

DIET COMPOSITION AND OVERLAP OF SYMPATRIC AMPHIBIANS IN PADDY FIELDS OF NEPAL

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Abstract.—Many studies have documented dietary habits and resource competition among sympatric amphibians, but few have focused on anuran diets in paddy fields. We studied the dietary habits of five sympatric amphibian species in the lowland paddy fields of Nepal. We extracted 685 prey items belonging to 13 major prey categories. The most frequent prey belonged to Hymenoptera, Coleoptera, Lepidoptera, Diptera, Hemiptera, and Orthoptera. Among them, Hymenoptera and Coleoptera were the most abundant prey categories. We recorded the highest prey abundance from the Asian Common Toad (*Duttaphrynus melanostictus*). We compared dry and rainy season diets and found similar compositions with high dietary overlap among the focal species: *D. melanostictus*, Common Skittering Frog (*Euphlyctis cyanophlyctis*), Jerdon's Bullfrog (*Hoplobatrachus crassus*), Asian Bullfrog (*Hoplobatrachus tigerinus*), and Terai Cricket Frog (*Minervarya teraiensis*). The differences in prey consumption we observed were associated with variation in body size and feeding strategy (e.g., sit and wait vs. active search) and may facilitate co-existence. Our findings highlight the need for a detailed study on the ecology of rice paddy amphibians and their diets.

Key Words.—dietary niche; frogs; feeding ecology; feeding strategy; farmlands

INTRODUCTION

Trophic interactions are a central theme of community ecology in which species co-existence occurs along a spectrum of competing for resources, to resource partitioning and niche differentiation (Pianka 1974; Schoener 1974). Availability of food and the degree of dietary overlap help define the trophic niche and ecological function of a population within a community (Marques-Pinto et al. 2019). Diet preferences play a significant role in resource competition (Lawlor 1980) and predator-prey interactions (Richter-Boix et al. 2007), which in turn shapes the community structure (Duellman and Trueb 1994). Therefore, diets are key indicators of the structuring and functioning of an ecosystem (Duffy et al. 2007).

Amphibians are widely distributed on every continent except Antarctica (Duellman and Trueb 1994), and due to their generalist nature, consume a wide range of prey including arthropods, annelids, mollusks, and sometimes small vertebrates such as frogs and snakes (Freed 1982). Many amphibian species have a high degree of resistance to human disturbance and persist

in human-dominated landscapes (Schleich and Kastle 2002). For example, frogs are widespread in rice paddy fields (Bambaradeniya et al. 2004) and these semi-aquatic agro-ecosystems can harbor relatively high amphibian diversity, up to 13 species (Khatiwada et al. 2016). Lowland Nepal provides important habitat for amphibians (Khatiwada and Haugaasen 2015; Khatiwada et al. 2017, 2019) and is also a rice-growing region. Here, we examine key aspects of amphibian ecology in paddy fields of lowland Nepal (elevation range 145–210 m elevation).

Many studies have documented dietary habits, trophic resource availability, and resource competition between sympatric amphibians in a variety of settings (Hirai and Matsui 2002; Santos et al. 2004; Sabagh and Carvalho-e-Silva 2008; Quiroga et al. 2009; Piatti and Souza 2011). Frequently, such studies find high niche overlap among sympatric amphibian species (Toft 1980; da Rosa et al. 2002; França et al. 2004; Almeida-Gomes et al. 2007; Piatti and Souza 2011); however, amphibians could avoid niche overlap among co-occurring individuals on the basis of microhabitat use and foraging time (Lawlor 1980). In addition, prey

selection may be influenced by morphology of head and limbs (Toft 1980; Duellman and Trueb 1994) or ability to capture prey.

Some detailed studies of anuran diets have been conducted in rice paddy fields (Hirai and Matsui 2002; Yousaf et al. 2010; Piatti and Souza 2011), but few have been conducted in Nepal (Khatriwada et al. 2016). Information on the natural history and the biology of most amphibians of lowland Nepal is poorly known. Because prey consumption and diet composition of predators are essential for the formulation of conservation strategies, we aimed to improve the knowledge of prey eaten by exploring the dietary habits of amphibians inhabiting lowland paddy fields.

MATERIALS AND METHODS

Study area.—We conducted fieldwork in western Nepal covering three lowland districts (81°34'E, 28°6'N to 80°30'E, 28°39'N): Bardiya (152–1,457 m elevation), Kailali (109–1,950 m elevation), and Kanchanpur (160–1,528 m elevation; Fig. 1). The area has a subtropical climate. January is the coldest month (mean = 16.2 °C) and the warmest months are May to August (mean air temperature = 36.5 °C) as measured near Dhangadi airport (<http://www.mfd.gov.np>). The area receives the majority of monsoon rainfall June–September (total annual rainfall = 1,563 mm; <http://www.mfd.gov.np>). Rice is the major staple crop in the area and is planted once a year. Besides rice, farmers in the area cultivate wheat, maize, mustard, vegetables, and keep livestock (Central Bureau of Statistics 2011). The study

area is human-dominated, with settlement scattered in the croplands; population densities of Bardiya, Kailali, and Kanchanpur districts are 572.4/km², 240.0/km², and 329.0/km², respectively (Central Bureau of Statistics 2011).

Surveys.—We sampled amphibians between June and August 2018 using nocturnal time-constrained Visual Encounter Surveys (Campbell and Christman 1982). Four people systematically walked at a slow pace for 30 min along transects (100 × 4 m) using headlamps from 2000 to 2300 (for details see Khatriwada et al. 2019). We placed transect routes at an interval of 500 m. We surveyed three transects per site with a total of 24 transects in eight sites. To avoid recapture, we captured amphibians encountered in each transect and kept them in a 5-L cotton bags. We recorded the number of species and individuals encountered in each transect and we identified the species using field guides (Schleich and Kästle 2002; Shah and Tiwari 2004).

Study species.—We focused on five species (Fig. 2): the Asian Common Toad (*Duttaphrynus melanostictus*), also called the Asian Toad, Black-spectacled Toad, and Common Sunda Toad, the Common Skittering Frog (*Euphlyctis cyanophlyctis*), also called Skipping Frog due to its habit of skipping over the water surface (<https://amphibiaweb.org/species/4703>), Jerdon's Bullfrog (*Hoplobatrachus crassus*), Asian Bullfrog (*H. tigerinus*), also called Indian Bullfrog, Bull Frog, Golden Frog, Tiger Frog, Tiger Peters Frog (<https://amphibiaweb.org/species/4715>), and the Terai Cricket Frog (*Minervarya*

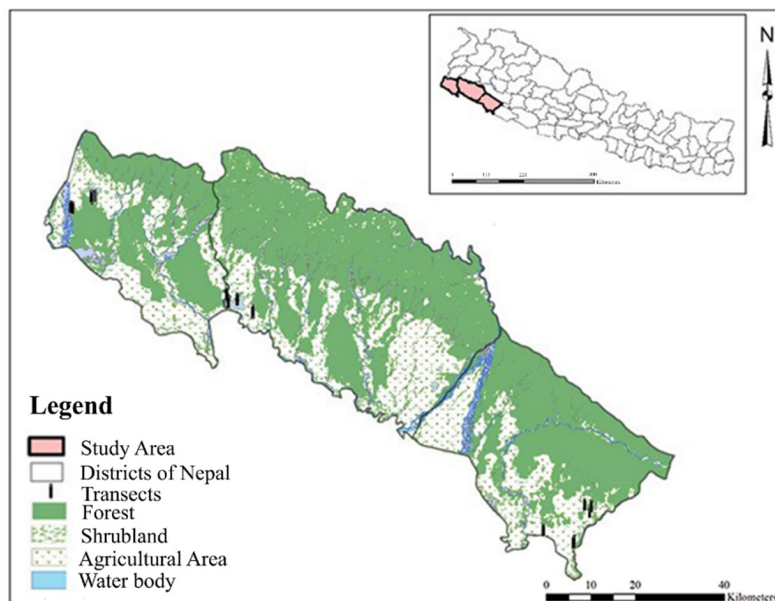


FIGURE 1. The study area showing sampling sites in three districts (Bardiya, Kailali, and Kanchanpur, from right to left) of western lowland Nepal.

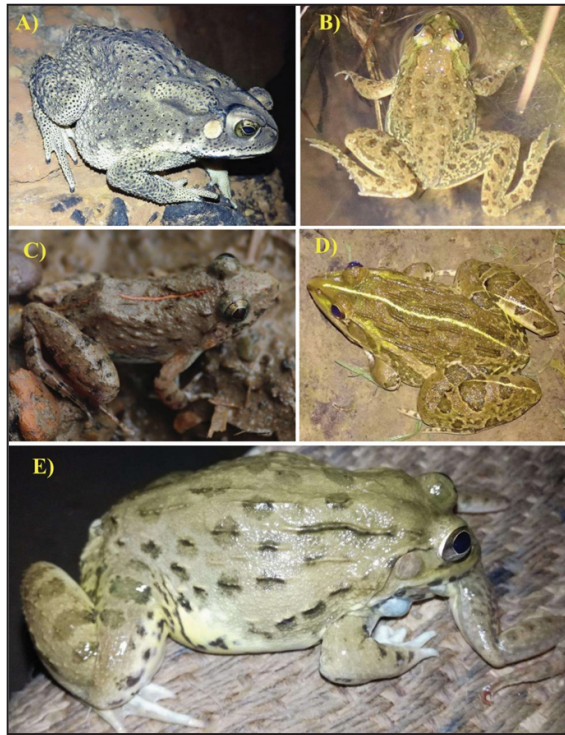


FIGURE 2. Rice paddy frogs of lowland Nepal studied for diet analysis. (A) Asian Common Toad (*Duttaphrynus melanostictus*), (B) Common Skittering Frog (*Euphlyctis cyanophlyctis*), (C) Terai Cricket Frog (*Minervarya teraiensis*), (D) Asian Bullfrog (*Hoplobatrachus tigerinus*), and (E) Jerdon's Bullfrog (*Hoplobatrachus crassus*) eating another frog. (Photographed by Suman Sapkota).

teraiensis). The range of *D. melanostictus* spans across Asia (van Dijk et al. 2004) up to 1,800 m elevation (van Dijk et al. 2004). It is commonly found in the human-dominated agricultural and urban areas of lowland and is uncommon in the forest. It breeds near slow-flowing rivers, ponds, lakes. The larval stage of the toad occurs in slow-flowing and stagnant water. The females are larger than the males with the snout-vent length (SVL) ranging from 50.8 mm to 90.6 mm for males and from 58.0 mm to 107.1 mm for females (Guo et al. 2019).

Euphlyctis cyanophlyctis is the most common and widely distributed species occurring in India, Afghanistan, Bangladesh, Bhutan, Iran, Malaysia, Myanmar, Nepal, Pakistan, Sri Lanka, below 1,800 m elevation (Khan 1997). This nocturnal frog is a voracious feeder on aquatic insects, beetles, dragonflies, grasshoppers, fish fry, tadpoles, etc. They normally breed after the first rain (Khatriwada et al. 2015). The SVL of males ranges from 30 to 50 mm and females from 35 to 56 mm (Ali et al. 2020).

Hoplobatrachus crassus occurs throughout south Asia in Nepal, India, Bangladesh, and Sri Lanka below 600 m elevation (<https://www.iucnredlist.org/>). It is very

common in agricultural fields and human settlements. The SVL of males ranges from 60–90 mm, whereas females range from 70–135 mm (<https://amphibiaweb.org/species/4711>). It breeds after the monsoon begins and breeding and larval development take place in slow-flowing water, ponds, lakes, and paddy fields (Yousaf et al. 2010). *Hoplobatrachus tigerinus* is distributed in most of the wetland areas of Nepal, Indian, Pakistan, and northern central Myanmar (Padhye et al. 2008) and occurs up to 2,000 m elevation (Khatriwada et al. 2015). This nocturnal frog forages terrestrially at water margins and does not stay submerged for prolonged periods (Padhye et al. 2008). The breeding activity is during the monsoon (Khatriwada et al. 2015). Body size (SVL) ranges from 42–116 mm for males and 51–123 mm for females (Kundu et al. 2020).

Minervarya teraiensis is widely distributed in Nepal, Bangladesh, and India, up to 2,440 m elevation (Bordoloi et al. 2016). The SVL of males is 33–51 mm and of females is 41–56 mm (Khatriwada et al. 2021). It is mostly aquatic and nocturnal in water-logged paddy fields, ponds, and slow-moving rivers (Khatriwada et al. 2021), and breeding occurs early March to June.

Diet sample collection and classification.—We captured amphibians during transect surveys in the paddy fields and carried them to a nearby dry place and measured SVL, to the nearest 0.01 mm using a digital caliper, and body mass to the nearest 0.01 g using a digital scale. We used individuals > 15 mm SVL, for diet extraction. We identified males based on secondary sexual characteristics such as the presence of black pigment on the throat (vocal sac) and nuptial pads, and gravid females based on enlargement of the coelomic cavity.

We used a non-lethal stomach flushing technique described by Solé et al. (2005). We used a 50 ml syringe with a 20 cm long surgical plastic tube (2 mm in diameter) attached to flush stomach contents. We used a plastic spatula to open mouths of amphibians, and carefully introduced the soft surgical plastic tube through the oesophagus and then into the stomach. Once the tube was inserted, we slowly squeezed 50 ml of potable water from the attached syringe into the stomach and collected any content flushed from the stomach (Fig. 3). We repeated the stomach-flushing procedure three times or continued until no contents remained in the stomach. Food items present in the oral cavity after flushing were carefully removed using entomological forceps. We preserved the stomach contents in 70% ethanol for identification and measurement in the laboratory. We released individuals at the location of capture approximately 30 min after flushing.

We used filter paper to remove moisture from the stomach contents and we weighed contents to the nearest 0.01 g. We spread prey items in a Petri dish and



FIGURE 3. (A) Process of stomach flushing of Asian Bullfrog (*Hoplobatrachus tigerinus*) and examples of stomach contents (B, C, and D) from rice paddy frogs of lowland Nepal. (Photographed by Janak Raj Khatriwada).

observed them under a stereoscopic microscope. We prepared reference slides of wings, antenna, and legs of prey items. We identified all the prey items to the lowest possible taxonomic level (usually Order or Family) in the Central Department of Zoology, Tribhuvan University, Kathmandu, Nepal.

Data analysis.—We tested the normality of the data using Shapiro–Wilk test and found that the total number of prey items consumed by the different species of amphibian were non-normally distributed ($W = 0.719$, $P < 0.001$). Hence, we used Kruskal–Wallis to test the difference in the total number of prey consumed by the amphibian species. We used `kruskal.test()` function at 0.05 significance level of α in R (R Core Team 2021). We also used Spearman’s Rank Correlation to relate total mass of prey consumed and frog SVL.

We used Non-metric Multidimensional Scaling (NMDS) ordination to compare the diet overlap between amphibian species using the Bray–Curtis Distance Metric with presence/absence transformations (Clarke and Gorley 2001). We also tested for differences in diet composition among species using Similarity Analysis (ANOSIM). ANOSIM is a nonparametric procedure similar to Analysis of Variance, where randomization techniques (number of permutations = 1,000) are used to calculate the dissimilarity among and within groups with the help of the Global-R statistic. The R-value ranges between -1 and +1; R close to 1 indicates that all samples within groups are more similar to each other than to any other samples from different groups (perfect separation). Values close to -1 indicate that differences

are greater among individuals of the same species than between different species. We performed NMDS and ANOSIM analysis using PRIMER version 5.0 (Clarke and Gorley 2001).

RESULTS

We flushed the stomachs of 188 individuals (110 males and 78 females), of which 19 had empty stomachs or completely digested food (Fig. 3). We excluded individuals with empty stomachs or completely digested foods from further analysis. Of the remaining 169 individuals (99 males and 70 females) of the five species, we found 685 prey items from 13 prey categories (mean per individual = 4.05 ± 3.52 standard deviation; range, 1–21; Table 1). We excluded algae and plant parts from dietary habit descriptions and comparisons, assuming that they were ingested incidentally. Overall, Hymenoptera (mostly ants) was the most abundant food group in the stomach contents, comprising 35.77% of the total number of prey items, followed by Coleoptera (32.42%) and Lepidopteran larva (6.13%; Tables 1 and 2).

The number of prey taxa differed significantly among groups ($H = 37.75$, $df = 13$, $P < 0.001$). *Duttaphrynus melanostictus* consumed the highest number of prey of all the species (Fig. 4). Overall, diet composition did not differ significantly among amphibian species (Global R = -0.013, $P = 0.702$), and there was a high degree of dietary overlap among amphibian species in the paddy fields (Fig. 5). There was a significantly positive correlation between SVL

TABLE 1. Relative abundance (%) of prey categories consumed by five amphibian species in the paddy fields of lowland western, Nepal: Asian Common Toad (*Duttaphrynus melanostictus*), Common Skittering Frog (*Euphlyctis cyanophlyctis*), Jerdon's Bullfrog (*Hoplobatrachus crassus*), Asian Bullfrog (*Hoplobatrachus tigerinus*), and Terai Cricket Frog (*Minervarya teraiensis*). The abbreviation n = total number of prey items found in each amphibian.

Prey categories	<i>D. melanostictus</i> n = 148	<i>E. cyanophlyctis</i> n = 88	<i>H. crassus</i> n = 247	<i>H. tigerinus</i> n = 180	<i>M. teraiensis</i> n = 22
Hymenoptera	45.95	36.36	37.25	24.44	40.91
Orthoptera	0.00	10.23	3.64	6.67	9.09
Odonata	0.68	1.14	2.02	1.11	4.55
Diptera	2.70	7.95	3.64	4.44	0.00
Coleoptera	42.57	27.27	25.51	36.11	31.82
Lepidopteran adults	0.00	1.14	1.21	0.00	0.00
Lepidopteran larvae	2.70	9.09	6.07	7.78	4.55
Spider	3.38	3.41	2.43	5.00	0.00
Snail	0.00	0.00	4.86	8.89	0.00
Crab	0.00	0.00	0.81	2.78	0.00
Blattodea	1.35	1.14	5.67	1.11	4.55
Earthworm	0.68	2.27	5.67	1.67	4.55
Frog	0.00	0.00	1.21	0.00	0.00

and the body mass of captured amphibians ($r_s = 0.177$, $P = 0.022$) and the largest species in the study was *H. tigerinus* (mean SVL = 81.30 ± 28.03), followed by *H. crassus* (59.68 ± 16.29). The average body sizes of *E. cyanophlyctis*, *M. teraiensis* and *D. melanostictus* was 37.74 ± 10.04 , 42.89 ± 8.07 , and 45.95 ± 13.03 , respectively. The total mass of prey consumed was

not correlated with the body size of amphibians ($P = 0.089$), although large-sized amphibians generally consumed more prey by weight (Fig. 6).

DISCUSSION

We studied the dietary habit of five sympatric amphibian species and found that Hymenoptera and Coleoptera were the preferred insect prey. Hymenoptera (mostly ants) was the dominant prey taxa in the diet of all amphibian species, except *H. tigerinus*. Eating ants may have important ecological and evolutionary implications in amphibians (e.g., use of ants as alkaloid precursors; Daly et al. 1994) and this dietary activity also can help to understand the prey capture strategy (e.g., active vs. sit and wait). Most frogs are generalist predators and consume a wide spectrum of food (Marques-Pinto et al. 2019; Piatti and Souza 2011). The large proportion of invertebrate fauna in the diet of many sympatric species of amphibians suggests that they can co-exist in a similar area (Quiroga et al. 2009; Piatti and Souza 2011). Similar to our study, Clarke (1974) reported that ants and beetles play an important role in the diet of amphibians.

Duttaphrynus melanostictus consumed higher absolute numbers of prey items compared to the other amphibians, and ants and beetles were their dominant food. Our finding is consistent with other studies of Bufonids showing that they are ant-specialist predators (Toft 1980; Flowers and Graves 1995; Hirai and Matsui 2001; da Rosa et al. 2002). Similarly, Hymenopterans were also the most common prey of Bufonids in our

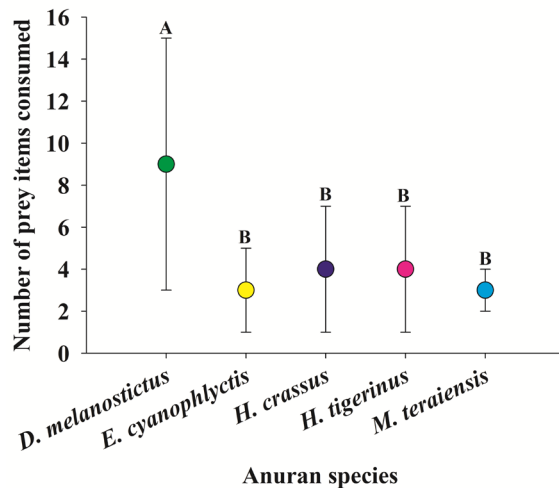


FIGURE 4. Average number of prey items consumed / individual (mean \pm standard error) by rice paddy frogs in lowland Nepal: Asian Common Toad (*Duttaphrynus melanostictus* n = 19), Common Skittering Frog (*Euphlyctis cyanophlyctis* n = 30), Jerdon's Bullfrog (*Hoplobatrachus crassus* n = 71), Asian Bullfrog (*Hoplobatrachus tigerinus*, n = 54) and Terai Cricket Frog (*Minervarya teraiensis* n = 14). Species that do not share the same letter are significantly different.

TABLE 2. Sex (M = male, F = female), weight (g), snout-vent length (SVL in mm), and dietary profiles of rice paddy amphibians from western lowland Nepal: Asian Common Toad (*Duttaphrynus melanostictus*), Common Skittering Frog (*Euphlyctis cyanophlyctis*), Jerdon's Bullfrog (*Hoplobatrachus crassus*), Asian Bullfrog (*Hoplobatrachus tigerinus*), and Terai Cricket Frog (*Minervarya teraiensis*). Values are mean \pm standard deviation, and n = number of individuals that were stomach-flushed.

Species	Sex	n	Weight (g)	SVL (mm)	Prey per stomach	Prey types	Dominant prey taxa
<i>D. melanostictus</i>	M	11	27.28 \pm 17.61	58.04 \pm 12.93	8.9 \pm 5.19	8	Hymenoptera
	F	8	34.01 \pm 18.78	63.45 \pm 11.71	8.43 \pm 5.96	3	
<i>E. cyanophlyctis</i>	M	19	6.45 \pm 4.08	36.78 \pm 11.01	2.98 \pm 1.90	10	Hymenoptera
	F	11	7.43 \pm 1.79	38.61 \pm 9.05	3.19 \pm 1.78	8	
<i>H. crassus</i>	M	38	30.65 \pm 18.79	60.26 \pm 16.39	4.63 \pm 4.30	13	Hymenoptera
	F	33	37.48 \pm 18.39	68.35 \pm 15.78	2.84 \pm 1.43	9	
<i>H. tigerinus</i>	M	32	74.88 \pm 53.69	79.24 \pm 30.43	4.24 \pm 2.30	11	Coleoptera
	F	22	82.08 \pm 60.70	84.82 \pm 25.33	2.79 \pm 1.40	11	
<i>M. teraiensis</i>	M	9	6.50 \pm 3.2	43.91 \pm 6.5	2.34 \pm 1.38	7	Hymenoptera
	F	5	7.67 \pm 3.41	51.98 \pm 8.5	2.68 \pm 1.25	3	

study and elsewhere (Bonansea and Vaira 2007; Santana and Juncá 2007; Sabagh and Carvalho-e-Silva 2008; Quiroga et al. 2009; de Carvalho Batista et al. 2011). Consumption of beetles and ants may reflect the greater availability of these insects in the paddy fields of this region. The consumption of a specific prey (specialization) can also be associated with the need to obtain a compound or a group of compounds essential for survival (defense, metabolism, etc.). This type of competition avoidance was noted by Clarke (1974). These species are also regarded as peri-anthropic species (living alongside humans), often found near human settlements, and tolerate some domestic pollution (Schleich and Kästle 2002). Interestingly, Orthoptera were not consumed by *D. melanostictus* in our study.

Euphlyctis cyanophlyctis is regarded as a perianthropic anuran found around small ditches, ponds, and puddles, and the feeding habits showed great similarity with Bufonids. *Hoplobatrachus tigerinus* is mostly aquatic and found in the paddy fields with perennial water and their diet contained a higher percentage of Coleoptera followed by Hymenoptera. The high consumption of these two orders of prey may be explained by the preference of these insects for wet environments with plenty of vegetative covers, which provide feeding and hiding grounds for the insects.

We did not find that amphibian body size (SVL) was correlated with the mass of prey consumed. Volume and mass of prey are often taken as suitable parameters of prey size analysis and few species show a positive

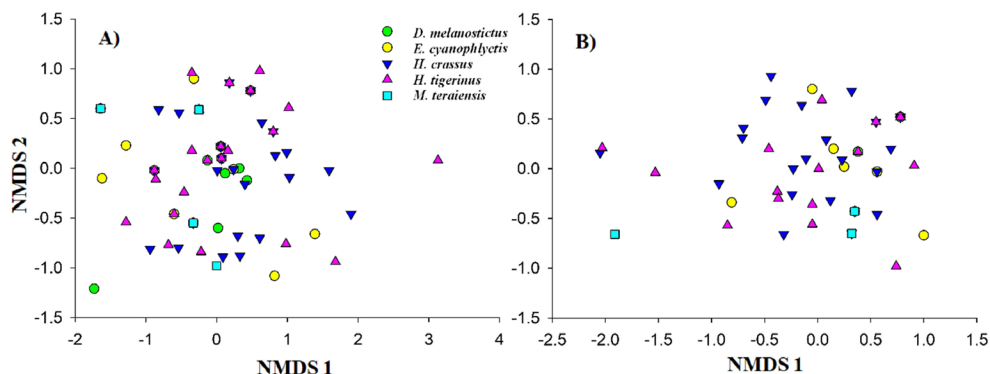


FIGURE 5. Multidimensional scaling (NMDS) ordination of diet overlap for five species of rice paddy frogs in western Nepal: Asian Common Toad (*Duttaphrynus melanostictus*), Common Skittering Frog (*Euphlyctis cyanophlyctis*), Jerdon's Bullfrog (*Hoplobatrachus crassus*), Asian Bullfrog (*Hoplobatrachus tigerinus*) and Terai Cricket Frog (*Minervarya teraiensis*). (A) Males and (B) Females. Closer symbols indicate greater similarity in diet than symbols that are further apart. Stress configuration = 0.11. Each symbol represents one individual.

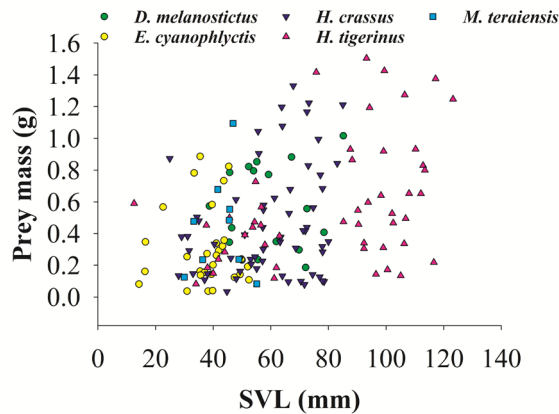


FIGURE 6. The relationship of body weight of studied amphibians with total weight of prey consumed for Asian Common Toad (*Duttaphrynus melanostictus*), Common Skittering Frog (*Euphlyctis cyanophlyctis*), Jerdon's Bullfrog (*Hoplobatrachus crassus*), Asian Bullfrog (*Hoplobatrachus tigerinus*) and Terai Cricket Frog (*Minervarya teraiensis*) combined.

correlation between body size and mass of prey items (Hirai and Matsui 2001). In a few cases, cannibalism was observed in *H. crassus*. They consumed frog species up to about half of their body size. Generally, large amphibians consumed large-sized prey items like crab, other frogs (anurophagy), and odonates (Table 2). Amphibians might consume a smaller number of large prey or a very high number of small prey items due to their generalized feeding habit (Vignoli and Luiselli 2012). Large sized amphibians such as *H. tigerinus* and *H. crassus* consumed large-sized prey, for example crabs (Decapods) and small amphibians. These frogs were mostly nocturnal and aquatic and showed a similar dietary pattern with other sympatric species in our study. Adult moths and their caterpillars were the most frequently consumed prey of these two species of frogs.

We found that amphibian species consumed a wide spectrum of invertebrate and vertebrate prey in the paddy fields. As expected, our results revealed a high degree of dietary overlap among the five amphibian species found in the study area despite the fact that only the two bullfrog species consumed snails, crabs, and other frogs. Given these findings, there could exist strong interspecific competition if food resources were limiting, and adverse effects of one amphibian species on another in terms of prey depletion could occur. Interspecific competition is likely to be more intense in closely related species (such as the congeneric bullfrogs, *H. tigerinus* and *H. crassus*), and morphologically similar species (*E. cyanophlyctis* and *M. teraiensis*) and can lead to either resource partitioning or dietary overlap (Toft 1980; Griffiths and Jaeger 1998). Similarly, the feeding physiology and behavior of species also likely contribute to determining the degree of partitioning relative to dietary overlap. Our study suggests that

amphibians with similar dietary requirements exhibited greater levels of dietary segregation. For instance, we recorded *H. tigerinus* and *H. crassus* most often from paddy fields that were usually filled with water, and they consumed 13 and 11 categories of prey, respectively. We also recorded the highest prey diversity in the diet of these two species, but because we also had the most captures of these two species, the greater diversity of prey could reflect a sampling bias to encompass more prey categories. In conclusion, despite the great dietary overlap that suggests the possibility of competition, amphibians in the paddy fields co-existed, with all five study species present at all sites. Co-existence is likely due to a combination of abundant food resources and some differences in foraging modes.

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