DIETARY ASSESSMENT AND NICHE OVERLAP OF ANURANS IN WESTERN LOWLAND NEPAL

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Batch: 2073

A thesis submitted in partial fulfillment of the requirements for the award of the Degree of Master of Science in Zoology with special paper Ecology and Environment

Submitted To

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

September, 2019

DECLARATION

I hereby declare that the work presented in this 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 author(s) or institution(s).

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Mr. Suman Sapkota entitled **"DIETARY ASSESSMENT AND NICHE OVERLAP OF ANURANS IN WESTERN LOWLAND 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.

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ACKNOWLEDGEMENTS

First of all, I would like to express my deepest gratitude and respectable appreciation to Dr. Bishnu Prasad Bhattarai for his expert guidance, constant encouragement and valuable suggestions for the completion of this study and to bring out the best in this thesis work.

Respectfully, I would like to appreciate Dr. Janak Raj Khatiwada for his guidance in overall field design, field work, data management and financial support. I would also like to express my deep sense of indebtedness and profound gratitude to Prof. Tej Bahadur Thapa, head of my department for providing me academic support to carry out this thesis. I would like to give sincere thanks to Department of Forests and Soil Conservation for granting permission to carry out this dissertation.

I am immensely obliged to all my respected teachers and administrative staffs, library staffs of Central Department of Zoology, for extending their helping hands in all the possible ways.

My sincere thanks go to Mr. Madan Raj Mishra and Jagan Nath Adhikari for their continuous support to carry out field survey. I wish to reiterate my acknowledgement to my friend Ms. Aarati Nepali for helping me during thesis preparation.

Last but not the least, I would like to express my sincere thanks to my parents and all the family members for their continuous help and encouragement to produce this thesis work. All the people who have helped me directly or indirectly in course of this study deserve heartfelt gratitude.

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LIST OF ABBREVIATIONS

Abbreviated form	**Details of abbreviations**
DFO	Division Forest Office
gm	Gram
GPS	Global Positioning System
IRI	Index of Relative Importance
IVI	Important Value Index
masl	Meters above sea level
mm	Millimeter
NMDS	Non-Metric Multidimensional Scaling
SVL	Snout Vent Length

ABSTRACT

Diet of organism plays important role to understand the ecology, behaviour and overall life processes. Present study was carried out in three different habitat types (cropland, forest and forest edge) of Western Terai of Nepal to explore dietary habit and niche overlap among the anurans. Nocturnal time-constrained visual encounter line transect method was employed for anuran surveys and the diet of the captured individuals were collected by using non-lethal stomach flushing technique. Nineteen (10.11%) individuals out of 169 stomach flushed were found with empty stomach. The diet contained 685 prey items which were categorized into 13 taxonomic groups. Hymenoptera (35.79%) and Coleoptera (32.41%) were highly abundant preys, found in all captured anurans. Anurophagy was observed only in *Hoplobatrachus crassus*. The relation between the body size and the weight of prey found significantly positive ($R^2=0.103, p<0.002$). Among all species, *H. tigerinus* showed the positive relationship between both body size and body weight with prey weight. There was no significant relationship between the habitat types and dietary preferences. The result from Non-Metric Multidimensional Scaling revealed that there was high degree of dietary niche overlap between *H. crassus, H. tigerinus, Minervarya teraiensis, Euphlyctis cyanophlyctis* and *Duttaphrynus* among them, *M. teraiensis* had the highest niche breadth (B_A = 0.501). Similarly, the niche breadth of *H. crassus* and *Duttaphrynus*; *H. tigerinus* and *E. cyanophlyctis* was quiet similar. In other hand, dietary niche overlap was found the highest in medium sized (SVL < 50 mm) frogs (O_{jk} = 0.97) whereas the large sized frogs (SVL > 50 mm) had comparatively lower niche overlap (O_{jk} = 0.89). Prey preferences with regards to different body size of anurans might help in co-existence of various species in same habitats. Furthermore, this study suggests the need of detailed study on amphibians and their diets. Such studies help for the conservation of amphibians including other wetland dependent fauna.

1. INTRODUCTION

1.1 Background

Amphibians are one of the diverse vertebrates in animal kingdom, comprised of more than 7664 species worldwide (Frost 2017) and 54 species in Nepal (Shah and Tiwari 2004). The class Amphibia include the orders Anura (Frogs and Toads), Caecilians (limbless amphibians) and Caudata (Salamander and Newts) where Anura alone comprises 51 species (Shah and Tiwari 2004). Amphibians generally have soft, scale less and moist skin and have life stage of egg, tadpole (larva) and adult.

Eastern Nepal represent one of the global biodiversity hotspot of Anurans and is the part of the Eastern Himalayas (Myers et al. 2000). Western Nepal is dry as compared to Eastern part and frog diversity in this region is less explored. Anurans are distributed in wide range of habitat and elevation range but overall species richness and abundance is seen declining with increasing elevation (Khatiwada and Haugaasen 2015). Temperature and vegetation cover are the major environmental variables affecting the distribution and abundance of frogs while seasonality influences the distribution of certain anuran (Contreras 2018). Amphibians are facing serious threats due to habitat loss and degradation, invasion, pollution, disease and global climate change (Gibbons et al. 2000). The worldwide increase in temperature may not be more harmful rather cooling can be a major threat for the persistence of amphibians (Araujo et al. 2006). Amphibians provide wide range of ecosystem services but unfortunately they are experiencing major declines and humans may be losing associated ecosystem services (Hocking and Babbitt 2014).

Anurans are described as generalist predators feeding upon variety of invertebrates, including molluscs, annelids, centipedes, millipedes, arachnids, crustaceans and especially insects (Anderson et al. 1999). Few studies of diets of Anurans from Nepal shows the largest proportion of insect pests of crop with high seasonal variation (Parajuli et al. 2005, Khatiwada et al. 2016) although no more evidences are found. Some of the major Anuran diet consists of insects of order Coleoptera, Lepidoptera, Orthoptera, Homoptera and Hemiptera (Mahan and Johnson 2007) but Hymenoptera was abundant followed by Coleoptera and Lepidoptera in Nepal (Khatiwada et al. 2016). Large frogs also feed upon small fishes and other frogs (Duellman and Trueb 1986), cannibalism is frequently seen among frogs (Crump 1992). Some small sized frog may also consume other frogs which even secret bufotoxin, the term called Batracophagy (Ceron et al. 2018). Frog diet is not only limited to the crop pest, they also feed upon variety of insects which are known to be important vectors of zoonotic diseases (Khatiwada et al. 2016) and also acts as important biological control agent for pests and helps in ecosystem management (Chowdhary et al. 2018).

All the frogs have almost similar feeding habitat and clear niche overlap can be observed which suggest that competition for food resource is not only the major driver to determine the frog distribution and community structure (Piatti and Souza 2011). The positive relationship is seen between predator-prey body size. Feeding strategies like ambush predation and combination of active search and sit and wait strategy supports the consumption of variety of prey by different species (Mohanty and Measey 2018). Sit and wait foragers may consume larger, mobile prey and few food item per time unit while opportunistic feeders consume greater number of smaller food items (Sole and Rodder 2010). Diet contained in different types of frog is affected by various factors like distance to foraging ground, hunting strategy, feeding behaviour, duration and time of foraging.

Niche overlap is the degree to which two species share various resources (Pianka 1988) and this would provide additional information on interspecific competition. Niche overlap helps to predict the relationship between several species competing in one dimensional continuum of resources like food (May and Mac Arthur 1972). However, the differential use of food resources between similar species could reduce competition and would therefore allow their coexistence (Pianka 1973). Microclimates, food, shelter and predators vary spatially and provide opportunities for resource partitioning, and hence there is niche differentiation among potentially competing species (Melville and Swain 1997). A community with more resource sharing or greater niche overlap may support more species than that with less niche overlap (Rusterholz 1981). In case of dense population of closely related species of frogs, interspecific competition for resources is predicted to be high (Crawford et al. 2009) which leads in consumption of similar prey by multiple species. For the terrestrial species, microhabitat resource partitioning and body size discrepancy among species may be the factors which influence the dietary patterns and helps in species coexistence (Vignoli et al. 2009). Although frogs are generalist/opportunistic feeders but species mobility and active foraging determines the dietary niche and feeding pattern in different species (Franca et al. 2004).

1.2 Rationale of the Study

Diet plays an important role for the exploration of habitat and ecology of organisms. Most of the researches are focused on diet analysis of large animals such as tiger, leopard, wild ungulates, etc. Frog plays a vital role on ecosystem services as they mainly uptake variety of small invertebrates including vector of the diseases and crop pests as food. In spite of these importance, very few research had been carried out on diet analysis of frogs in Nepal. The feeding habit of anurans helps to discover the benefits of frog in ecosystem as well as ecosystem services. Information on anurans from the Western Nepal is lacking as frog diversity in this region is less explored. Though the Western Nepal is dry as compared to Eastern part of Nepal but this region may also harbor the different species of anurans that are adapted to the particular environment in that region. Only few studies of diet analysis of frog have been documented till now in Nepal which is not adequate to assess conservation needs of the anurans. Hence, this study is designed to explore the dietary niche overlap between different species of frog which would contribute to better understanding of distribution, survival and the feeding ecology of anurans in different and similar habitats.

1.3 Objectives

1.3.1 General Objective

The general objective of the study was to assess diet and niche overlap of anurans in Western lowland Nepal.

1.3.2 Specific Objectives

- To assess the dietary composition of different anuran species in Western lowland Nepal.
- To determine dietary niche overlap between different anuran species in Western lowland Nepal.

2. LITERATURE REVIEW

Dietary Composition

Dietary analysis of anurans in Nepal is rarely carried out although there are diverse group of anurans. Khatiwada et al. (2016) examined dietary habits of anuran species in croplands of Chitwan, Nepal and found that the diet of frog includes a high proportion of crop pest. The study was conducted approximately 3-4 weeks after rice plantation. Further, the diet differed in rainy and dry seasons among different species and even among different individuals of similar species. The result revealed that frog diet also consists of insects which are known to be important vectors 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 prey size varied from 3 to 56 mm. While comparing the dietary content of anurans in different habitat types: sandy coastal plains, lowland forest and island, no variation was observed in prey type and volume but ants were dominant in all habitats (Mageski et al. 2019). Based on IVI the most important prey categories of anurans from eastern Amazon, Brazil were Hymenoptera (32.2) and Hemiptera (13.8). No correlation between SVL and volume of prey consumed (Sanches et al. 2019). Sole et al. (2019) studied diet of *Leptodactylus spixi* from cacao plantation in Brazil. A total of 168 prey items were obtained from 69 stomach flushed individuals. Orthoptera was the most dominant prey category which were followed by Acarina, Formicidae and Diplopoda. *L. spixi* feeds on majority of invertebrates and follows sit and wait strategy and is also a generalist predator.

Asrafuzzaman et al. (2018) carried out the study on dietary assessment of five species of Anuran Tadpoles from Northern Odhisha, India. The stomach contents of 75 tadpoles belonging to five different anuran species (*Duttaphrynus melanostictus*, *Euphlyctis cyanophlyctis*, *Fejervarya orissaensis*, *Polypedates maculatus* and *Microhyla ornata*) belonging to four families namely Bufonidae, Dicroglossidae, Rhacophoridae and Microhylidae were examined which revealed that the diets of tadpoles included mostly detritus, followed by phytoplankton represented by 5 classes and 54 genera. First ever Batracophagy in the diet of *Leptodactylus policipnus* was recorded from South Pantanal, Brazil. Despite of small sized frog *Leptodactylus podicipinus*, their diet contained the abundant post metamorphic stage of *Rhinella schnederi* found in same area. *R. schneideri* secrets bufotoxin which may cause nausea and vomiting after ejection. The result suggests that the sit and wait strategy of *L. policipnus* may lead in batracophagy (Ceron et al. 2018). Coleopteran was found to be most dominating prey followed by Orthroptera based on the IRI (Index of Relative Importance) and frequency values. Park et al. (2018) conducted study on diet composition of Japanese tree frog in South Korea. The study found empty stomachs in 71% of calling males during the reproductive period. All prey items obtained from the stomachs of frog belonged to the phylum Arthropoda, from eight orders of Insecta followed by one order of Arachnida. Among insect prey, the most common items in the stomachs were adults of beetles, flies and bugs, and larvae of butterflies and moths. There was a significant positive correlation between the body mass of Japanese tree frogs and the volume of prey items.

Dietary composition differs among those similar species of anurans thriving in different habitat types. Although similar type of prey category Orthopterans was dominant in both rainforest and cave population of *Craugastor alfredi* the dietary diversity and feeding intensity was found to be higher in rainforest population. This might be due to the small body size, direct development and semi arboreal living of the species inside the cave (Manzona and Bautista 2017). Beside other factors different foraging strategies of anurans also help in different feeding nature and habits. Castro et al. (2016) carried out dietary assessment of *Dendropsophus branneri* in cocoa plantation in Brazil. The low number of prey per stomach was found in *D. branneri* which suggest this species uses “sit and wait” strategy for foraging. One interesting finding from this study was that stomach flushing can successfully apply to those frogs whose size is not less than 14.4 mm. Norval et al. (2014) carried out study on the diets of five amphibian species from southwestern Taiwan. Three thousand four-hundred and six prey items, from 21 orders of 6 classes were recorded, and ants (Formicidae) were the most numerous prey items in the diets of all five anuran species. *Fejervarya limnocharis* had the broadest dietary niche breadth, followed by *Duttaphrynus melanostictus*, *Microhyla fissipes*, *Micryletta stejnegeri*, and *Microhyla heymonsi*. There were also substantial dietary overlaps among the studied anurans.

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 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.

Dietary Niche Overlap

Most of the anurans are generalist predators feeding mainly on invertebrates and small vertebrates hence, dietary niche overlap can be higher among different species and habitat types. Moser et al. (2019) conducted study on diet and trophic niche overlap of *Boana bischoffi* and *Boana marginata* in Southern Brazil. There was high trophic niche overlap (0.90) between these two frogs as they both feed on similar type of prey: Araneda and Coleopteran being dominant. The result also suggests the generalist feeding behaviour of these two species as the niche breadth varied from 0.35 to 0.42. This might be different while comparing between large sized and small sized anurans. Generally large sized anurans can consume large sized prey than that of the small anurans resulting in dietary portioning. Mohanty and Measey (2018) conducted study on diet and trophic impact of invasive *Hoplobatrachus tigerinus* on Andaman. They found that small vertebrates were the major diet of *H. tigerinus* while other frogs consume small invertebrates. The dietary niche overlaps with *Limnonectes* sp. but not with *Fejervarya* sp. which were found in same habitat.

Body size and microhabitat use by the species also influences the dietary niche between anurans. Positive relationship can be seen between predator-prey body size which results in preference to different prey categories thus minimizing the niche overlap. The study conducted to investigate the influence of body size and microhabitat use on seasonal variation of the trophic ecology of two sympatric hylids (*Pseudis minuta* and *Scinax squalirostris*) on the estimates of prey availability. During the study, *Pseudis minuta* exhibited larger body size and mouth width and revealed broader use of microhabitats mostly within and near major water bodies, whereas *S. squalirostris* had smaller body size and mouth gape and was found exclusively within or near *Phytotelmata*. *P. minuta* had a more diverse diet than *S. squalirostris* (Huckembeck et al. 2018). 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. Generally, all other frogs are generalist and feed upon similar type of prey items showing dietary niche overlap.

Beside other facts foraging strategy is also equally responsible for the various degree of dietary niche overlap. Anurans generally feed rapidly on small sized preys in active search while sit and wait strategy allows to consume less prey in long time interval. Narvaez et al. (2014) carried out study on diet and trophic ecology of *Leptodactylus fragilis* and *Dendropsophus columbianus* in Southwestern Colombia. Trophic niche overlap was found to be 68% which was due to the foraging strategy: active foraging behaviour and sit and wait behaviour respectively. Mostly all the anurans have high dietary niche overlap as they have preference to almost similar prey categories. Study on diet composition and trophic niche overlap between two sympatric species of *Physalaemus* in a sub temperate forest of Southern Brazil where higher prey categories were found in the diet of *P. lisei*. Formicidae was the most important prey category in the diet of both species, followed by Coleoptera and Araneae. Despite the high importance of ants in the diet of both species, Coleoptera presented the highest volumetric contribution. Both species had a similar trophic niche breadth and a high diet overlap (Moser et al. 2017). Both *Xenopus* species were found to consume large amounts of tadpoles belonging to different amphibian species, including congeners, with an overall higher incidence of anurophagy than previously recorded which shows greater niche overlap between species of same genera (Vogt et. al. 2017).

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 on food niche overlap between two sympatric leaf litter frog species from Central Amazonia. 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. Although the frequency of Coleoptera, Hymenoptera, larvae of Hexapoda, Hemiptera, Diptera and Orthoptera was higher niche overlap was not larger than expected by chance in the rice fields of Pantanal region, Brazil (Piatti and Souza 2011). Cazade et al. (2010) conducted study on the trophic ecology of *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 trophic niche overlap between 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).

Nepal is the home for diverse group of Anurans. Diet analysis and dietary niche overlap including ecology of anurans is not well known. This study provided additional information on feeding ecology and resource use by anurans in Nepal. Furthermore, the anuran diversity in Western Terai is even more unknown and therefore this study focused on Western Terai region of Nepal.

3. MATERIALS AND METHODS

3.1 Study Area

The study was conducted in three districts of Western Terai of Nepal (Figure 1): Bardiya, Kailali and Kanchanpur ($81^0$34'E, $28^0$6'N to $80^0$30'E, $28^0$39'N). Bardiya district lies in Province 5 whereas Kailali and Kanchanpur in Far-Western Province. The Western Nepal is characterized by rugged terrain and high seasonal climatic variability. Summer monsoon rainfall from Bay of Bengal strongly affect the seasonal temperature and precipitation in this region. The mean annual temperature varies between 7 °C and 26 °C (Vetaas 2000).

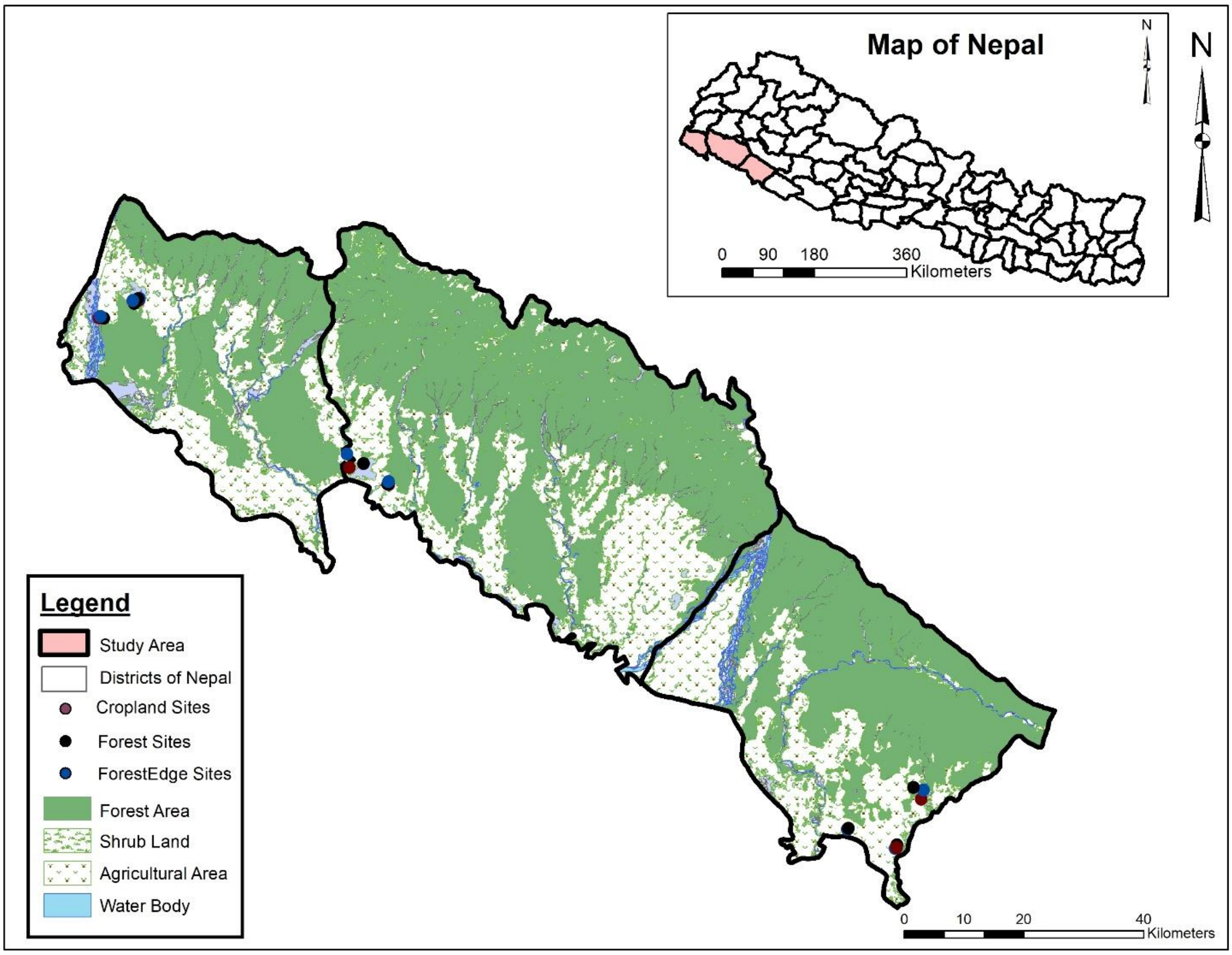

Figure 1: Map of study area showing land cover types and sampling sites.

The major vegetation of the study area includes Sal (*Shorea robusta*), Khair (*Acacia catechu*) and Sisoo (*Dalbergia sissoo*). Kanchanpur, Bardiya and Kailali districts have the moderate climate prevailing**.** The average annual temperature for Kanchanpur is 32°C. The highest average temperature in Kanchanpur is 41°C in April**.** The average annual rainfall is about 513 mm. It is dry for 249 days a year with an average humidity of 53%

(DFO Kanchanpur 2017). The average annual temperature for Bardiya is 27° C with the highest average temperature in 37°C in June and the lowest in 22°C in January. The annual rainfall is 765 mm of rain in a year. It is dry for 186 days a year with an average humidity of 55% (DFO Bardiya 2017) The average annual temperature for Kailali is 33°C.The highest average temperature in Kailali is 45°C in June and the lowest is 25°C in January. The annual rainfall is 578 mm. It is dry for 248 days a year with an average humidity of 49% (DFO Kailali 2017).

Three different categories of habitats were surveyed: cropland, forest and forest edge.

Cropland habitat: Agricultural paddy plantation area were taken as cropland. The mean temperature of substrate in cropland was 25.2^0C. Anurans were surveyed in microhabitats like trail between fields, small ditches, inside the field and associated terrestrial habitats.

Forest habitat: Small patches of forest around the study area were chosen for anuran survey. The dominant vegetation in forest were Sal (*Shorea robusta*) and Khair (*Senegalia catechu*). The mean temperature of substrate in forest was 27.4^0C. Anurans were surveyed in microhabitats like leaf litter, tree branches, ditches and associated grasslands.

Forest edge habitat: The outer area of forest was taken as forest edge. This includes the outer boundary of forest patches in the study area. The mean temperature of substrate in forest edge was 27.2^0C. Anurans were surveyed in microhabitats like walking trail, leaf litters, ditches and tree branches.

3.2 Materials

- GPS: Garmin Etrex 10
- Camera: Canon IXUS 145
- Field Guide Books (Schleich and Kastle 2002, Shah and Tiwari 2004)
- Three-digit Weighing Machine: Sartorius LC 1201S
- Torch Light
- Diet Extraction Set
- Measuring Tape

3.3 Methods

3.3.1 Field Sampling Design

The study was started by conducting the preliminary survey during June, 2018 to gather the necessary information about the study area and the feasibility of the research. Based on the field observation, habitats were divided into three categories as forest, cropland and forest edge and sampling design was made accordingly. A total of 24 transects were made with eight transect representing each habitat. Sampling was done in two phases in three different districts of Western Terai which included Kanchanpur, Bardiya and Kailali.

During first phase, a total of 9 transects were made in the study area. Survey was carried out from 9-12 July, 2018 in three above mentioned habitats in Majhgaun, Salghari and Baagphanta of Kanchanpur district. Similarly, during second phase, a total of 15 transects were made; 5 in each three habitats. Survey was carried out from 18-26 August, 2018 in Bardiya and Kailali districts. Three different sites which included Sitalabazar, Malangsarobar and Bansgadi in Bardiya while two different sites in Dhangadi, Kailali were surveyed. Anuran surveys were carried out during the months of July and August, 2018. Equal sampling effort was used in terms of time and manpower in all three habitat types.

3.3.2 Anuran Survey

Nocturnal, time-constrained visual encounter survey (Campbell and Christman 1982) was employed for the anuran survey. Anurans were surveyed at night along transects (100 x 4 meter) for 30 minutes using torches, walking at a slow pace from 20:00 to 23:00 hrs. Transects were placed at the interval of 250 m. The number of species and individuals encountered in each transect were recorded. Anurans encountered in transect were captured and kept in small cotton bag to avoid the repetition The species were identified by using the field guide books (Schleich and Kastle 2002, Shah and Tiwari 2004). Beside these, the physiochemical parameters such as temperature of substrate and water, conductivity, p^H and humidity of each transect were also recorded.

3.3.3 Diet Extraction

For the diet extraction, each captured individual in the transect was taken to nearby dry area and measured snout-vent length and weight along with sex differentiation. Frog diet was collected using a non-lethal stomach flushing technique described by Sole et al. (2005). The stomach contents of each frog was flushed by using 50 ml syringe with attached surgical plastic tube (20 cm long and 2 mm in diameter). Thumb was used to open the frog's mouth and the soft surgical plastic tube was introduced carefully through the esophagus into the stomach. Then, 50 ml of ordinary tap water was slowly squeezed from the attached syringe into the stomach and any content ejected from the stomach was then collected. The stomach-flushing procedure was repeated up to three times to ensure the complete removal of stomach content. Stomach contents were then preserved in 70% ethanol for further identification and measurements. Frogs were then released at the captured location approximately 30 minutes after flushing.

3.3.4 Diet Analysis

Stomach contents of individual frog were dried on filter paper and weighted by using 3-digits weighing machine. Prey items were placed in the petri dish and observed under a stereoscopic microscope. Reference slides of wings, antenna and legs were used to identify the prey items. Possible aquatic and terrestrial prey species were collected with the help of sweeping net in the study area. Antenna and legs were used to prepare the reference slides from collected prey items. All the prey items were identified to lowest possible taxonomic level at the lab of Central Department of Zoology, Tribhuvan University, Kathmandu. Those prey items which were completely digested could not be identified and excluded from the analysis. Other items like stone, grass and mud that might be accidently entered to the frog's stomach were excluded. Those prey items found as whole specimen in stomach were used for analysis. Prey items were classified up to orders in case of Insecta and rest were categorized as their type such as snail, earthworm, crab, larva, spider and anurans.

3.4 Data Analysis

The data were arranged, organized and entered in Ms-Excel for further analysis. The total number of each prey item and prey category was summed and percentage was obtained.

Linear Regression Model was used to describe the relationship between body size of anurans and weight of their stomach content. The model was tested for each species and those species showing significant relationship were only included in the result. Linear Regression Model was performed in Ms-Excel 2016 with the help of Regression in Data Analysis Tool pack and graph was prepared. One-way-ANOVA was used to test dietary preferences of anurans in three different habitat types.

Non-Metric Multidimensional Scaling (NMDS) was used to find out the dietary niche overlap between different species. Bray-Curtis Similarity Index was employed to find the similarity between prey categories. The final graph was prepared in PAST 3.25 (Hammer et al. 2001).

Levins measure was used to quantify the niche breadth (Levins 1968) of different anuran species by using formula,

$$B = {}^{1}\!/_{\sum pi^2}$$

Where, B is the Levins measure of niche breadth and pi is the proportion of individuals found using resource i. Further, the values of niche breadth were standardized to range of 0 to 1 by using the formula,

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

Where, B_A is the standardized niche breadth, and n is the total number of food items for the species.

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

$$O_{jk} = \frac{\sum P_{ij} P_{ik}}{\sqrt{\sum P_{ij}^2 P_{ik}^2}}$$

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 188 anurans were captured out of which diet was extracted only from 169 anurans and remaining 19 (10.11%) were found with empty stomach (Table 1).

Table 1: Species that were stomach flushed.

S. N.	Name of species	Individuals with stomach content	Individuals with empty stomach
1	*Hoplobatrachus crassus*	65	5
2	*Hoplobatrachus tigerinus*	49	5
3	*Euphlyctis cyanophlyctis*	29	1
4	*Minervarya teraiensis*	9	2
5	*Duttaphrynus melanostictus*	9	1
6	*Duttaphrynus stomaticus*	8	1
7	*Polypedates maculatus*	0	4
	Total	**169**	**19**

The prey items after analysis, were classified into 13 categories (Table 2). Prey items from class Insecta was further classified upto order level whereas other prey items were categorized according to their type. A total of 685 identifiable prey items were identified from the recorded seven species of anurans belonging to three families (Appendix 2).

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

S.N	Prey Categories	No. of Prey	Percentage contribution
1	Hymenoptera	245	35.79
2	Coleoptera	222	32.41
3	Larva	42	6.13
4	Orthroptera	32	4.67

5	Diptera	28	4.09
6	Snail	28	4.09
7	Spider	23	3.36
8	Earthworm	21	3.07
9	Blattodea	20	2.92
10	Odonates	10	1.46
11	Crab	7	1.02
12	Lepidoptera	4	0.58
13	Anurans	3	0.42
	Total	**685**	**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 only in three individuals of *Hoplobatrachus crassus*.

4.2 Dietary Profile of Individual Species

Hoplobatracus crassus

Hoplobatracus crassus was the most abundant frog in the study area consisting of 70 individuals. Out of 70 individuals, 65 individuals were found with stomach content while 5 individuals (7.14%) with empty stomach. The stomach content of this species contained all 13 prey categories including 247 prey items. This is the only species which shows anurophagy. Hymenoptera was the most dominating prey category consisting of 92 items which was followed by 63 items of Coleoptera while crab and Lepidoptera being very rare (Figure 2).

Hoplobatracus tigerinus

Hoplobatrachus tigerinus was the second most abundant frog with 54 individuals. Out of 54 individuals, 49 individuals were found with at least one prey item while five individuals (9.26%) were found with empty stomach. Out of 13 prey categories, this species contained 11 prey categories including 180 prey items. The most dominating prey category was

Coleoptera including 65 items which was followed by 44 individuals of Hymenoptera. Blattodea and Odonates each represented only 1% of dietary composition in *Hoplobatrachus tigerinus* (Figure 2).

Euphlyctis cyanophlyctis

A total of 30 individuals of *Euphlyctis cyanophlyctis* were stomach flushed out of which 29 individuals were found with stomach content while a single individual (3.34%) was found with empty stomach. The diet of this species consisted of 88 prey items belonging to 10 prey categories. Similar pattern was seen in this species that Hymenoptera being dominant consisting 32 items which was followed by 24 items of Coleoptera. The least dominating prey categories was comprised of Blattodea, Lepidoptera and Odonates each representing only 1% of total dietary composition in *Euphlyctis cyanophlyctis* (Figure 2).

Minervarya teraiensis

Eleven individuals of *Minervarya teraiensis* was stomach flushed out of which diet was extracted only from nine individuals and remaining two individuals (18.18%) were found with empty stomach. A total of 22 prey items belonging to 7 prey categories were found. The most dominant prey category was Hymenoptera consisting of 9 items which was followed by 7 items of Coleoptera while Larvae was the least dominant prey category that was contained in *Minervarya teraiensis*. (Figure 2).

Duttaphrynus

Duttaphrynus consists of two species: *D. melanostictus* and *D. stomaticus*. Both species were kept in single group in this study. Total number of stomach flushed individuals were 19 out of which two individuals (10.53%) were found with empty stomach. The diet of *Duttaphrynus* consisted of 148 prey items belonging to 8 categories. The dominant prey type was Hymenoptera consisting of 68 items which was followed by 63 items of Coleoptera while the odonates represented only 1% of total diet being the least dominant prey category (Figure 2).

Polypedates maculatus

This species was the least abundant among all of the above. Total of 4 individuals were found and stomach flushed. All the individuals were found with empty stomach and it was not kept in data analysis.

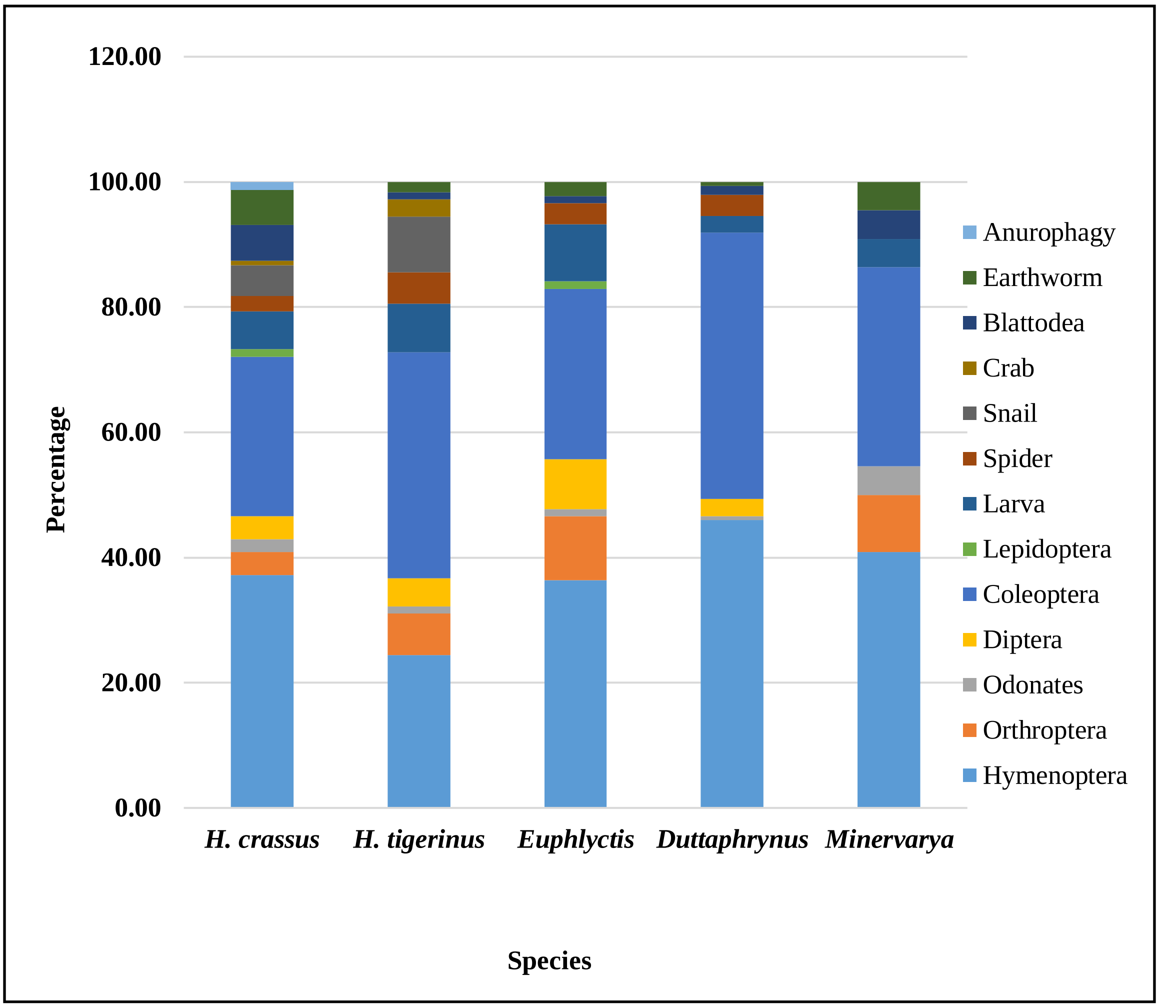

Figure 2: Stacked bar graph showing dietary composition of recorded species.

4.3 Effect of Body Size on Prey Consumption

The average body size (SVL) of all the stomach flushed anurans was 61.2 mm and the average body weight was 40.9 gm. The average weight of stomach content after being dried was 0.519 gm. There was a positive relation between SVL and body weight of captured frogs ($R^2 = 0.54$, $p < 0.001$). The total number of prey consumed was not affected by body size of frog. There was no relationship number of prey consumed was not affected by body size of frog. There was no relationship between total number of prey consumed with SVL and the body weight of frog ($p = 0.249$).

The largest frog in the study area was *H. tigerinus* with average SVL of 81.30±28.03 which was followed by *H. crassus*, 59.68±16.29. The average body size of *E. cyanophlyctis*, *M. teraiensis* and *Duttaphrynus* was 37.74±10.04, 42.89±8.07 and 45.95±13.03 respectively.

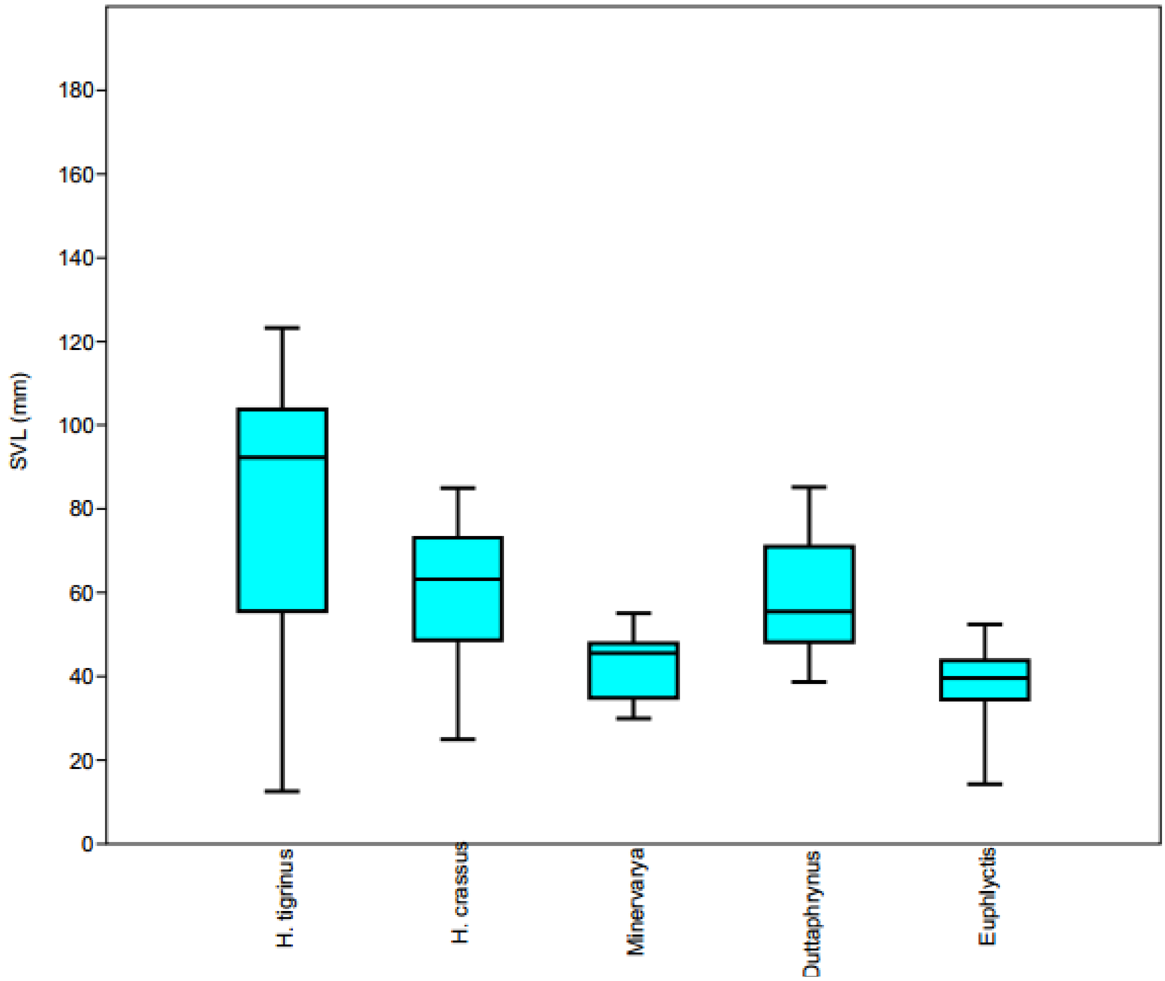

Figure 3: Box plot showing the SVL (Mean ± SD) of five species in the study area in 95% interval.

The linear regression analysis showed the positive relationship (R^2 = 0.103, p < 0.002) between the total weight of prey consumed with the body size of frog. It was seen that large sized frogs generally consumed more prey (Figure 4). In case of individual species, no significant relationship was observed between body size of frogs and total weight of prey consumed. *Hoplobatrachus tigerinus* was the only frog which showed positive relationship between both SVL with prey weight (R^2 = 0.165, p = 0.036) and body weight with total weight of prey (R^2 = 0.140, p = 0.008) (Figure 5). The larger body size of *H. tigerinus* consumed more prey by weight.

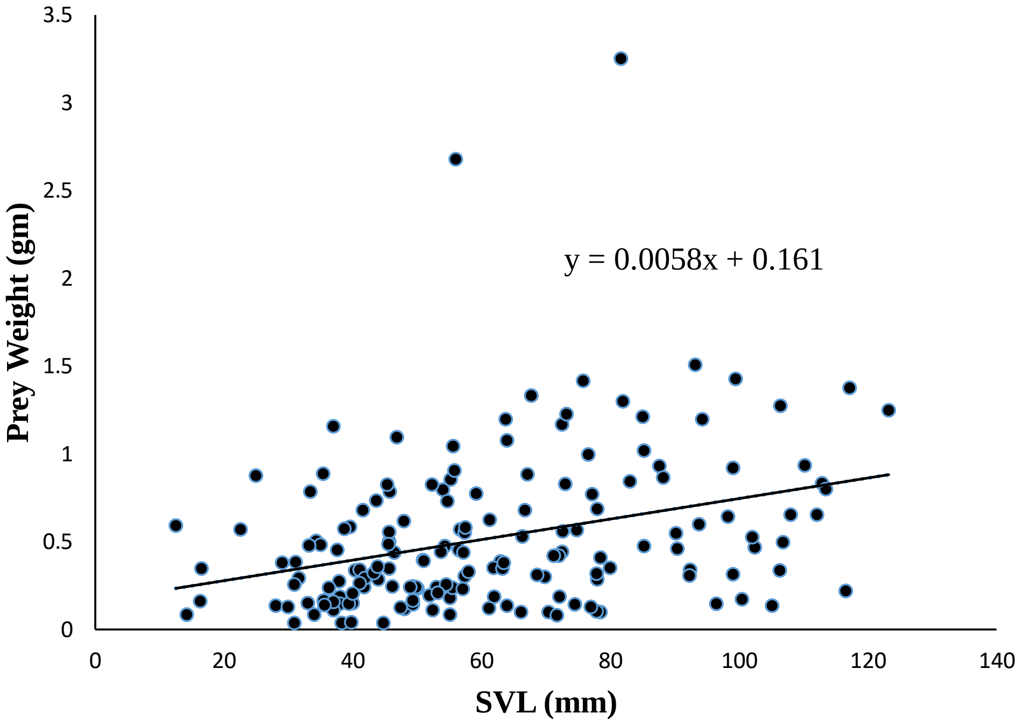

Figure 4: Linear Regression Analysis showing the relationship of body weight of frog with total weight of prey consumed.

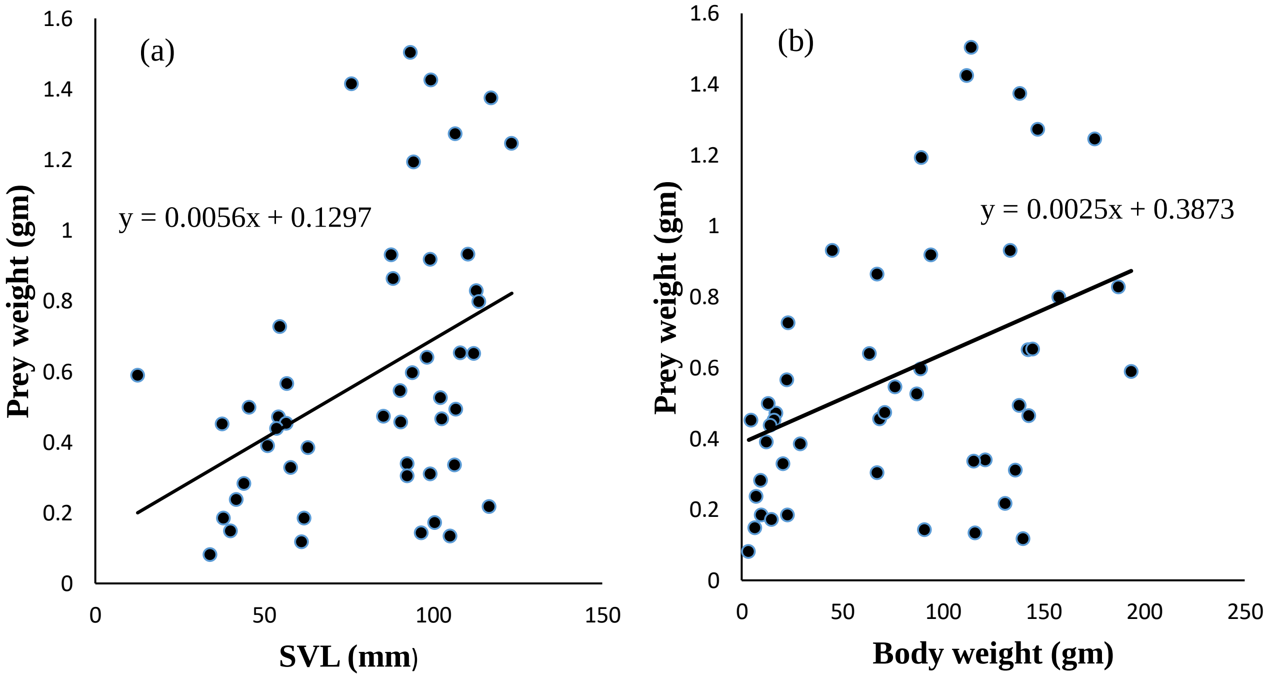

Figure 5: Linear regression analysis showing the relationship between SVL (a) and body weight (b) of *Hoplobatrachus tigerinus* with total weight of prey consumed.

4.4 Effect of Habitat Type on Dietary Habit

The result from one-way ANOVA revealed that there were no significance effects of habitat on dietary preferences/habit of frogs ($p = 0.932$). The differences 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

Table 3: Niche breadth of five anuran species.

S.N.	Name of species	Levin's Niche Breadth (B)	Standardized Niche Breadth (B_A)
1	*Hoplobatrachus crassus*	4.54	0.294
2	*Hoplobatrachus tigerinus*	4.67	0.366
3	*Euphlyctis cyanophlyctis*	4.28	0.364
4	*Minervarya teraiensis*	3.51	0.501
5	*Duttaphrynus*	4.67	0.366

Minervarya teraiensis was found to have highest niche breadth with 0.501 suggesting it is the most specialized predator among five different species. Niche breadth of 0.366 in *Duttaphrynus* and *Hoplobatrachus tigerinus* revealed that these two species are almost similar in terms of dietary preferences. The lowest niche breadth was 0.294 which was observed in *H. crassus*, made it more generalist in diet among five different species in the study area (Table 3).

Dietary niche overlap was tested according to the body size of species captured. They were grouped in two categories: large sized frogs which includes *H. tigerinus* (average SVL = 81.30 mm) and *H. crassus* (average SVL = 59.68 mm) and medium sized frogs *E. cyanophlyctis* (average SVL = 37.74 mm) and *M. teraiensis* (average SVL = 42.58 mm) (Figure 3). The dietary niche overlap between medium sized frog was seen very high O_{jk} 0.97 whereas dietary niche overlap between large sized frog was found to be slightly low Ojk 0.89. *Duttaphrynus* was not used to find the dietary niche overlap since it already consists two different species under same category.

The feeding habit and dietary preferences of all the five species was found to be similar. All the species were generalist predators and fed on almost similar type of prey categories. There was a high dietary niche overlap between all the species. The result obtained from Non-Metric Multidimensional Scaling (NMDS) clearly showed the high degree of dietary niche overlap. *Hoplobatrachus crassus* was observed with larger dietary niche followed by *H. tigerinus*. Almost all of the prey category was seen clumped in one place and in the convex hulls of all species (Figure 6). Final stress value in 2-D was 0.354 hence graph was prepared in 3-D to reduce the final stress to 0.248.

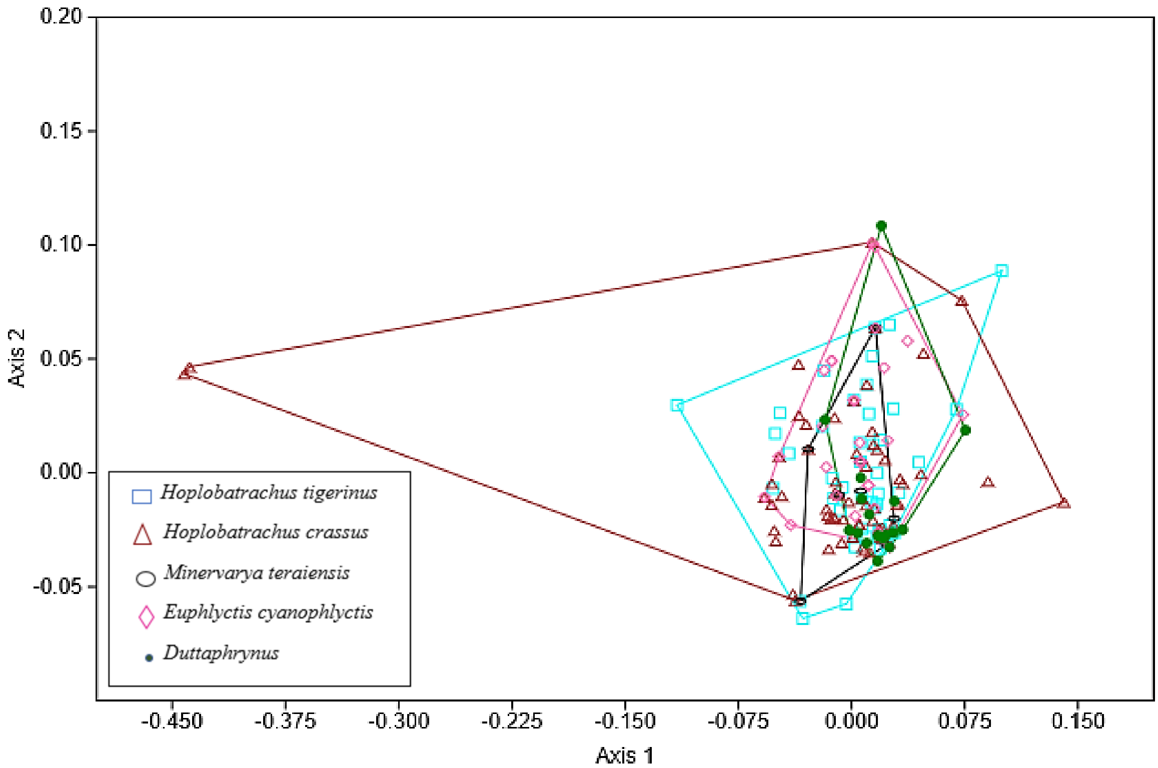

Figure 4: Dietary niche overlap between five different frogs in western Terai of Nepal. The graph is obtained from NMDS in 3-D, Bray-Curtis Similarity Index was used and convex hulls is shown for different species.

5. DISCUSSION

5.1 Dietary Composition

The diet of all the frog species contained Hymenoptera (35.79%) as a dominant group which is followed by Coleoptera (32.41%). This was similar to the result found by (Khatiwada et al. 2016) in rice fields of Chitwan, Nepal. Hymenoptera was the dominating prey comprising 35.5% which was followed by Coleoptera 22%. The diet of Indian Rice Frog consisted 94.8% of arthropods in their diet dominating ants (26.9%) and large proportion of earthworms (Hirari and Matsui 2001). The diet of frogs was mainly composed of terrestrial vertebrates mostly belonging to arthropod groups (Balint et al. 2008), usually Formicidae and Orthoptera (Santos-Pereira et al. 2015), Formicidae and Coleoptera (Olivera et al. 2015, Caldart et al. 2011). Frogs also consume large sized prey like crab, snail, earthworm etc. The large invasive anurans were often found consuming other small frogs and small vertebrates (Measey et al. 2015, Hirai 2004). Thirteen different prey category was found in the present study suggesting the generalized feeding behaviour of frogs. Very few individuals were found with empty stomach (10.11%), this might be due to the time when they were captured. The presence of stones, mud and grass in the stomach of frog might be due accidental inhalation along with their prey.

In the present study tree frog were captured in minimum number and were found with empty stomach during the study period. Tree frogs generally have specialized feeding habit and consume only selective prey items from available ones (Vieira et al. 2018). Capturing tree frogs in less number and being specialized in diet might be the reason behind empty stomach of *Polypedates maculatus*. Three individuals of *Hoplobatrachus crassus* was found consuming smaller frogs in the study area. This might be due to the large body size of frog and also due to sit and wait foraging strategy (Sole and Rodder 2010) and opportunistic predation (Ceron et al. 2018). In global scale, frogs eating anurans is not unusual (Measey et al. 2015), large frogs consume smaller ones. The dietary preference and composition may differ in different sexes (Balint et al. 2008) and in different age group or life form (Caldart et al. 2011).

5.2 Effect of Body Size on Prey Consumption

Positive relationship was seen between frog body size (SVL) and weight of prey consumed. Volume/weight of prey are often taken as suitable parameters of prey size analysis and few species showed positive relationship between body size and weight of prey items (Hirai and Matsui 2001). But this trend was not seen in all species of the study area. Few individuals of *Hoplobatrachus crassus* was found consuming about the half of their body sized frogs. Large frogs were found consuming large sized prey items like crab, other frogs and odonates. But there was no any relationship between the weight of frog with prey weight they consumed. The overall analysis of frogs showed positive relationship between body size and prey weight. Only single species (*Hoplobatrachus tigerinus*) was found to show positive relationship between both body size and body weight with prey weight. This might be due to the size of the species. *H. tigerinus* was the largest frog in the study area with average SVL of 81.30 mm and maximum of 123.25 mm. The bigger size of this frog might help to consume larger prey items and more food by weight. Similar trend was found on the study conducted by (Mohanty and Measey 2018) in which *H. tigerinus* showed strong positive relation between prey size and volume with the body size but *Limnonectes* and *Fejervarya* sp. did not show the same pattern in same area. In some anurans, it was seen that there was strong positive relationship between body size with prey length and also a limit for maximum length of prey (Azid 2018). But there was no any relationship between the total number of prey consumed by frog with its body size. This might be due to the size of prey consumed by frog. Frog might consume less number of large prey and very high number of small prey items due to its generalized feeding habit (Vignoli and Luiselli 2012). This makes very hard to predict the dietary pattern and prey selection by number in anurans.

5.3 Effect of Habitat on Feeding Habit

Three different habitat types viz. cropland, forest and forest edge were surveyed and there was no significant difference in prey selection among habitat types. This might be due to the feeding habit and generalist predation by all the anurans found in the study area. Generally, anurans forage at night and the insects might also follow the same pattern so they are active during the night in all three habitat types. Floristic composition, forest type, time, temperature and habitat heterogeneity could affect the diversity of beetles and other insects (Chung et al. 2000). All the cropland in the study was rice plantation area and tree composition was also almost similar in all the sites of study area. This might be the reason in similar type of insects being dominant causing similarity in the diet preferences. The diet of anurans in forest patches showed the similar pattern to those reported in other habitat types (Herp 2010). Further (Menin et al. 2015) also reported no difference in diet of anurans in agro ecosystem and forest remnants, rather they consumed all the prey items available in the environment. The feeding intensity or consumption proportion might differ between different habitats but overall dietary pattern showed similarity in anurans thriving in different habitat types (Manzano and Bautista 2017). 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.

5.4 Dietary Niche Overlap

High degree of dietary niche overlap was seen among the five species of anurans found in the study area. The diet of all frogs contained almost similar type of prey category, Hymenoptera and Coleoptera being dominant. Feeding habit and consuming similar type of prey might be due to their foraging strategy. The combination of active search and sit and wait strategy resulted in consumption of similar type of prey category (Mohanty and Measey 2018). Foraging strategy plays important role in amphibian trophic ecology but the study should be repeated in different season and in different microhabitats to explain the precise dietary niche (Vignoli and Luiselli 2012). The anuran species recorded in the study area were common (Bhattarai et al. 2018) and found in all three habitat types which might result in foraging upon similar prey items. The abundance of anurans in many habitats or with high mobility could generate better opportunities for prey selection and large range of prey consumed suggesting generalist or opportunistic feeders (Franca et al. 2004). Sympatric anuran species generally showed high dietary niche overlap which could be explained by high prey availability and similar foraging habits (Piatti and Souza 2011). Two species of frogs (*Hoplobatrachus tigerinus* and *H. crassus*) had comparatively larger body size than rest of the recorded species. Larger individuals consume larger prey as well as smaller ones but similar-sized individuals or species fed on almost similar prey category showing higher dietary overlap (Marango and Souza 2011).

H. crassus was the only species which consumed all 13 prey category found in study area which suggest it can consume every possible prey found in the environment. It was followed by *H. tigerinus* with 12 prey category. *H. tigerinus* was not found consuming other frogs but with all other prey category. *Euphlyctis cyanophlyctis, Minervarya teraiensis* and *Duttaphrynus* are medium sized frogs and they feed on small sized prey items only. High degree of dietary niche overlap was due to the reason that large sized frogs also consume large number of small sized prey in which small sized frog rely on. High degree of competition for similar type of prey category was observed among frogs in study area. Generally, large frogs focus on larger prey but due to opportunistic and sit and wait foraging strategy, resulting in similar type of prey content in different type of species. Although anurans feed on similar type of prey category, the prey limitation, different mouth width and microhabitat use helps in co-existence in similar habitat (Santos-Pereira et al. 2015). In the study area *Duttaphrynus* generally preferred ants, *H. crassus* and *H. tigerinus* could consume larger prey size, *E. cyanophlyctis* and *M. teraiensis* depended on smaller prey items.

The niche breadth of *H. crassus* and *Duttphrynus* was almost similar in the study area. *H. crassus* is one of the large sized frog while *Duttaphrynus* is ant specialist predator. The different in body size and dietary preference might be the reason for their coexistence in similar environment. Similarly, the niche breadth of *H. tigerinus* and *E. cyanophlyctis* was found to be similar. *H. tigerinus* being larger in size than *E. cyanophlyctis* feeds on larger prey showing the resource portioning between these two species. Larger species forage at larger trophic level as compared to smaller individuals of same species and other smaller species. This could reduce the competition for food within similar species and between different species to facilitate co-existence (Cloyed and Eason 2017). Further, the difference in niche breadth, absence of some prey category in some species might also help to co-exist in similar habitat (Santos-Pereira et al. 2015). The niche breadth might vary according to different seasons and different life stage of anurans (Leivas et al. 2018). However, the present study was only focused on the niche breadth of the recorded anuran species rather to observe the seasonal variation in niche breadth of the anurans. The dietary niche overlap between two medium sized frog was more than two large sized frog. This might be due to the small head width and gape size of medium sized frog which enables them to consume the prey of limited size and shape. But in case of large sized frogs, their large gape size might help them to consume different prey categories. The high niche overlap among anurans might suggest the fact that diet composition might be affected by other factors like microhabitat use, gape size and time of activity (Woolrich-Pina et al. 2017). The prey item preference in species level might also differ among five different anuran species. In the current study, the prey availability in the study area was not studied and dietary analysis was not done in microhabitat level, which makes difficult to predict a particular reason for the species co-existence in spite of their high degree of dietary niche overlap.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The most prey categories that was preferred by anurans were Hymenoptera and Orthoptera followed by Odonatata, Diptera, Coleoptera, Lepidoptera, Blattodea, Larva, Spider, Snails, Crab and Earthworm. Furthermore, positive relationship was seen between the body size (SVL) of anurans and the weight of consumed prey. This phenomenon was seen more in large sized frogs like *Hoplobatrachus tigerinus.* However, anurans did not show any relationship between total number of prey items and their body size. While comparing dietary preferences or habits, no significant difference was seen in cropland, forest and forest edges. Thus, anurans being generalist predator can feed on every available prey items in the habitat. Dietary niche showed high degree of overlap among five species. All the anurans in the study area dependent on similar type of prey category showing no variation in dietary preferences. But the feeding habit might vary according to body size of anurans. Anurophagy was also reported from the study which supports that large anurans can also consume smaller frogs found in the same habitat. Niche breadth of different anurans revealed their trophic ecology. All the species has almost similar niche breadth; *Miniervarya teraiensis* possessing the broadest 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 suggest that there is a strong competition for the food resources however, the competition for food alone can't explain about their co-existence in similar habitat.

6.2 Recommendations

Some of the recommendations based on the present study are:

1. Since the study was conducted during one season only, complete feeding ecology could not be explored. Dietary assessment should be done in pre-monsoon, monsoon and post-monsoon to understand feeding ecology in detail.
2. The prey items should be identified up to species level to find out the precise niche overlap between different species.

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8. APPENDICES

1. Dietary composition of five species of anurans.

Prey categories → / **Species ↓**	Hymenoptera	Orthoptera	Odonates	Diptera	Coleoptera	Lepidoptera	Larva	Spider	Snail	Crab	Blattodea	Earthworm	Anurophagy
Haplobatrachus crassus	92	9	5	9	63	3	15	6	12	2	14	14	3
Haplobatrachus tigerinus	44	12	2	8	65	0	14	9	16	5	2	3	0
Minervarya teraiensis	9	2	1	0	7	0	1	0	0	0	1	1	0
Euphlyctis cyanophlyctis	32	9	1	7	24	1	8	3	0	0	1	2	0
Duttaphrynus	68	0	1	4	63	0	4	5	0	0	2	1	0
Total	245	32	10	28	222	4	42	23	28	7	20	21	3
Percentage	35.77	4.67	1.46	4.09	32.41	0.58	6.13	3.36	4.09	1.02	2.92	3.07	0.44

2. Anuran species recorded during field survey

S. N.	Family	Name of species	CL	F	FE
1	Dicroglossidae	*Euphlyctis cyanophlyctis*	1	1	1
		Minervarya teraiensis	1	1	1
		Hoplobatrachus crassus	1	1	1
		Hoplobatrachus tigerinus	1	1	1
2	Bufonidae	*Duttaphrynus melanostictus*	1	0	1
		Duttaphrynus stomaticus	1	1	1
3	Rhacophoridae	*Polypedates maculatus*	0	1	0

CL = Cropland F = Forest FE = Forest Edge

1 and 0 represents the presence and absence of species in different habitat types.

9. PHOTO PLATES

Diet of *Hoplobatrachus tigerinus*

Diet of *Hoplobatrachus crassus*

Diet of *Duttaphrynus*

Anurophagy