific diet composition. Role of each species to the ecosystem could be describe by species specific dietary composition. Hence, species specific diet composition recommends for the study.
- ❖ Prey items should be identified up to the species level to calculate precise niche overlap between different species recommended to study.

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APPENDICES

Table 4: Dietary composition of eight species of anurans

Anuran species / Prey Categories	*Hoplobatrachus tigrinus*	*Euphlyctis cyanophlyctis*	*Hoplobatrachus crassus*	*Minervarya teraiensis*	*Polypedates maculatus*	*Duttaphrynus*	*Uperodon taprobanicus*	*Polypedates taeniatus*	Total	Percentage (%)
Hymenoptera	188	27	18	26	4	12	2	1	278	62.90
Orthroptera	6	4	0	1	0	0	1	0	12	2.71
Hemiptera	11	1	0	0	0	2	0	0	14	3.17
Diptera	8	2	1	0	0	0	0	0	11	2.49
Coleoptera	24	9	1	5	4	3	0	0	46	10.41
Lepidoptera	1	0	0	0	0	0	0	0	1	0.23
Larva	7	12	1	4	1	0	0	0	25	5.66
Spider	6	4	2	0	0	0	0	0	12	2.71
Snail	1	1	0	0	0	0	1	0	3	0.68
Snake	1	0	0	0	0	0	0	0	1	0.23
Blattodea	2	0	0	0	0	0	0	0	2	0.45
Earthworm	3	0	0	0	0	0	0	0	3	0.68
Mariopoda	1	0	0	0	0	0	0	0	1	0.23
Isopoda	1	0	0	0	0	0	0	0	1	0.23
Tricoptera	1	1	0	0	0	0	0	0	2	0.45
Anurophagy	17	9	4	0	0	0	0	0	30	6.79