# DIET COMPOSITION OF ANURANS IN RICE FIELD CHITWAN, NEPAL

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# RECOMMENDATIONS

This is to recommend that the thesis entitled "**Diet composition of Anurans in Rice Field Chitwan, Nepal**" has been carried out by **Mr. Subarna Raj Ghimire** for the partial fulfilment of Master's Degree of Science in Zoology with special paper Entomology. 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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On the recommendation of supervisor "Prem Bahadur Budha" this thesis submitted by Subarna Raj Ghimire entitled "**Diet composition of Anurans in Rice Field Chitwan, Nepal**" is approved for the examination and submitted to the Tribhuvan University in partial fulfilment of the requirements for Master's Degree of Science in Zoology with special paper Entomology.

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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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# ABSTRACT

The stomach flush technique of 398 individuals of frogs and 46 species of toads in the rice field of Bachhauli, Chitwan was conducted to find their food composition. Frogs and Toads are secondary consumers in food web and consume significant amount of insects and non-insect prey items. The stomach contents of frogs and toads contained 72 % prey within Hymenoptera, Coleoptera and Lepidoptera. Other insect orders were Orthoptera, Homoptera, Hemiptera, Dermaptera, Odonata, and Isoptera. Non-insect prey includes millipeds, spiders, and prawns including earthworms and snails. Coleoptera was the most abundant insect order and was reported in the diet of most of anuran species. There was no any specialization of prey items in anuran species. They were found preying on any prey items which are available on their way. Anurans consumed significantly higher number of insect prey than non-insect prey ($t=14.07$, $df= 892$, $p=<0.001$). There was no significant difference between consumption of insect prey by male and female frogs. However female consumed significantly higher number of non-insect prey compared to male (Man-Whitney U-test = 22013.50, $p = 0.028$).Female anurans were comparatively bigger than male and there was significant difference between the weights of diets between them ($p=0.001$).

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| Abbreviated form | Details of abbreviations |
|---|---|
| VDC | Village Development Committee |
| m | Meter |
| asl | Above the sea level. |
| ADO | Agriculture District Office |
| SVL | Snout Vent Length |
| Hr. | Hour |

# 1. INTRODUCTION

Amphibians are the first group of vertebrates occupying two different habitats (i.e. aquatic and terrestrial) in their lifetime and start terrestrial life by developing lungs and enabled them to breathe on land. They are globally widespread except in Polar Regions of Antarctica and Greenland and especially concentrated in the Neotropical countries. They are nocturnal, ectothermic, regulating their body temperature with behavior as they rely on ambient environment for their body temperature. They have soft, permeable skin with many glandular properties. Their skin is protected with a layer of mucus which also helps to absorb environmental oxygen (Gascon et al., 2007).

Class Amphibia is classified into three orders namely Anura, Caudata (Urodela) and Gymnophiona. Anurans include frogs and toad which are the most diverse and widespread containing about 5208 extant species (www.amphibiaweb.org). Toads have dull, dry, wrinkled, watery or pimply skin and slow movement whereas frogs have moist, bright, smooth skin. Toads are more terrestrial than frogs, although they also occasionally visit water to avoid from getting dry. Frogs rarely wander far from water except in rainy seasons.

In Nepal Anurans are represented by single genus (*Bufo*) of toad and four species and 47 species of frogs within four families of 17 genera (Shah and Tiwari, 2004). They are widely distributed from 60-5490 m asl and throughout the country from Mechi to Mahakali including Terai to Mid-Hills and higher Mountainous Regions (Shah and Tiwari, 2004).

Frog and toads are typically found within the vicinity of sources of freshwater that can be used for breeding as well as feeding. Lentic freshwater like seasonal wetlands, semi-permanent or permanent wetlands, ponds, lakes etc. and lotic freshwater likes springs, streams, rivers provides suitable habitat for them. Seasonal wetlands with fewer fishes provide suitable habitat for amphibian species (Gascon et al., 2007). There are large numbers of lakes, ponds, rivers, marshy lands, agricultural land in Nepal. The agricultural landscape provides foraging and other habitats for variety of anurans and invertebrates.

Frogs and toads are important component of natural ecosystem. They are secondary consumers in the food chain preying on wide range of insects (Duellman and Trueb, 1994; Lannoo, 2005; Parajuli et al., 2005) and non-insect preys (Parajuli et al., 2005; Hirai, 2008), small or juvenile vertebrates (Hirai, 2008; Sole et al., 2005) and larval forms (Kupferberg, 1997). They transfer energy from primary consumer to tertiary consumers. Many amphibians play important role in agro-ecosystem by controlling number of insect populations. Understanding feeding relationship in amphibian communities is of fundamental interest to herpetologist and ecologists because of the pivotal role that amphibians may play in an aquatic ecosystem. Many amphibian species occupy intermediate position in food chain,

being an important predator on invertebrates an important prey for larger vertebrates (Hirai and Matsui, 2000). Frogs are recognized as key bio-indicators of environmental health due to their sensitivity to water pollution (Meschersky, 1997).

The paddy agroecosystem is man-made environment which include human, animals, rice plants and other plants (Kiritani, 2000). Rice is mainly grown in Tarai and inner valleys of Nepal including irrigated land of mid-hills. The rice fields provide potential habitat for many low-land anurans and many species aggregate near water sources (Naniwadekar and Vasudevan, 2007). The permanent water sources near in or around rice fields act as breeding habitat for anurans. The female release large number of eggs in a large gelatinous string and the male release the sperm to spray over the eggs for fertilization. The eggs may be freely suspended or attached to submerged vegetation in quite or stagnant water at ponds, poodles etc. (Duellman and Trueb, 1994).

Rice fields are important ecosystems where variety of invertebrates and vertebrates interact each other. Frogs and toads are important component of rice field ecosystem. They consume large number of insects, annelids, molluscs, fishes, and other small amphibians and reptiles. Traditionally they are generalist predators, with opportunistic foraging behavior. The main diet items in adult diet consists invertebrates viz. molluscs, annelids, centipedes, millipedes, arachnids, crustaceans and insects (Toft, 1980; 1981; Duellman and Trueb, 1994; Lima and Magnusson, 1998; Van Sluys and Rocha, 1998; Anderson et al., 1999). Most tadpoles of anurans are omnivorous feeding on microorganism, algae, protozoan, larvae of insects, shrimps, eggs and young ones of other amphibians. The dietary patterns of anurans vary with seasons and species; they follow two major dietary patterns, the ant specialists feeding on slow moving chitinous arthropods and non-ant specialist feeding on larger less chitinous species (Tuft 1980; 1981). Rice is attacked by more than 100 species of insects from different orders like Diptera, Lepidoptera, Coleopteran, Orthoptera, Hemiptera, Thysanoptera, Homoptera etc (Neupane, 2009). Among these orders some adult representatives and some larval form are the pests. Anurans are one of the most efficient natural enemies of pest species and 50 frogs can keep an acre of a paddy field free of insects (Ted, 2007).

The study of the diet is important for the conservation actions for these threatened animals. Study of diet of anurans was generally performed by killing the collected species (Toft, 1981; Flowers and Graves, 1995) for its stomach content. For the purpose of maintaining biodiversity alive some researcher developed and applied stomach-flushing techniques in salamanders (Frasser, 1976) and in frogs (Sole et al. 2005).

Insects belong to the class Insecta of the Phylum Arthropoda, which is largest class and includes beneficial as well as harmful species. Most of the larvae of Lepidoptera are phytophagous feeding on economically important plants. They include beneficial species such as bombycids which is predatory in nature and some species feeding on weeds so can be

used as biological control agents. The studies on feeding habit of anuran species are still scare in Nepal. Harmful insects include different pest species. Ants, beetles, flies, grasshoppers, aphids, leafhoppers and molluscs are preferred by *Hoplobatachus tigerinus*, *Limnonectes* (=*Zakerana*) *nepalensis*, *Bufo* (=*Duttaphrynus*) *stomaticus* (Parajuli et al., 2005). The present study includes following toad and frog species:

*Duttaphrynus melanostictus* (Schneider, 1799) is a widely distributed species of lowland habitat from river bank to human dominated agricultural and urban areas. It breeds in still and slow-flowing rivers, temporary and permanent ponds, pools etc. Adults are terrestrial and may be found under ground cover (rocks, leaf-litter, and logs). *Duttaphrynus stomaticus* (Lutken, 1862) is found in wide range of habitats like grassland, forest, agricultural land and human habitations. It breeds in permanent and seasonal pools, seasonal streams and slow flowing streams. Adults hide under rocks, crevices and adaptable in human houses.

*Euphlyctis cyanophlyctis* (Schneider, 1799) is widely distributed near water bodies such as marshes, pools, river, streams and other man-made wetlands. It has also reported from brackish water in southern Sri Lanka. Adults are generally found basking at the edge of water bodies. The species breeds and larva develops in suitable freshwater water bodies. The species may be found in all modified habitats like rice fields, seasonal ponds where suitable wetland habitat is available.

*Zakerana nepalensis* (Dubosis, 1975) is present in Nepal, India, Bangladesh and generally found below 500 m asl. It occurs in grasslands, agricultural land especially paddy field and close to human settlements. Breeding takes place in temporary and permanent pools, marshes and paddy fields.

*Zakerana syhadrensis* (Annandale, 1919) is widely distributed in India, Nepal, Pakistan and Bangladesh. It is found at elevation below 2000 m asl. It is associated with paddy fields and other suitable wetlands. Non-breeding individuals may be found hiding in crevices, vegetation cover and other ground cover.

*Zakerana teraiensis* (Dubois, 1984) is known from southern Nepal where it is restricted below 500 m asl. It probably occurs more widely in open grasslands close to permanent pools and streams. Breeding takes place in temporary and permanent pools and paddy fields.

*Hoplobatrachus crassus* (Jerdon, 1853) is a terrestrial species found in seasonally flooded grasslands, open plain and arid areas. Adults are often found in burrows and aestivate during dry seasons. Its breeding and larval development takes place in many different types of water bodies. They are present in cultivated areas around human settlements. *Hoplobatrachus tigerinus* (Daudin, 1802) is found mostly throughout the wetland areas of Nepal, India, Bangladesh. It is mainly aquatic, inhabiting mainly in freshwater wetlands. It is mostly solitary and nocturnal, found in holes and bushes near permanent water body.

The present study focuses on the diet composition of above species of frogs and toad particularly in an agricultural field of Chitwan, Central Nepal.

### 1.1 Objectives of the study

The general objective of the study is to explore insect and non-insect food items consumed by different frog and toad species but specific objects are to:

1. Find composition of insect prey items in the diet of frog and toad species found in paddy fields.
2. Compare weight of diet in male and female of different species of frogs and toad.
3. Study prey preference and species richness or species diversity in diet of toad and frogs.

### 1.2 Rationale of the study

Frogs are recognized as key bio-indicator of environmental health due to their sensitivity to water pollution (Bridges et al. 2002). Anurans play an important role as natural enemies of insect pests in the rice field. Rice is attacked by more than 100 species of insects and 20 of them cause economic damage (Pathak and Khan, 1994). Rice fields are preferred habitat for amphibians and act as natural biocontrol agent of many insect pests (Bambaradeniya and Amerasinghe, 2004). However the knowledge about the role of amphibians in the ecosystem is poorly studied in Nepal. Probably, Parajuli et al. (2005) were first to study of food habits of anuran in Nepal including two species of frog *Hoplobatrachus tigerinus* and *Limnonectes nepalensis*, and a toad species *Bufo stomaticus.* Diet composition of many other species of anurans are severely lacking in Nepal. This study aims to generate further data which will be useful for researchers, educators, conservationists and agriculturists.

### 1.3 Limitations of the study

The study is primarily focused on the diet composition of Anuran species and insect and non-insect prey items. Due to lack of advanced knowledge on taxonomy and proper identification of collected prey items upto genus and species level was too difficult task within short period of time. Thus, the present study identified the order level of different prey items with some exception of some families of some orders. This study also focused on the conservation of frogs and toad so the direct stomach flush technique was applied and caught individuals of frog and toad were released without harming in the nature.

# 2. LITERATURE REVIEW

## 2.1 Faunal diversity of Anurans

Amphibians are unique group of vertebrates including over 5743 currently known species worldwide Anura is the largest order consisting 5208 living species currently recognized. Anurans are reported from sea level to as high as 5244 m above the sea level. The Caudata contains 535 described salamanders and newts representing about 10% of the number of Amphibians. Gymnophiona is the smallest order with only 172 described species. Its population is declining and nearly about 32% of world's amphibians are threatened. They have existed on earth for over 300 million years, but in last two decades there have been alarming number of extinctions. Nearly 168 anuran species are believed to have gone extinct and at least 2469 more frog and toad populations are declining (Stuart et al., 2004).

South Asia is rich in amphibian and reptilian diversity including several unique and endemic species. Nepal is relatively richer in herpetofauna in comparison to other South Asian countries inhabiting 206 species and sub-species. Fourteen species of herpetofauna are endemic to Nepal 17 herpetofauna species are enlisted as threatened of which six species are globally threatened (Bhuju et al., 2007, Pokhrel et al., 2011)

The widespread use of Benzene hexachloride and parathion for controlling rice insect pest causes contamination of the environment. It causes disruption of natural enemies like spiders and parasitoids (Kiritani, 2000). Production of rice had increased by environment- formative and environment adaptive technologies. Traditional earth canals were replaced by U-shaped concrete canals reduces the variety of habitats of aquatic species. Conversion of ill-drained wet paddy fields to well drained fields raised both land productivity and diversity of aquatic organisms (Fujioka and Lane, 1997). As a result two insect species from Hemiptera and Coleoptera along with five bird species, one fish species and one amphibian species are listed in endangered category (Kiritani, 2000).

## 2.2 Insect diversity

Nepal as a Himalayan country in South Asia has unique geographical features with topographic and climatic variation. It supports to flourish high insect diversity. Northern belt of Nepal is well furnished with the glacial high Himalayas (above 4,000 m), mid belt consist Alpine and Temperate climate (4,000-2,000 m) and Southern Siwalik and Tarai belt (upto 2,000 m). The different level of precipitation from east to west in decreasing order and duration of snow cover lesser in Western regions supports high insect diversity in East than in West (Thapa, 2015).

Thapa (2015) compiled the list of 12,136 species of insects from Nepal including 3,883 endemic species. The insect diversity in rice field is composed of resident and migratory species corresponding to the continuous cropping of rice in same field. Paddy field is used by different species for feeding and breeding ground (Gewali, 2013). The paddy fields are artificial wetlands which are used by many aquatic organisms. Due to reduction of natural wetlands different aquatic organisms use paddy field fully or partially for completing their life cycles (Kiritani, 2000). Thirty-one species of Odonata or 30% of insect species utilize paddy field for oviposition (Ueda, 1998). Many aquatic Hemiptera and Coleoptera including giant water bugs, water scropions, dytiscid beetles and fireflies reproduce in paddy fields.

Rich wetlands of Nepal support high faunal diversity including amphibians. The amphibian fauna associated with paddy fields plays a significant role in controlling harmful insects/pests (Lockie and Carpenter, 2010). The rice field in Chitwan gets severe outbreaks of different pest species like plant hoppers on early rice field from Feb/march- June/July, in 1996 (Pokhrel et al., 1988), white flies in August-September, 2003( Pokhrel and Pokhrel, 2011).

### 2.3 Diet composition of Frog and toad

Diet is an important life-history component of ecological niche. In communities, species feeding habits can play a key role in shaping competition dynamics (Lawlor, 1980) and predator-prey interactions (Richter-Boix et al., 2008). Particularly among anurans, community structure is often associated with a relationship between species habitat and diet (Duellman and Trueb, 1994). Some species of anurans can be considered specialists (Simon and Toft, 1991; Santos et al., 2004; Biavati et al., 2004), but some species are reported as generalist and opportunistic feeding habits (Parajuli et al., 2005; Schaefer et al., 2006; Mahan and Johnson, 2007). Feeding habits have reported a high niche overlap among syntopic and sympatric anuran species (Toft, 1981; Rosa et al., 2002; França et al., 2004; Almeida-Gomes et al., 2007). For generalist anurans, aspects such as phylogeny, foraging mode, prey availability and abundance, and morphological constraints for capture and ingestion of a given prey type can be related to resource partitioning among species (Lima, 1998; Lima and Magnusson, 1998; França et al., 2004; Santos et al., 2004). Furthermore, community niche differentiation can be influenced by human induced environmental changes, which may affect ecological interactions (Caldwell and Vitt, 1999; Albrecht and Gotelli, 2001; Rocha et al., 2008).

Frogs, in general, are generalist predators and consume wide variety of prey items in response to prey availability in the environment (Duellman and Trueb, 1994). Some frogs, however, are known to selectively feed on particular prey. Many Dendrobatid and some Bufonid species are myrmecophagous (anteater). These frogs are specialized for eating ants, and consume them in a higher proportion than found in surrounding environments (Toft 1980; 1981; Lieberman, 1986). Species of liter frogs form a continuum from species that

specialized on ants and mites. Different frog species shows different foraging habits *Atelopus* and *Bufo* eat many prey primarily ants and mites per day. *Colostethus* eat ants but to a lesser degree and have a more sedentary foraging behavior.

The stomach contents of frogs also consists plant remains, shed-skin fragments, animals and animal fragments. Plant parts were frequently observed in most frog species examined although in small amount (Almeria and Nuneza, 2013). *Fejervarya cancrivora* (Crab-eating frog), *Limnonectes leytensis* (Swamp frog), *Rhinella marina* (Cane toad), *Polypedates leucomystax* (Common tree frog) and *Kaloula conjuncta meridionalis* (Philippine Narrowmouth toad), have broad dietary diversity, indicating a generalist active feeding and opportunistic foraging behavior. *Rhacophorus appendiculatus* (Rough-armed tree frog) and *Occidozyga laevis* (Puddle frog) consumed fewer food items implying that these species are specialist passive feeders. The most consistently observed diet items in the frog samples in Agusan marsh were Hemiptera (bugs) and Hymenoptera (ants) found in all frog species examined. Coleoptera (bettles) was found in 90% of the samples while Orthoptera (grasshoppers), Isoptera (termites) and Diptera (mosquitoes) were found in 70% of the samples examined. Gastropoda, Decapoda, Siphonoptera, Scolopendromorpha, Mecoptera, Dermaptera, and Protozoa were seldom observed. The results indicate that anurans that are specialist feeders need to be monitored especially the vulnerable *R. appendiculatus* because any alteration of the habitat can affect the animals particularly the invertebrate prey of this threatened frog (Almeria and Nuneza, 2013).

Anurans have sexual size dimorphism with significantly larger female than male however the mean length of prey item in stomach did not differ significantly. The frequency of empty stomach, mean number of prey item and mean volume don't show significant difference. Diet composition is similar between both sexes with high dietary overlap. The diet composition is directly correlated with the relative abundance of prey in their foraging habitat (Hirai and Matsui, 2000). They are classified into explosive or prolonged breeders on the basis of their breeding habits. The explosive breeders like *Rana nigromaculata* and *Bufo japonicus* don't forage but prolonged breeder like *Hyla japonica* forage during breeding season. The smaller body size in male of prolonged breeders due to lack of foraging time by different reproductive activities like advertisement, territorial defense, agonistic behavior etc. and by loss of energy for such activities (Woolbright, 1983).

### 2.4 Anuran diversity in rice fields

The total rice field in Chitwan district is 30198 ha with annual production of 90594 mt (ADO 1990, Pokhrel and Thapa, 2011). The most part of eastern and western Chitwan have year round irrigation along with highly fertile humus enriched soil which is suitable for rice cultivation (Pokhrel and Pokhrel, 2011). Irrigated rice fields being temporary aquatic habitats with generally predictable dry phase can be scientifically defined as agronomically managed

temporary wetland ecosystem (Bambaradeniya, 2000). They are seasonal and temporary aquatic habitat managed with variable degree of intensity (Halwart, 1994). The rice ecosystem consists of two physically and morphologically distinct habitats: the rectangular or similar shaped flooded field comprising mainly of rice plants and surrounding bunds which harbors weeds. Under irrigated condition this mosaic system is connected with irrigation canals and ditches while sump ponds, marshes and tank serves as contiguous aquatic habitats. Physically, the aquatic phase has a shallow fluctuating water depth 5- 30 cm (Bambaradeniy, 2000).

Rice fields play important role as a substitute habitat for wetland species and support to enrich ecosystems. Agricultural modernization has altered the rice paddy environment, and many previously common species are now endangered. It is urgently necessary to evaluate rice paddies as habitats for conservation. Among the species living in rice paddies, frogs are representative and are good indicator species, so we focused on frog species and analyzed the influence of environmental factors on their habitat use. For frog conservation in rice paddies, we need to choose favorable rice paddy in relation to surrounding land use and apply suitable management for target species (Naito et al., 2012).

The Japanese tree frog (*Hyla japonica*) did not differ in abundance in the rice fields, but the Japanese brown frog (*Rana japonica*) and the Tokyo daruma pond frog (*Rana porosa porosa*) preferred the old-style rice fields. These findings suggest that the status of *Rana* species has been adversely affected by the conversion of rice fields to the new irrigation system. Modification of the deep, U-shaped concrete ditches and the water management regime during the spawning season is needed to safeguard these species (Fujioka and Lane, 1997).

The food of anurans has been studied in Holoarctic and Ethopian region by number of workers including Hamilton (1948) and Tyler (1958) but in Oriental region little work has been done by Berry and Bullock (1962). The gut content of *Bufo melanostictus* showed that this toad feeds wide range of invertebrates of which insects form greater part. Feeding was confined to ground dwelling forms but was apparently unselective (Berry and Bullock, 1962).

*Rana nigromaculata* was found to consume a wide variety of prey species in rice fields. Arthropods constitute the bulk of its diet both numerically and volumetrically. Frequencies of the major prey taxa fluctuated seasonally and were significantly correlated with prey availability estimated by a sweeping method. This frog feed opportunistically depending on encounter rates of prey. The diet is more strongly influenced by prey availability than by prey selectivity because this anuran is generalist predator (Hirai and Matsui, 1999). The anuran family Ranidae includes many species and some have peculiar food habits.

*Bufo* species live in areas characterized by both vegetative cover and open patches and are generally nocturnal feeding on ground dwelling arthropods generally ants and termites. Insects make up the major portion of frog's diet while arachnids are of lesser importance for most of frogs (Moore and Strickland, 1954). Some studies show ants and beetles comprise 81% of all the food item eaten which are omnipresent taxa and other available arthropods makes worldwide success of toads (Raymond, 1974). Diet examination of *Limnonectus* indicated the frogs' high dependence on eight orders of invertebrate fauna dominated by Orthoptera (Fabricante and Nuneza, 2012).

### 2.5 Anurans as bio-indicators

Anurans are indicators of ecosystem health and have important functions in natural food webs. Amphibians are recognized as key bio-indicators of environmental health due to their sensitivity to water pollution. Nepal harbors high amphibian diversity, but amphibians inhabiting agricultural lands remain poorly known. The agricultural landscape provides foraging and other habitats for amphibians. The rice field may be particularly important since they are often filled with water and the majority of amphibians frequently aggregate near water sources. Rice fields therefore provide potential habitat for many low-land amphibians. However, farmers in the area lack the knowledge about the role of amphibians in the ecosystem despite of their important role in managing insect pests. Indiscriminate use of pesticides has become a regular phenomenon. Amphibians in the agricultural landscapes are therefore at high risk due to excessive use of insecticides and pesticides. It is thus very important to highlight the role of amphibians as crop pest controllers for farmers in the region.

Amphibians have limited dispersal abilities and spatially separated breeding habitat (Smith and Green, 2005). The limited dispersal ability may further increase the vulnerability of amphibians to change in climate. Slight change in water level in breeding ponds can cause reproductive failure, causing severe drop in the population of short-lived species and persistent change can lead to extinction of species (Araú Jo et al., 2006).

The habitat of amphibian is not stable over the time due to climate change and succession. Climate change alters temperature and rainfall pattern causing negative impact on amphibian population. It increases pressure on many amphibian species in different ways like reduction of soil moisture, reduction of wetlands etc. Many breeding habitats are altered by ecological succession. Widespread amphibian extinctions show the vulnerability of biodiversity to climate change (Gascon et al., 2007).

### 2.6 Frogs, toad and crop pests

Frogs are an important part of the agroecological system with the role of pest control agent. The major insect pest of rice crops includes root feeders like termites, mole cricket, and rice

root weevil; stem borers like yellow stem borer, stripes stem borer, dark headed borer etc; plant hoppers, leaf hoppers, defoliators like caterpillar and grasshopper and grain suckers (Neupane, 2009).

The agricultural landscape provides suitable foraging habitat for anurans. The stomach content of *Hyla* captured from soybean field is dominated by aphids and leaf beetles, catarpillars (Hirai 2008). The stomach content of *Rana* from rice fields of India contains many rice pests like *Spodoptera mauritia* (Boisd.), *Pelopidas mathias* (F.) (*Parnera mathias*), *Rhinyptia* and *Holotrichia* sp., *Melanitis leda ismene* (Cram.) (*M. ismene*), *Hieroglyphus banian* (F.) and *Scirpophaga incertulas* (Wlk.) (*Tryporyza incertulas*). The land crab *Parathelphusa* sp., a major rice pest was also found in its diet.

The use of frogs in control of harmful insects like pests and vectors is not studied properly. Frogs as an important part of ecosystem plays an important role for control of mosquito population. Amphibian predators like frog and toads live in close association with human and which can live over land and water. Adult frogs feed on adult stages of mosquito and tadpoles feeds on mosquito larvae and can be effective mosquito control agent. Among different mosquito control methods, the biological control using frogs have low ecological impacts, reduced side effects on humans and gives significant positive results (Sarwar, 2015; Russell et al., 2001).

# 3. MATERIALS AND METHODS

## 3.1 Study Area

This study was carried out extensively in cultivated agricultural landscape of Bachhauli Village Development Committee of Chitwan, Central lowland Nepal (Figure 1). It lies between 27.6030° to 27.5490° northing and 84.4680° to 84.5510° easting. The area lies in the Buffer Zone of Chitwan National Park which has high biological diversity and recognized as the world heritage site by the UNESCO.

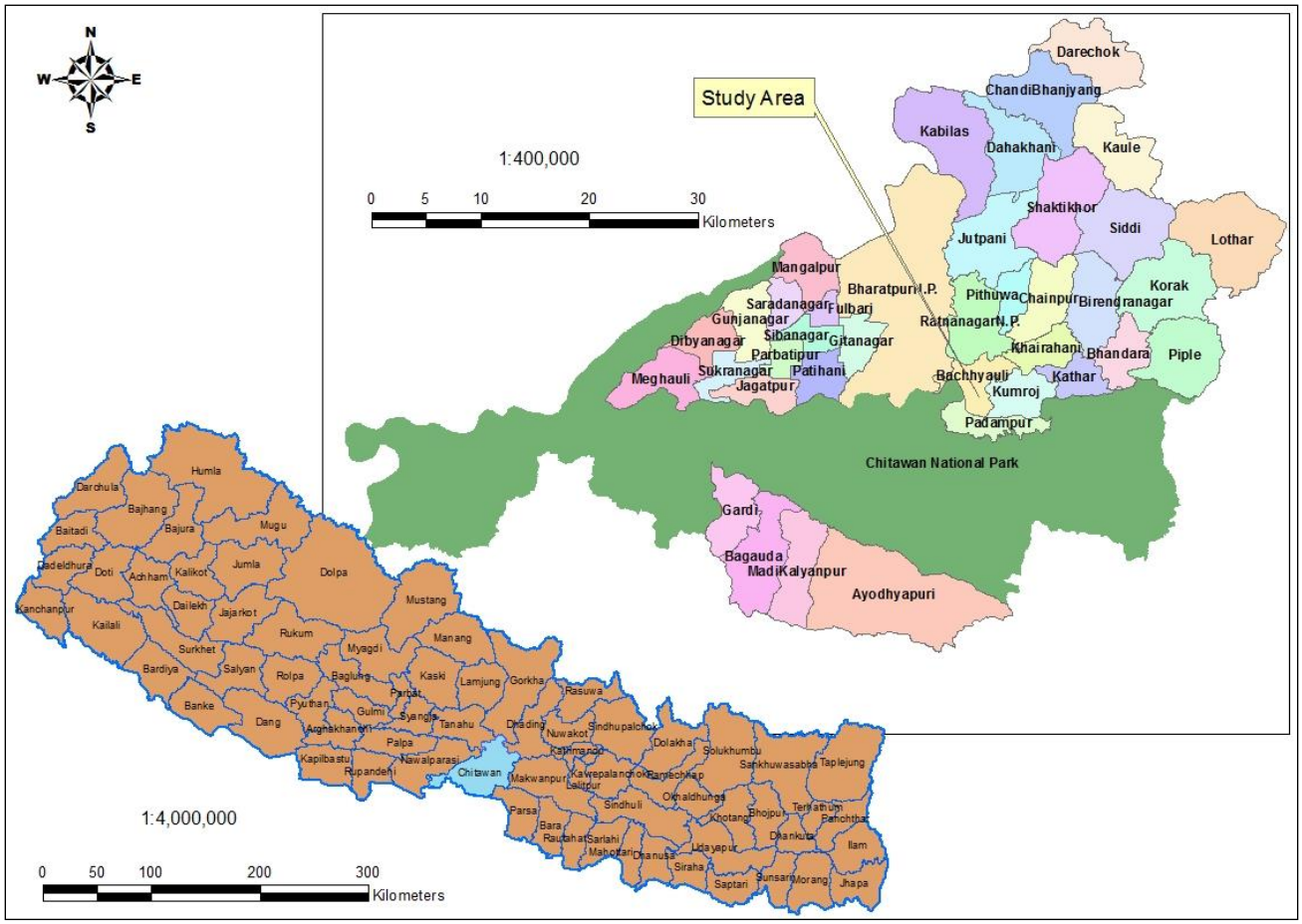


Figure 1: Map showing study area.

## 3.2. Frog survey and collection of the diet contents

Anuran surveys were carried out at night along transects of length 250 m and width 4 m between 19.00 to 23.00 hr. The field survey was carried out during June, July, August and September, which coincides with the monsoonal rainfall, rice planting and breeding season of frogs (Schleich and Kästle, 2002). A total of 44 transects were surveyed during four month study period. Transects were surveyed by four people with the speed of walking 500 m/ Hr.

by using torches. The distance of transect was placed at least 250 m apart from eachother. The number of individual species encountered in each transect were captured, recorded and were identified in the field with the help of field guide books (Schleich and Kästle, 2002; Shah and Tiwari, 2004). All captured individuals were released in the same habitat after physical measurements (snout-vent length, body weight, and mouth width), stomach flushed and taking photographs.

A minimum number of five individuals (if possible) of each species encountered in each transect were collected for diet analysis. A stomach flushing technique was followed by Sole et al. (2005). A rubber tube attached to a syringe and other free end was introduced through the oesophagus into the stomach. Once the tube is inserted, a small amount of water squeezed from the attached syringe into the stomach and the content that is forced out from mouth were collected. The stomach-flushing procedure was repeated until no further stomach content is produced. The stomach contents were preserved in 70% ethanol for further identification and measurements in the laboratory. The mortality of anurans in this experiment was about 1% only (Sole et al., 2005). The collected insect diet was identified at least order level with the help of standard textbook of entomology (Borror et al., 1954; Rochards and Davies, 1997)

**3.2.1 Monitoring the Transect:** After doing stomach flushing of anuran species they were released in the same site. Again we have visited the same transect next day to monitor whether there were any dead anuran species or not. We didn't find any dead frog or toad around these transects.

### 3.3 Taxonomic Identification

All the diet of individual Anurans collected from field were sorted out into different groups on the basis of its major morphological characters by following different textbooks on insect identification Borror et al., 1954; Rochards and Davies, 1997. Permanent and temporary slides of different available parts like antenna, leg and wings were prepared which were examined under microscope and trinocular microscope. Photographs of image of temporary slides were taken for identification process.

Taxonomic identification was done based on the structure of antennae, legs and wings fragments observed under microscopes. There are some typicalantennae of some insect groups viz. geniculate (Annex 5 IA) clubbed antenna of (Annex 5 I,B1 and B2) of Hymenoptera, filiform (Annex 5, IIC), serrate (Annex 5 IID), clubbed (Annex 5 IIG) of Coleoptera.

Similarly shape and size of fore, mid and hind legs have variations on number of tarsal segments of different order and family of insects. Ants (Hymenoptera) bears of six tarsal segments along with feather like tibial spur in fore legs and needle like in mid and hind legs

(Annex 5 VA, B, and C). Some Hymenoptera also bears four five segmented tarsi (Annex 5 II and III). Order Coleoptera is highly diverse having variations on tarsal segments and tibia. The tibia is much larger and horny in comparison with tarsal segments (Annex 5 IX F, G, and H). There is presence of tibial spur of different shape and size (Annex 5 III D and E; IX G and H). Hymenoptera also bears different structures in tibia (Annex 5 V A and C; VII A) and tibial spurs (Annex 5 VIII D, E and F).The tarsal segments also show different modifications like hairs and spines (Annex 5 III D, III F, IV G) in aquatic Coleoptera.

There are modifications on wings which are also important taxonomic characters for identifying different group of insects. The hard leathery wing i.e elytra (Anenx 5 XIII B) is characteristics of Coleoptera, half-leathery and half-transparent wing or hemi-elytra (Annex 5 XIIIA) is characteristics for Hemiptera. Hymenoptera bears wings with less number of veins and with distinct wing coupling (Annex 5 XIV B) for interlocking fore and hind wings.

### 3.4 Data analysis

Sigma-plot version 12.5 was used to analyses the proportion of diet by male and female individuals of sampled frog and toad species. The species diversity index of insect in Anuran's diet was calculated by using Shannon and Weiner diversity index.

Shannon and Weiner Diversity Index (H') = - $\Sigma$ ni/N Log (ni/N)**.**

# 4. RESULTS

## 4.1 Prey composition in Anuran diet:

Stomach of 444 frogs/toad individuals were flushed and a total of 1417 (1223 insect and 194 non-insect) items were recorded in their diet (Table 1). Among the diet of Anurans insects constitute 86.3 % and non-insect constitute 13.7% by volume. Out of the total prey items toad species comprised 97.95% of insect and 2.05 % of non-insect items. Similarly frog species comprised 83.25 % of insects and 16.75 % of no insects (Table 1). The mean number of prey is 3.2 with average number of preys 6.6 and 2.8 in toad and frog respectively.

Table 1: Insect and non-insect diets of Anuran species.

| **Anuran Category** | **No. of species** | **Total prey items** | **Insects** | **Non-insects** | **Mean no of prey** |
|---|---|---|---|---|---|
| Toad (n=46) | 2 | 294 | 288 (97.95) | 6(2.05) | 6.6 |
| Frog (n=398) | 6 | 1123 | 935(83.25) | 188(16.75) | 2.8 |
| 444 | 8 | 1417 | 1223(86.3) | 194(13.7) | 3.2 |

(Numbers in parenthesis represents percentage)

Among the dietary composition of some individual frog and toad species, the largest group by numeric proportion (n %) was Hymenoptera (35.57%) followed by Coleoptera (23.39%), and Lepidoptera (12.98%) respectively.

Table 2: Comparison of different prey items in the diet of toad and frog.

| **Prey items** | | **Prey item consumed by anurans** | | | **% in individual group** | |
|---|---|---|---|---|---|---|
| | | **Toad** | **Frog** | **Total** | **Toad** | **Frog** |
| Insects | Coleoptera | 73(5.15) | 257(18.14) | 23.39 | 24.83 | 22.89 |
| | Lepidoptera | 19(1.34) | 165(11.64) | 12.98 | 6.46 | 14.69 |
| | Hymenoptera | 176(12.42) | 328(23.15) | 35.57 | 59.86 | 29.21 |
| | Orthoptera | 0(0) | 69(4.87) | 4.87 | 0 | 6.14 |
| | Homoptera | 0(0) | 50(3.53) | 3.53 | 0 | 4.45 |
| | Hemiptera | 3(0.21) | 33(2.33) | 2.54 | 1.02 | 2.94 |
| | Dermeptera | 1(0.07) | 21(1.48) | 1.55 | 0.34 | 1.87 |
| | Odonata | 0(0) | 6(0.42) | 0.42 | 0 | 0.53 |
| | Isoptera | 16(1.13) | 6(0.42) | 1.55 | 5.44 | 0.53 |
| Non-insects | Annelida | 2(0.14) | 21(1.48) | 1.62 | 0.68 | 1.87 |
| | Myripoda | 1(0.7) | 11(0.78) | 0.85 | 0.34 | 0.98 |
| | Mollusca | 1(0.7) | 63(4.45) | 5.15 | 0.34 | 5.61 |
| | Arachnida | 2(0.14) | 33(4.33) | 4.47 | 0.68 | 2.94 |
| | Crustacea | 0(0) | 60(4.23) | 4.23 | 0 | 5.35 |

Other prey groups were Orthoptera (4.87%), Mollusca (5.15), Crustaceans (4.23%), Homoptera (3.52%), Hemiptera (2.54%), Annelida (1.62%), Dermaptera (1.55%), Isoptera (1.55%), millipede (0.85%) and Odonata (0.42%) respectively as shown in table 2.

### 4.1.1. Insect and non-insect diet composition of toad species.

During the field study two toad species *D. melanostictus* and *D. stomaticus* were recorded and captured for study. A total of 99 preys (98 insect and 1 non-insect) were extracted and identified from 16 individuals of *D. melanostictus*, which consist Hymenoptera(51%), Coleoptera (41%), Lepidoptera (6%) and Dermaptera and Annelida were 1% each (Fig.2). Insect items cover Hymenoptera (50%), Coleoptera (42%), Lepidoptera (6%) and Dermeptera (1%) as shown in figure 4. Non-insect items include 100% annelida.

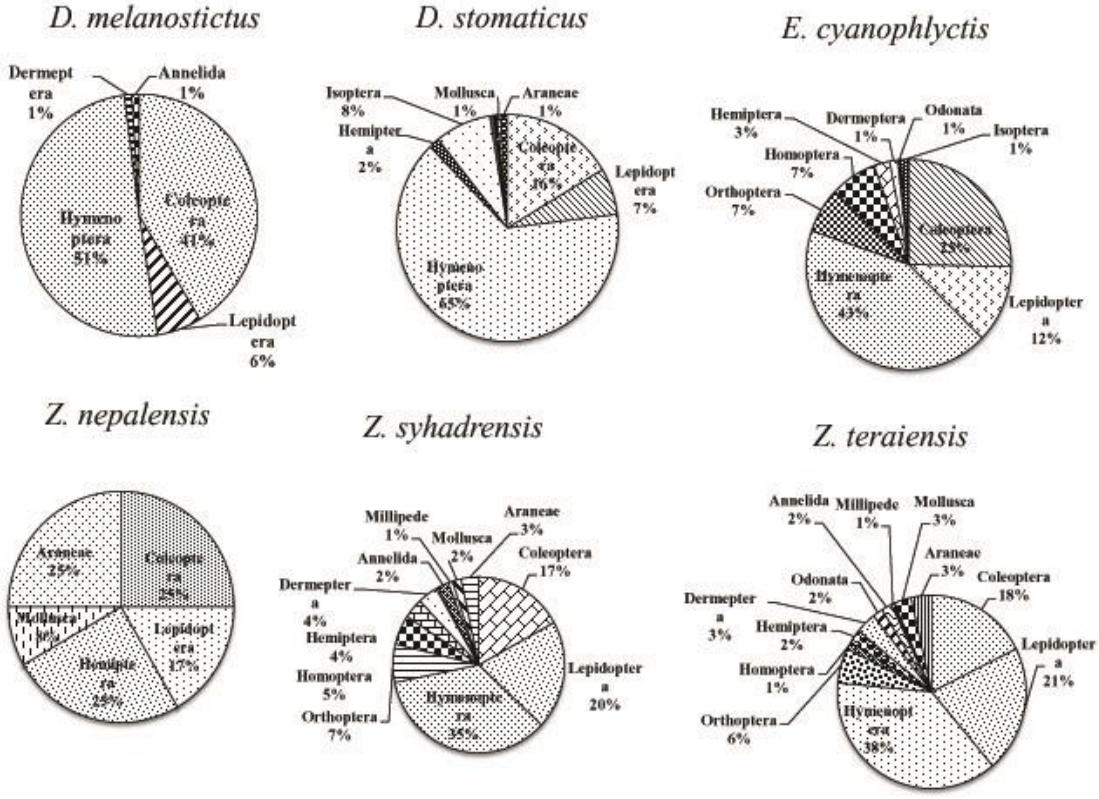


Figure 2: Total diet prey of individual Anuran species

A total of 195 (190 insect and 5 non-insect) preys were collected from stomach of 30 individual of *D. stomaticus*. Among them, Hymenoptera (65%) Coleoptera, (16%), Isoptera (8%), Lepidoptera (7%), Hemiptera(2%), Araneae (1%), Mollusca (1%) and remaining percentage by millipede and Annelid (Fig. 2). In the insect group, Hymenoptera (66%), Coleoptera (17%), Isoptera (8%), Lepidoptera (7%) and Hemiptera (2%) as shown in figure

4. In non-insect group, Araneae covers 40% and Annelida, Millipede and Mollusca 20% each (Fig.5).

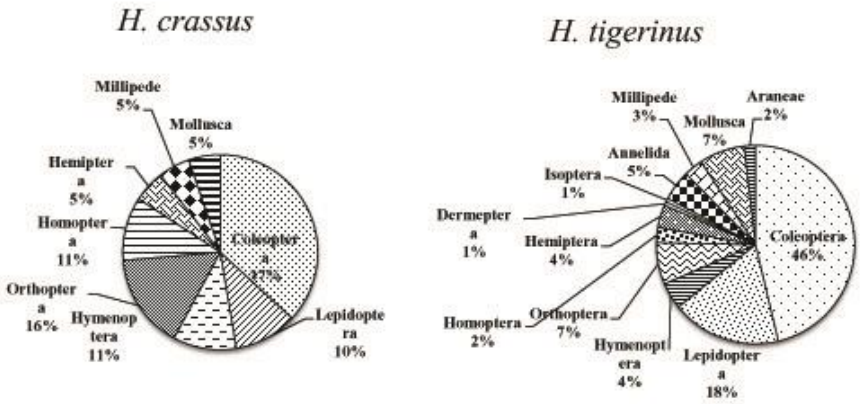


Figure 3: Total diet of *Hoplobatrachus* species

**4.1.2. Insect and non-insect diet composition of frog species.**

A total number of six species of frog namely *H. crassus, H. tigerinus, E. cyanophlyctis, Z. nepalensis, Z. syhadrensis and Z. teraiensis* were collected and flushed.A total of 19 preys (17 insect and 2 non-insects) were collected from six individual of *H. crassus*. The diet comprises Coleoptera (37%), Orthoptera (16%), Hymenoptera and Homoptera (11%) each, Lepidoptera (10%), Mollusca (5%), Hemiptera (5%) and millipede (5%) as shown in fig. 3. In insect category Coleoptera covers 41%, Orthoptera 17%, Hymenoptera, Homoptera and Lepidoptera 12% each and Hemiptera 6% (Fig. 4). Two non-insect groups Mollusca and millipede covers 50% each in its group as shown in fig. 4.

A total of 162 prey items (134 insect and 28 non- insect) were collected from 42 individual of *H. tigerinus*. The preys item includes 46% Coleoptera, 18% Lepidoptera, Orthoptera and Mollusca 7% each, Annelida 5%, Hymenoptera and Hemiptera 4% each, millipede 3%, Homoptera and Araneae 2% each, Isoptera and Dermaptera 1% each. In individual category of insect and non-insect, insects includes Coleoptera 56%, Lepidoptera 22%, Orthoptera 8%, Hymenoptera 5%, Hemiptera 4%, Homoptera 3% and Dermeptera,Isoptera 1% each. Non-insect includes Mollusca 43%, Annelida 28% , millipede 18% and Araneae 11% (Fig. 5).

A total of 519 prey items (399 insect and 120 non-insect) were collected from 173 individuals of *E. cyanophlyctis*, among them Hymenoptera comprises 33%, Coleoptera comprises 20%, Lepidoptera comprises 9%, Homoptera comprises 6% Orthoptera comprises 5%, Hemiptera 2%, Isoptera 1% and non-insect group covers 23% (Fig. 2). In non-insect category crustacean includes 50%, Mollusca 33%, Araneae 11%, Annelida 4% and millipede

2% (Fig. 5). In insects, Hymenoptera 43%, Coleoptera 25%, Lepidoptera 12%, orthoptera, Homoptera 7% each, Hemiptera 3% and Isoptera, Odonata, Dermaptera 1% each (Fig. 4).

A total of 12 prey items (8 insect and 4 non-insect) were collected form 8 individuals of *Z. nepalensis*. The diet profile comprises Coleoptera, Hemiptera and Araneae 25% each, Lepidoptera 17%, and Mollusca 8 (Fig. 2)%. Total insects diet covers Hemiptera 38%, Coleoptera 37% and Lepidoptera 25% (Fig. 4). Total non-insects diet consist Araneae 75% and Mollusca 25% (Fig. 5).

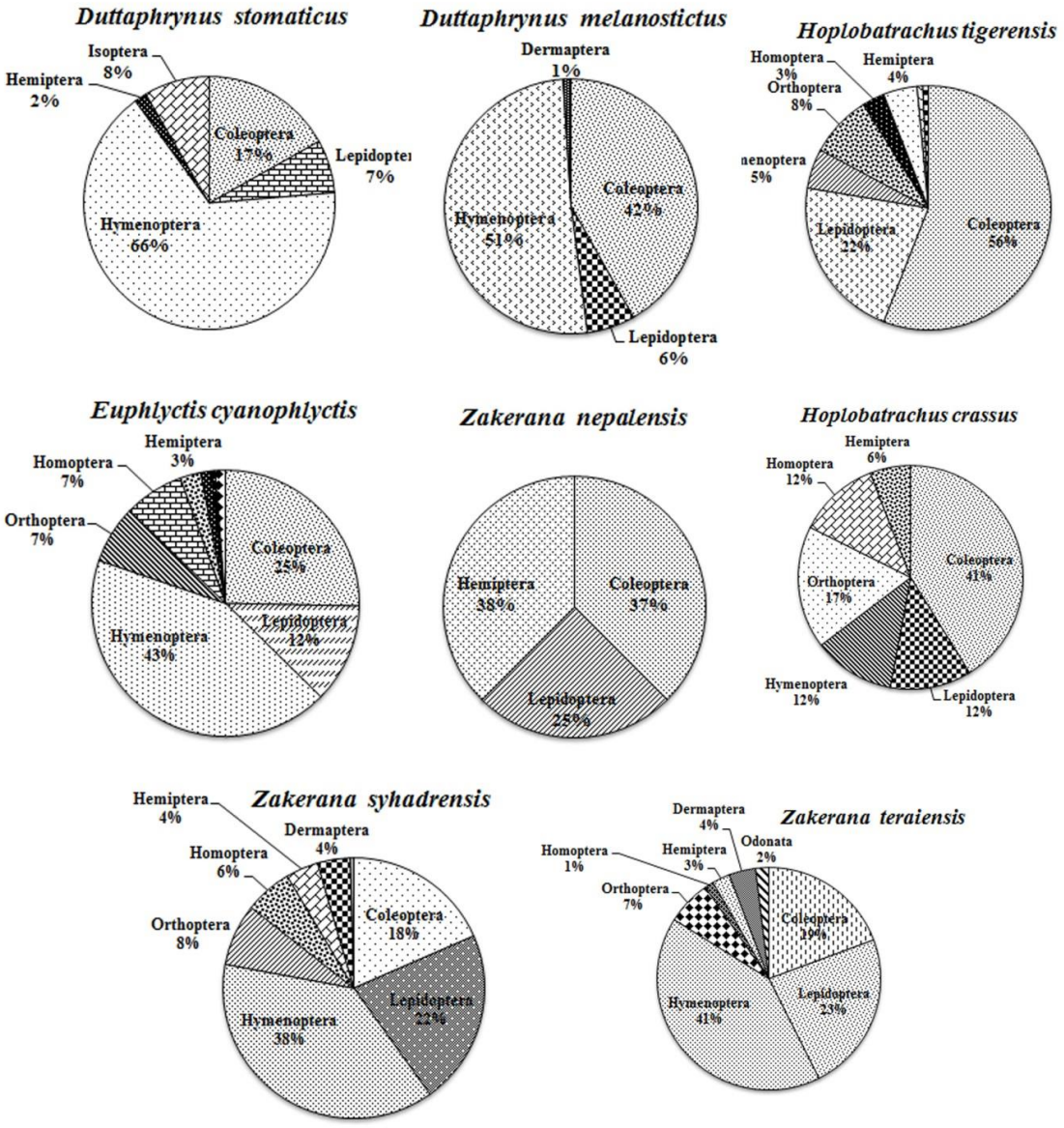


Figure 4: Insect prey items of Anuran species

A total of 241 prey items (222 insect and 19 non-insect) were collected from 104 *Z. syhadrensis*. The diet comprises 35% Hymenoptera, 20 % Lepidoptera, 17% Coleoptera, 7% Orthoptera, 5% Homoptera, 4% Hemiptera, 4% Dermaptera, 3% Araneae, 2% Annelida 2% Mollusca and 1% of millipede as shown in fig 2. Insect group comprises Hymenoptera 38%, Lepidoptera 22%, coleopteran 18%, Orthoptera 8%, Homoptera 6% and Hemiptera, Dermeptera 4% each (Fig. 4). Non-insect group comprises Crustacea (50%), Mollusca (33%), Aranea (11%), Annelida (4%) and millipede (2%) as shown in figure 5.

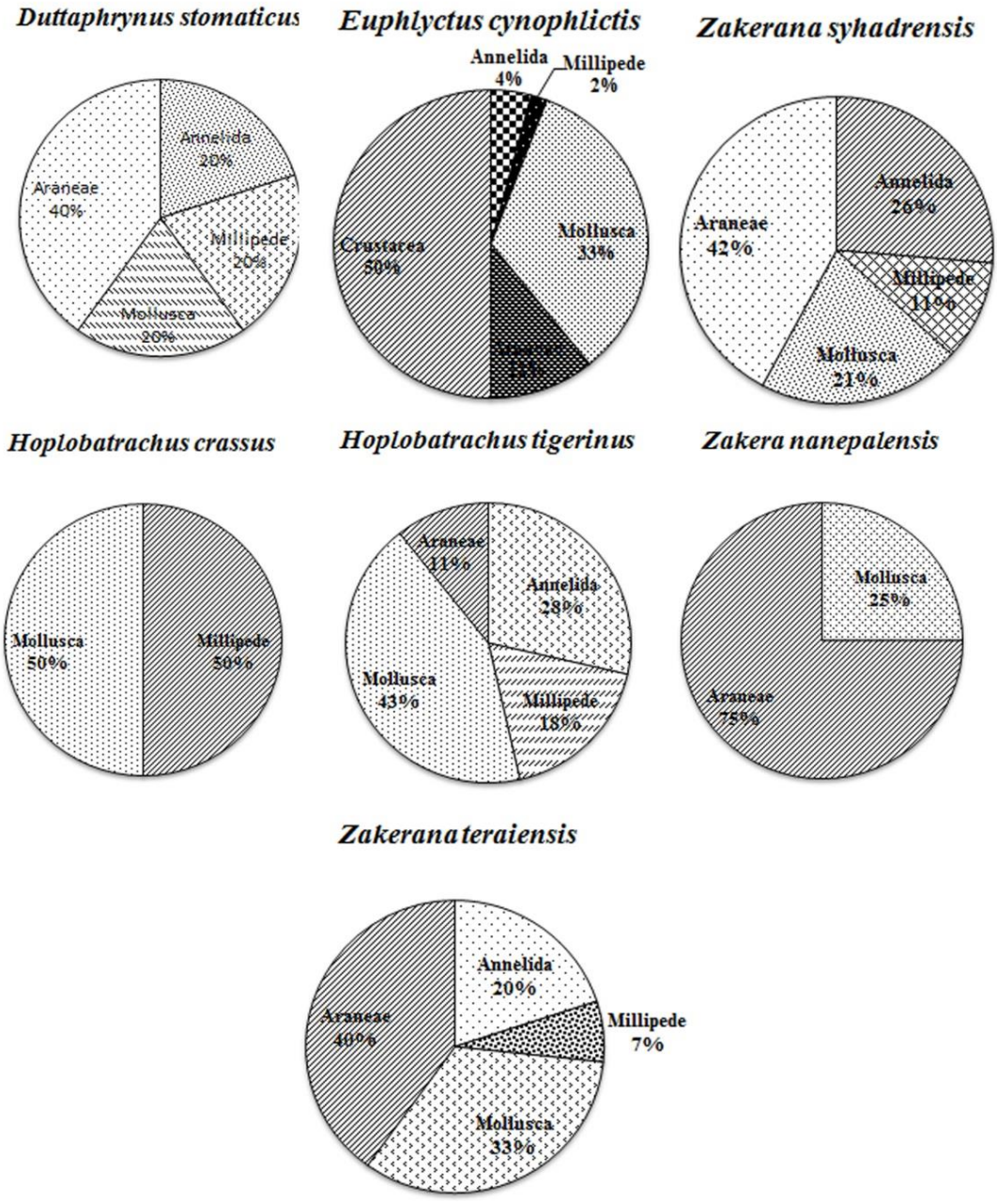


Figure 5: Non-insect diet items of Anuran species

A total 170 (155 insect and 15 non-insect) prey items were collected from 65 individuals of *Z. teraiensis.*. The prey items includes 38% Hymenoptera, 21% Lepidoptera, 18% Coleoptera, 6% Orthoptera, 3% Dermaptera, 3% Mollusca, 3% Araneae, 2% Hemiptera, 2% Odonata, 2% Annelida, 1 % Homoptera and 1 % millipede (Fig. 2). Among insect prey items, Hymenoptera covers 41%. Lepidoptera covers 23%, Coleoptera covers 19%, Orthoptera covers 7%, Dermeptera covers 4%, Hemiptera covers 3% and Homoptera covers 1% (Fig. 4). In non-insect group, Araneae (40%), Mollusca (33%), Annelida (20%) and millipede (7%) as shown in Fig. 5.

### 4.1.3. Analysis of insect and non-insect prey consumed by frogs.

Anurans in the rice field consumed significantly higher number of insect prey than non-insect prey ($t = 14.07$, $df = 892$, $p = < 0.001$) Fig 5. Insects are highly diverse organisms occupying aquatic as well as terrestrial habitats and are widespread groups than non-insect. Due to opportunistic feeding behavior of anurans and easy availability insects, insects were main components of diet.

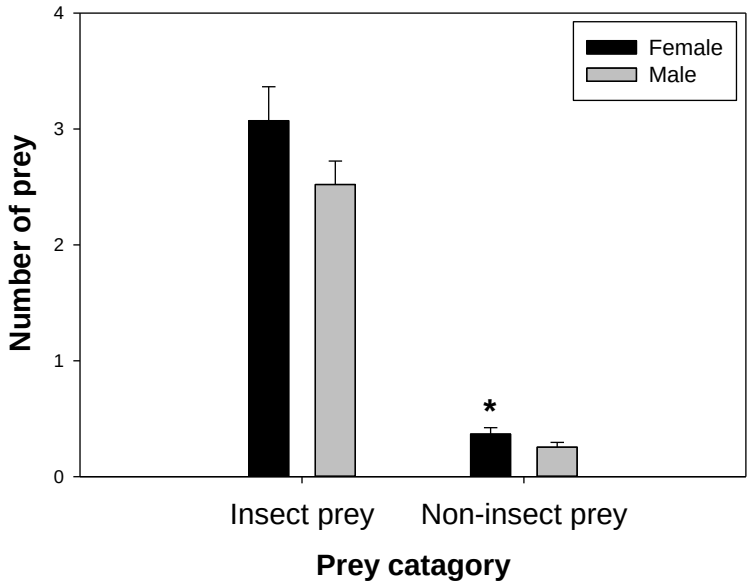


Figure 6: Comparison of insect and non-insect prey by male and female frogs.

### 4.2. Composition of prey items in male and female of frog and toad species.

Anurans have sexual dimorphism with larger females than male. The mean value of weight and SVL shows some difference between male and female species (Table 3). There was no significant difference between consumption of insect prey by male and female frogs.

However female consumed significantly higher number of non-insect prey compared to male (Man-Whitney U-test = 22013.50, p = 0.028).

The difference in the median values between the two groups was greater than would be expected by chance; there was a statistically significant difference between the weight of diet in male and female (P = 0.001).

### 4.3. Preference of prey as a diet by frog species.

A total of nine insect orders (Coleoptera, Hymenoptera, Orthoptera, Lepidoptera, Hemiptera, Homoptera, Dermaptera,Isoptera, Odonata, and four non-insect items (spiders, earthworms, snails and millipede) were recorded from diet of frogs (Table 4). Out of ten insects orders Coleoptera and Lepidoptera (many larval forms) were most preyed orders. In Isoptera termites were the diet of three different frog species like B. *stomaticus, E. cynophlictis* and *H. tigerinus.* Earwigs were the member of Dermaptera which were preyed by *Z. syhadrensis, Z. teraiensis, E. cyanophlyctis* and *H. tigerinus.* Order Hemiptera and Homoptera includes different bugs and leafhoppers respectively which were preyed by all *Zakerana* sp. *Hoplobatrachus* sp., *E. cyanophlyctis* and *D. stomaticus.*

Table 3: Comparision between male and female of anuran species

| Anuran Species | | Mean Wt. | Mean SVL | Mean prey Wt. | Mean No. of Prey |
|---|---|---|---|---|---|
| DM | Total | 25.53 | 56.87 | 0.23 | 5.7 |
| | Male/Female | 24.6(26.4) | 54.7(58.5) | 0.1(0.3) | 7(6.8) |
| DS | Total | 17.4 | 51.4 | 0.3 | 7.2 |
| | Male/Female | 17.2(17.5) | 51.6(51.3) | 0.3(0.3) | 5.8(8.05) |
| EC | Total | 6.6 | 39.2 | 0.1 | 2.9 |
| | Male/Female | 5.5(8.9) | 37.2(43.4) | 0.1(0.2) | 3.2(2.3) |
| HC | Total | 35.9 | 71.3 | 0.6 | 3 |
| | Male/Female | 33.3(38.6) | 66.6(76) | 0.01(1.1) | 1.3(4.7) |
| HT | Total | 63.03 | 79.2 | 0.5 | 3.8 |
| | Male/Female | 61.6(62.5) | 79.1(79.2) | 0.6(0.5) | 3.3(4.2) |
| ZN | Total | 6.03 | 37.4 | 0.1 | 1.6 |
| | Male/Female | 4.2(11.6) | 35.3(43.5) | 0.2(0.03) | 1.7(1.5) |
| ZS | Total | 9.7 | 44.7 | 0.2 | 2.4 |
| | Male/Female | 7.6(12.2) | 43.4(46.4) | 0.1(0.2) | 2.5(2.2) |
| ZT | Total | 5.7 | 37.8 | 0.1 | 2.7 |
| | Male/Female | 5.01(7.09) | 36.3(41) | 0.1(0.1) | 2.9(2.4) |

DS- *Duttaphrynus stomaticus,* DM- *Duttaphrynus melanostictus,* HT- *Hoplobatrachus tigerinus,* HC- *Hoplobatrachus crassus,* ZS = *Zakerana syhadrensis,* ZT- *Zakerana teraiensis,* ZN- *Zakerana nepalensis* and EC-*Euphlyctus cynophlicti*

Orthopterian insects like grasshoppers and molecrickets were on preference of *Zakerana* sp., *Hoplobatrachus* sp. and *E. cyanophlyctis.* Hymenoptera includes ants and different wasps out of it, mostly ants were preyed by *Duttaphrynus* sp. and other taxa were preyed by *Zakerana* sp., *Hoplobatrachus* sp. and *E. cyanophlyctis*. Order Diptera includes different flies and mosquitoes which were diets of *Zakerana* sp., *Duttaphrynus* sp., *Hoplobatrachus* sp. and *Euphlyctiis* sp. Some nymphs of dragon fly were also observed on diet of two frog species *E. cyanophlyctis* and *Z. syhadrensis.*

Other non-insect prey like earthworm (Annelida), spiders (Araneae), snails (Mollusca) and millipede were also reported. Earthworm was also frequently preyed by *D. stomaticus, Z. syhadrensis, Z. teraiensis E. cynophlictis* and *H. tigerinus.* Similarly snails by *Zakerana* sp., *Hoplobatrachus* sp., *Euphlyctis* sp. and *D. stomatics.* Spiders are also in the diet list of six different frog species *Zakerana* sp., *D. stomaticus, E. cynophlictis* and *H. tigerinus.* We have observed cannibalism in frogs, *H. tigerinus* preyed *Bufo sp.* (Annex 1).

Table 4: Percentage of individual prey items in toad and frog

| Prey items | Total | Percentage | Toad | Frog |
|---|---|---|---|---|
| Termites | 22 | 1.55 | 4.54(0.07) | 95.45(1.48) |
| Earwigs | 22 | 1.55 | 4.54(0.07) | 95.45(1.48) |
| Bugs | 36 | 2.45 | 2.77(0.07) | 97.22(2.47) |
| Homoptera | 50 | 3.52 | 0(0) | 100(3.52) |
| Grasshoppers | 69 | 4.86 | 0(0) | 100(4.86) |
| Hymenoptera | 504 | 35.56 | 34.92(12.42) | 65.07(23.14) |
| Insect Larvae | 184 | 12.98 | 10.32(1.34) | 89.67(11.64) |
| Dragonfly nymphs | 6 | 0.42 | 0(0) | 100(0.42) |
| Beetles | 330 | 23.28 | 8.48(1.97) | 91.51(21.31) |
| Earthworm | 23 | 1.62 | 8.69(0.14) | 91.30(1.48) |
| Spider | 35 | 2.47 | 5.71(0.14) | 94.28(2.32) |
| Milliped | 12 | 0.84 | 8.33(0.07) | 91.66(0.77) |
| Snails | 64 | 4.51 | 1.56(0.07) | 98.43(4.44) |
| Prawns | 60 | 4.23 | 0(0) | 100(4.23) |

(Value outside parenthesis indicates the percentage in individual prey items and value in paranthesis indicate the percentage in total prey items.)

### 4.4. Diversity Index of prey species

The Shannon and Weiner Diversity index of anuran by using above formula was calculated as H=0.849. The diversity index between frog and toad were 0.906 and 0.471 respectively. The diversity of species in diet of frog was found higher than toad.

Table 5: Diversity index of different anuran species

| Anuran Category | Name of Species | Diversity Index |
|---|---|---|
| **Toad (H'= 0.471)** | *D. melanostictus* | 0.42 |
| | *D. stomaticus* | 0.50 |
| **Frog (H'= 0.906)** | *E. cyanophlyctis* | 0.88 |
| | *H. crassus* | 0.79 |
| | *H. tigerensis* | 0.77 |
| | *Z. nepalensis* | 0.67 |
| | *Z. syhadrensis* | 0.83 |
| | *Z. teraiensis* | 0.79 |

The diversity index of *E. cyanophlyctis* was high as it feeds on most of all prey items in comparison with rest of all frog species. The *D. melanostictus* feeds on narrow range of insects showing lowest diversity index. The diversity of rest other species ranges from 0.50 to 0.88 as shown in table 5.

# 5. DISCUSSION

Our study of 444 anuran individuals (46 toads and 398 frogs) showed insects are the main diets covering 86.3 % of total diet. Non-insect covers 0.42% in toad and 13.26% in frog, which were mostly terrestrial. Toads were adapted for terrestrial habitat and its diet comprises mainly terrestrial species like ants, termites, ground beetles etc. We have reported 12.42% of ants, 1.97% ground beetles and 1.34% insect larva in the total biomass of stomach diet. Nocturnal foraging *Bufo* prey mainly on social insects like ants, termites and are habitat specific (Strüssmann et al., 1984).

We have observed fourteen groups of prey items from eight anuran species from rice field. Toad consumed ten different prey category and frog were found preyed upon fourteen prey items. Ants occupy more than half of its total diet. As Hymenoptera (Ants) were highly abundant in tropical and sub-tropical regions (Borror and DeLong, 1988) Dendrobatids and Bufonids prey upon hard-bodies, slow-moving Arthropods like ants and mites and Leptodactylids eat soft-bodied mobile Arthropods (Toft, 1980).

Rice field provided homogenous habitat for diversity of insect by providing suitable habitat. The prey abundance in irrigated rice fields causes low diversity (Piatti et al., 2010) of insects. Insect orders like Hymenoptera, Coleoptera and Lepidoptera constitute main component of diet. High abundance of these insects in terrestrial habitat adjacent to water body and opportunistic feeding behavior of anurans are the causes for dominance in diet of anurans. These insects and non- insects along their larval forms were also important constituents in earlier studies due to its habitat similarity (Pokhrel et al., 2011; Schaefer et al., 2006; Anderson et al., 1999).

Non-insect prey items like prawns, snails and spiders are important prey along with earthworm and millipede. Non-insect prey like crustaceans, spiders and earthworms constituted important supplement of most of anuran species. The dietary habit of *Rana macrocnemis* includes Arachnida, Diplopoda, Gastropoda, Chilopoda, Opiliones, Oligocheta and Amphibian group (Kuzmin and Tarkhnishvili, 1997; Meschersky, 1997; Uğurtaş et al., 2004).

All the anuran species were opportunistic predators having wide and generalist feeding behavior with mean number of preys 6.65 in toad and 2.83 in frog. Toads have narrow range of diet and feeds high number of prey items whereas frogs have wide range of prey category. Feeding behavior of toad on slow moving colonial organisms (Toft, 1980; Strüssmann et al., 1984) is due to its terrestrial habitat and behavior. Most of prey items were from terrestrial habitats than aquatic habitats. High number of terrestrial and presence of some aquatic forms

(Duellman and Trueb, 1994; Cogalniceanu et al., 2002; Sas et al. 2005, Cicek, 2011) shows their opportunistic feeding feeding behavior.

Female anurans are larger than males, with average body weight 11.17 of male and 18.93 of female. There were no any difference in the consumption of prey items in male and female. We observed high number of non-insect diet in females than male. The food composition of Ranids were associated with the surrounding prey items (Hirai and Matsui, 2000; Sas et al., 2005) but it doesn't totally dependent on surrounding prey in case of *R. macrocnemis* (Cicek, 2011; Cogalniceanu et al., 2000). Diet composition in male and female was similar as they inhabits in same place. There was no statistically significant difference between number of prey item in male and female's stomach (Cicek, 2011). The overlapping of food composition indicates that it doesn't vary by sex and age (Hirai and Matsui, 2000).

The toads and frog were opportunistic feeder and do not show any specialization on its prey item. The highly distributed insects like ants, beetles and non-insect like spiders are the preferred by most of studied frog species. I have reported some tadpoles on the diet of some frog species along with some fragments of plants. The presence of tadpole was a part of parental care or used as food is under discussion. I have reported cannibalism in *H. tigerinus*, preying on *Duttaphrynus sp.* The switching of insectivorous behavior to cannibalism reflects the high dry season densities of conspecifics and the scarcity of alternative prey due to dry weather (Pizzatto and Shine 2008). Besides invertebrates prey some anurans display cannibalistic behavior as intraspecific competition for food leads to cannibalism for survival (Polis, 1981; Meschersky, 1997; Kuzmin and Tarkhnishvili, 1997; Ugurtas et al., 2004) and the Giant African Bullfrog (*P. adspersus*) is carnivorous and cannibalistic in the juvenile stage (Du Preez, 2004).

The species diversity index in diet of frogs was slightly higher than toads. Frogs use terrestrial as well as aquatic habitats for feeding whereas toads are mostly terrestrial. Frogs consume diet from both aquatic and terrestrial habitats whereas toads were found to feed on terrestrial species only. B. melanostictus has broad, opportunistic diet (Berry and Bullock, 1962) but North American bufonids were highly selective (Flowers and Graves, 1995). It is nocturnal and preys exclusively on ground and litter-dwelling variety of invertebrates especially ants and termites (Berry and Bullock, 1962).Ant specialist such as dendrobatids was known to have toxic skin secretions that serve as a defensive role (Hirai and Matsui, 2000).

# 6. CONCLUSION AND RECOMMENDATIONS

## 6.1. Conclusion:

Frogs are secondary consumers in the rice field ecosystem. They were generalist feeders as they consume large number of prey species. There was no any selection of food between frog species but the toad (*Duttaphrynus* Sp.*)* consumes less diverse prey. Insects constitute main diet of frogs in comparison to non-insect prey. The size of prey was directly proportional to the size of frog species. As they do not have any special structure for capture of prey except protrusible tongue, the size of prey is also small in relation to its body size.

They consumed prey from 9 orders of insect and 5 categories of non-insect species. The insect prey item includes large portion of ants, beetles, grasshoppers, bugs, moth and larval forms of different insects. Frog also consumed some leafhoppers, termites, earwigs and nymph of dragonfly. The non-insect prey includes earthworm, snails, spiders, millipede etc. Some frogs were found feeding on other adult frog species. There was no any special food preference, the sluggish species like earthworm, different larval forms, snails were found in stomach content of most frog species.

## 6.2 Recommendations:

This study is only based on the diversity of insect prey of anuran. Anurans are mostly insectivorous organisms. So they may consume some pest species so the conservation of frogs may be beneficial for pest control. To conserve the frog species, application of chemical fertilizer and pesticides should be reduced. I recommend new researcher to focus on the pest and non-pest insect diet of different frog species.

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# ANNEXES

## ANNEX 1: PHOTO PLATE OF FIELD ACTIVITIES.

**Researcher during sample collection**

**Field study tools and equipments.** **Measurement of morphological characters.**

**Landform of study area**

**Cannibalism in frog**

## ANNEXE 2: DATA SHEET FOR RECORDING OF PREY SPECIES FOR FIELD STUDY

**Transect No.:** **Elevation:**

**GPS Location: ……..X…….**

| Date | Name of frog species | Sample ID | Sex | Weight | Weight stomach content | No. of prey species | Description of prey |
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## ANNEX 3: DATA SHEET FOR SORTING DIET DURING ANALYSIS OF FROG PREY SPECIES

| **Insect species** | **Sample ID** | **Frog species** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **E.C.** | **Z. T.** | **Z.S.** | **Z.N.** | **D.S.** | **D.M.** | **H.T** | **H.C** | **Remarks** |
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**DS-** ***Duttaphrynus stomaticus,*****DM-** ***Duttaphrynus melanostictus,*** **Ht-** ***Hoplobatrachus tigerinus,*****HC-** ***Hoplobatrachus crassus,*** **ZS-** ***Zakerana syhadrensis,*** **ZT-** ***Zakerana teraiensis,*** **ZN-** ***Zakerana nepalensis*** **and EC-***Euphlyctus cynophlictis*

## ANNEX 4: DESCRIPTION OF FROG SPECIES.

| Frog Species | IUCN Category | Geographic Distribution | Habitat and Ecology | Major Threats |
|---|---|---|---|---|
| ***D. melanostictus*** | Least Concern | - Found in Northern Pakistan, Nepal, Bangladesh, India, Sri Lanka, Thailand, Vietnam,Singapore and Indonesia. - Altitude range from sea level upto 1800 m asl. | - Lowland habitats, from river bank to human dominated agricultural and urban areas.<br><br>- Uncommon in close forests.<br><br>- Breeds in still and slow-flowing rivers and temporary or permanent ponds, pools.<br><br>Adults are terrestrial and may be found under ground cover (rocks, leaf litter, log) and human habitations.<br><br>- Larvae are found in still and slow-moving water bodies. | No major threats to this very adaptable species.<br><br>- It is sometimes found in the international pet trade.<br><br>- It is eaten locally in Thailand. |
| ***D. stomaticus*** | Least Concern | - Widely distributed in Pakistan, Nepal, India, and Bangladesh.<br><br>-Altitude range is from sea level to | - Found in wide variety of habitats including open plains, grasslands, forest, suitable agricultural land and human habitations.<br><br>- Breeding occurs in permanent and seasonal | - No major threats to this adaptable species.<br><br>-Localized threats includes loss of habitats, intensification |

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| | | 4500 m asl. | pools, seasonal streams and slow flowing streams. Adults hide under rocks and in crevices.<br><br>It is very adaptable species which is found in houses. | of agriculture, pollution of wetlands and land by agrochemicals, traffic mortality, long term drought etc. |
| ***H. crassus*** | Least Concern | - Bangladesh, India, Nepal and Sri Lanka<br><br>- Altitudinal range upto 600 m asl. | - Terrestrial species of seasonally flooded dry grasslands, open plain and arid areas<br><br>- Breeding and development takes place in many different types of water bodies<br><br>- Adults found in burrows during dry season, cultivated land and around human settlements in wet season | - Habitat loss through general development of infrastructure within the region<br><br>- Adult mortality through road accidents (In India).<br><br>- Over collection of adults<br><br>- Reclamation of wetlands in Sri Lanka. |
| ***H. tigerinus*** | Least Concern | - Throughout most wetlands of Nepal,<br><br>Bangladesh, India,Pakistan, Myanmar, | - Mainly aquatic, mostly freshwater wetlands, both natural and artificial (esp. paddy fields).<br><br>It is mostly solitary and | - Heavy collected for international frog leg trade.<br><br>- Loss of wetland habitats |

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| | | Afghanistan, Sri lanka.<br><br>- Principal low land species.<br><br>- Found at elevation between 25 - 800 m asl (2000 m asl in Nepal).<br><br>- introduced in Madagascar and Maldives. | nocturnal.<br><br>- Inhabiting holes and bushes near permanent water bodies.<br><br>- It produces large number of eggs but there is a high mortality rate among tadpoles, froglets and adults. | through infrastructure development, prolonged drought and<br><br>- Water pollution by pesticides, fertilizer and other agrochemicals |
| **E. cyanophlyctis** | Least Concern | -Most of South Asian countries like Afghanistan, Sri Lanka, Nepal, and Iran.<br><br>- It is present upto 2500 m asl. | - Aquatic species found in marshes, pools and various other water bodies.<br><br>It has been recorded from brackish water in Southern Sri Lanka.<br><br>- Adults are generally found basking at the edge of the water bodies and males call from within the water.<br><br>-Also found in modified habitats, usually where suitable wetland habitat is available. | - No major threats to this species as a whole.<br><br>The species is locally threatened by pollution of aquatic habitats by use of agrochemicals, prolonged drought and desiccation of wetlands. |
| ***Z. nepalensis*** | Least Concern | - Present in Nepal, Nagaland, | - Occurs in grassland, forest, agricultural land | - Loss of breeding habitat |

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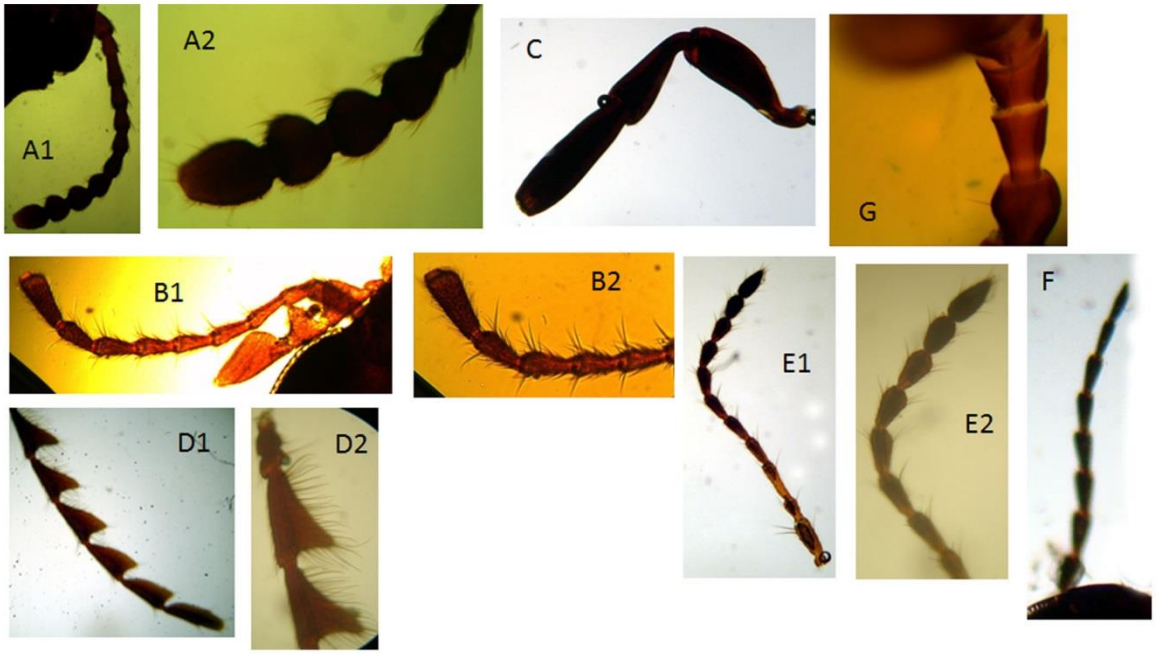
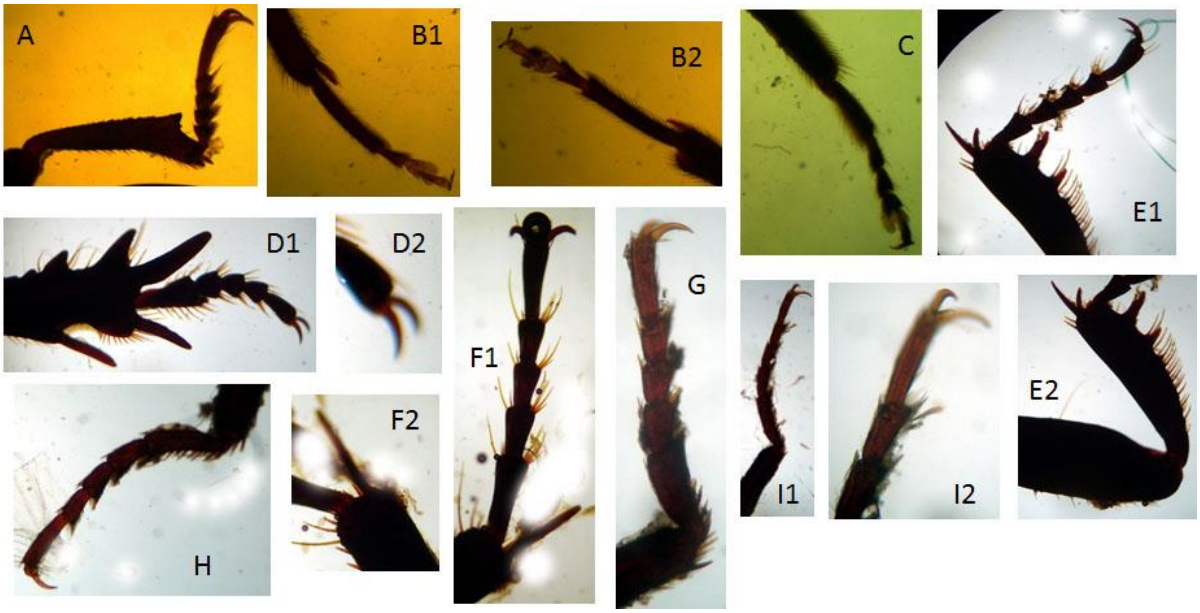
| | | Arunachal Pradesh of India, Southern and southeastern Bangladesh.<br><br>- It is generally found at elevation below 500 m asl. | (especially paddy field), and close to human settlements.<br><br>- Breeding takes place in temporary and permanent pools, marshes and paddy fields. | and feeding ground.<br><br>- Pollution and poisoning by agrochemicals.<br>- The over harvesting for subsistence use and medicinal use |
| ***Z. syhadrensis*** | Least Concern | - Widely distributed over much of central northern and western India, Southern Nepal, eastern Pakistan and Bangladesh.<br><br>- It is found at elevation below 2000 m asl. | - Associated with paddy field and similar habitats. - It breeds in paddy fields and other suitable wetlands.<br><br>- Non-breeding individuals may be found hiding in crevices and under vegetation or other ground cover. | The main threat to this species is the pollution of water bodies with agrochemicals. |
| ***Z. teraiensis*** | Least Concern | -Widely distributed in Nagaland (up to 2440 m asl), Arunachal India, Southern Nepal (Below 500 m asl) and southern to southeastern Bangladesh. | - Associated with open grassland close to permanent pools and streams.<br><br>- Breeding takes place in temporary and permanent pools and paddy fields. | Contamination of water bodies by agrochemicals.<br>- Harvesting for consumption and for religious purpose. |

## ANNEX 5: ANTENNA LEGS AND WINGS OF DIFFERENT PREY ITEMS


I. Antenna of: A-B: Formicidae(Hymenoptera); C: Siricoidea(Hymenoptera); D:Hebridae (Hemiptera); E: Dermaptera




II. Antenna of Coleoptera: A,B & G: Coleoptera; C: Carabidae; D: Anobiidae; E: Staphylinidae; F: Dytiscidae;

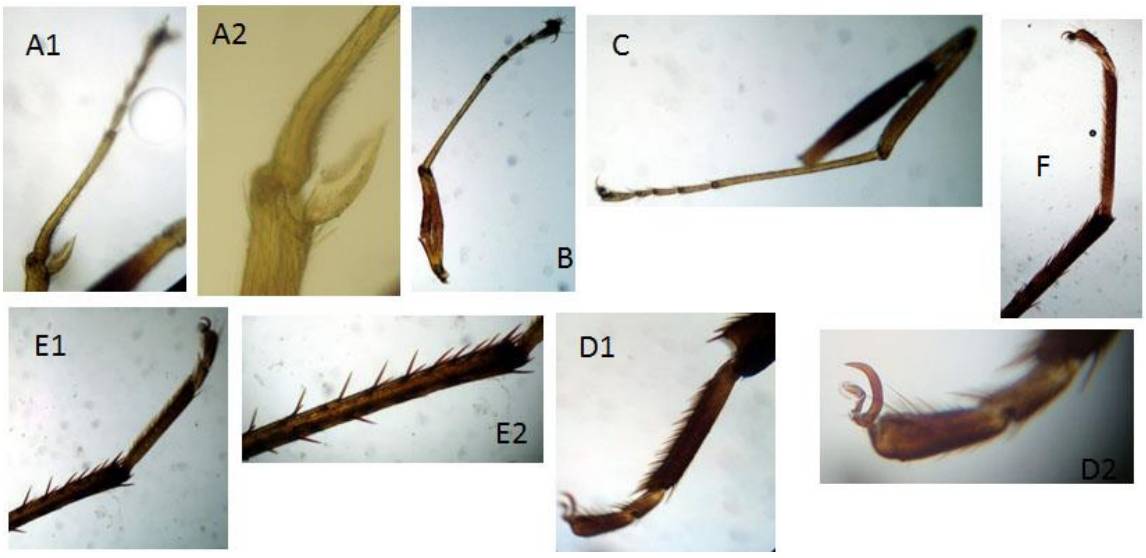



III. Legs of Coleoptera: A-C: Coleoptera; D-F: Carabidae; G-I: Elateridae


IV. Legs of Coleoptera: A-C: Anobiidae; D: Coleoptera; E-G: Dytiscidae

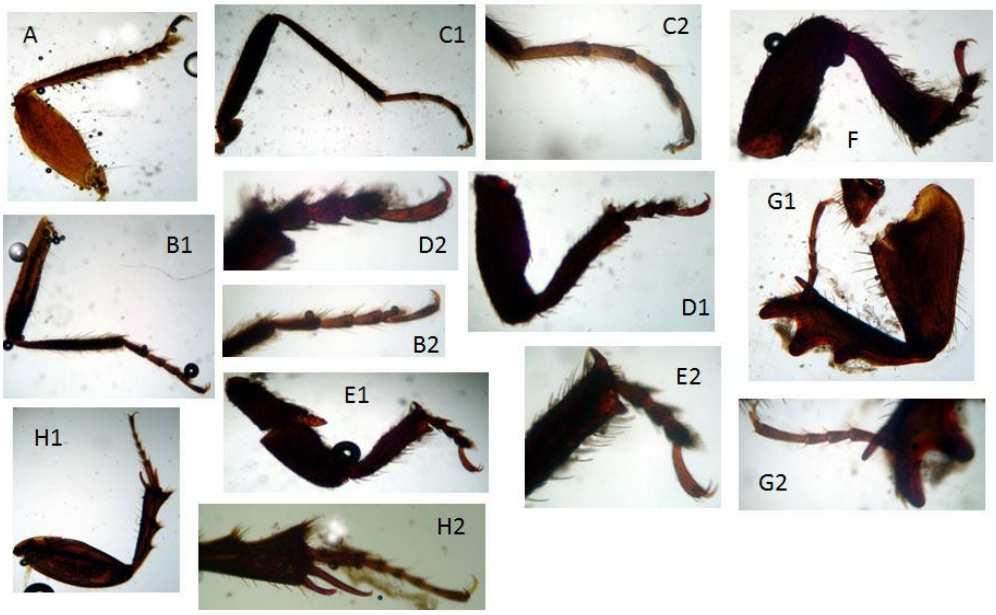

V. Legs of: A-C: Formicidae (Hymenoptera); D-F: Termitidae (Isoptera)


VI. Legs of: A-C& E-G: Formicidae (Hymenoptera); D:Unidentified; F: Termitidae (Isoptera)



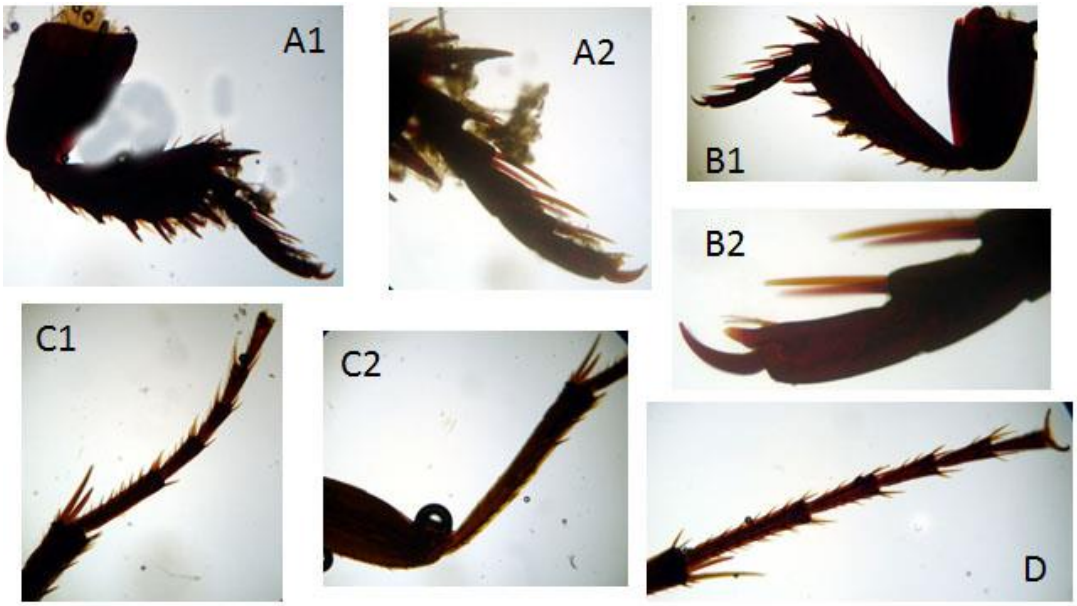

VII. Legs of: A-C: Pteromalidae (Hymenoptera); D-F: Siricoidea (Hymenoptera)


VIII. **Legs of:** A-C: Pteromalidae (Hymenoptera); D-F: Siricicoidea (Hymenoptera)

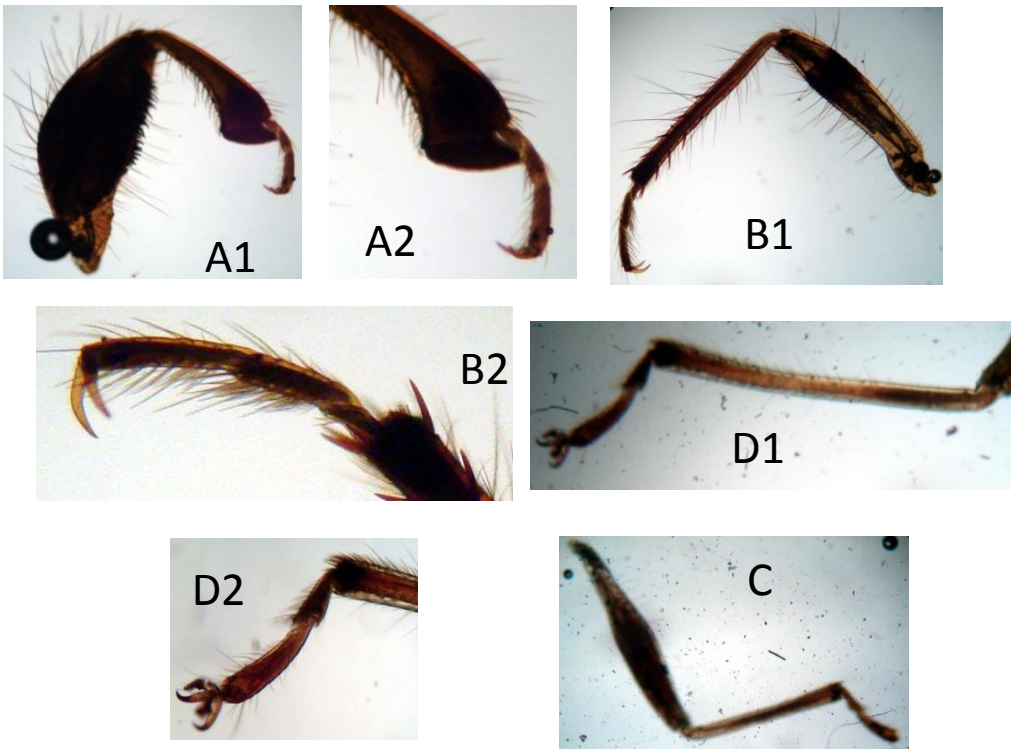



IX. Legs of: Staphylinidae (Coleoptera); D-H: Coleoptera.

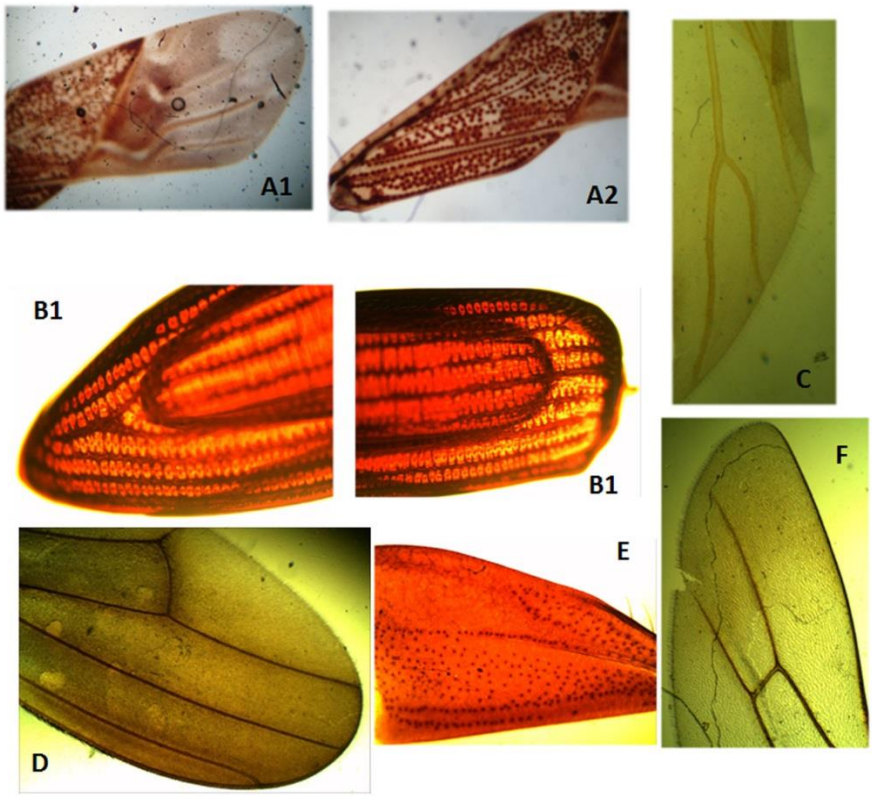

X. Leg of: A-C: Curculionidae (Coleoptera); D-F: Coleoptera



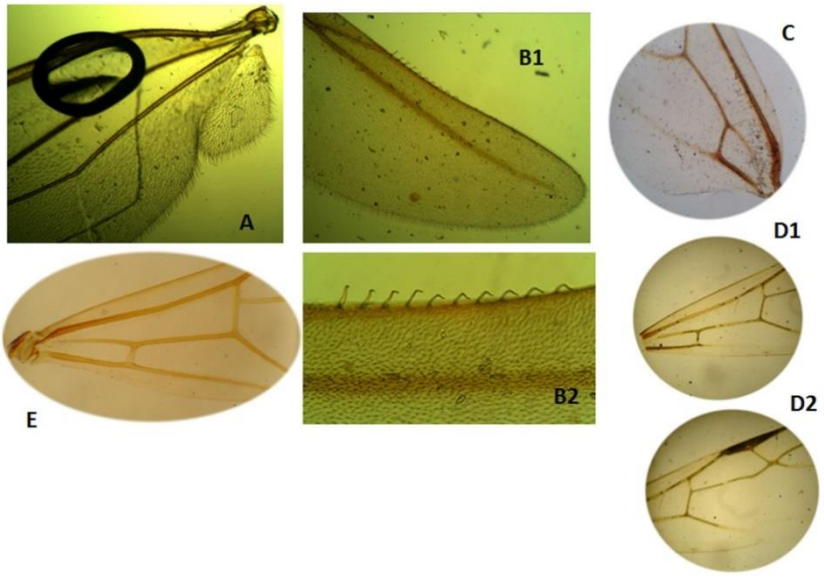

XI. Legs of Coleoptera



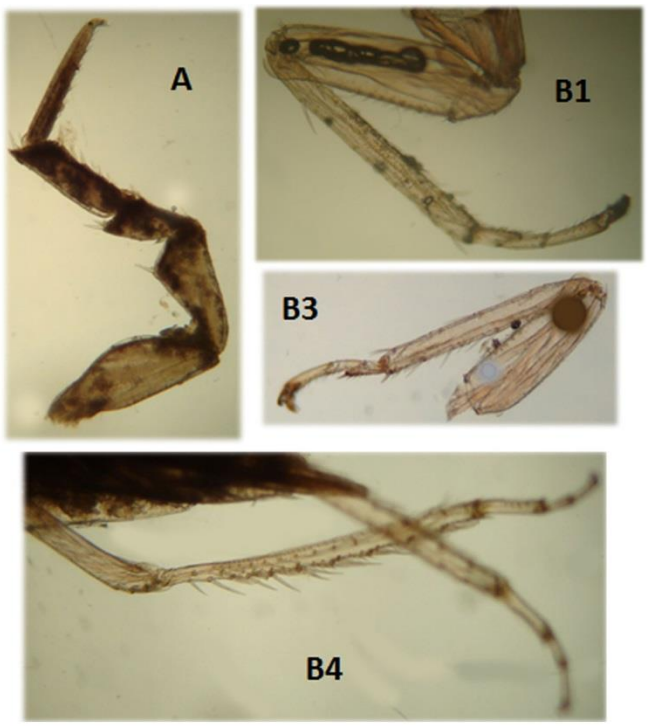

XII. Legs of**:** A-B: Naucoridae (Hemiptera); C-D: Dipsocoridae (Hemiptera)




XIII. Wings of A: Hemiptera  B- E: Coleoptera




XIV. Wings of Hymenoptera




XV. **Legs of** A: Unidentified; B: Leaf hopper.


XVI. **Legs of**: A: Dermaptera; B: Cricket; D: Moth

## ANNEX 6: POSTER PRESENTED IN INTERNATIONAL CONFERENCE ON BIODIVERSITY, CLIMATE CHANGE ASSESSMENT AND IMPACTS ON LIVELIHOOD, KATHMANDU

# DIET ANALYSIS OF ANURANS IN RICE FIELD CHITWAN, NEPAL

ICBCL 160

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## Introduction

Anurans play an important role as natural enemies of insect pests in the rice field. Rice is attacked by hundreds of insect species and some of them cause high economic loss. The study of amphibian diet is important for the conservation of amphibians and work as biological control agents for harmful pest species. In the present study, stomach food contents of Anurans (398 frogs and 46 toads) were surveyed along 250 m transects in the rice fields at Bacchauli, Chitwan in 2014. Stomachs contents of live frog and toad individuals were flushed, sorted and identified.

## Materials and Methods

- Frogs and toads were searched along 44 transects (250 m) by four people and stomachs of captured live individuals were flushed and released back to nature.
- Food items were sorted and identified based on structure of shells, animals, and antennae, legs and wings fragments (see figures below).

Study Area

Field tools and equipment

Flushing Technique

## Results

We recorded a total 1417 prey items from 14 groups of insects (n=1223) and non-insects (n=194) prey items covering 86.3% of Insects and 13.7% non-insect items. Frogs comprises 76.5% of insect food items while toad comprises 23.5%. Similarly among non-insect items frogs and toad consumed about 97 and 3 per cent respectively. Hymenoptera and Coleoptera were major proportion of the diet (see Table 1).

**Table 1. Prey item composition of Anurans**

| Prey items | | Prey items consumed by anurans | | |
|---|---|---|---|---|
| | | Toad | Frog | Total |
| Insects | Coleoptera | 73(6.0) | 257(21.0) | 27.0 |
| | Lepidoptera | 19(1.6) | 165(13.5) | 15.1 |
| | Hymenoptera | 176(14.4) | 328(26.8) | 41.2 |
| | Orthoptera | - | 69(5.6) | 5.6 |
| | Homoptera | - | 50(4.1) | 4.1 |
| | Hemiptera | 3(0.3) | 33(2.7) | 3.0 |
| | Dermaptera | 1(0.08) | 21(1.7) | 1.8 |
| | Odonata | - | 6(0.5) | 0.5 |
| | Isoptera | 16(1.3) | 6(0.5) | 1.8 |
| | **Total** | **288(23.5)** | **935(76.5)** | **100** |
| Non-insects | Annelida | 2(1.0) | 21(10.8) | 11.8 |
| | Myripoda | 1(0.5) | 11(5.7) | 6.1 |
| | Mollusca | 1(0.5) | 63(32.5) | 33.0 |
| | Arachnida | 2(1.0) | 33(17.0) | 18.0 |
| | Crustacea | - | 60(30.9) | 30.9 |
| | **Total** | **6(3.1)** | **188(96.9)** | **100** |


## Conclusions

Anurans are generalist feeders. They feed on insect and non-insect prey items. Hymenoptera and Coleoptera are the major food items in the diet of frogs and toad.

## Acknowledgements

We thank Janak Raj Khatiwada for guiding Subarna R. Ghimire during survey in the field and DNPWC provided research permission.