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Communication

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Abstract: The ecological diversity of insects and its predators like amphibians are important determinants in ecological balance. A total of 1,222 prey items in 84 specimens were examined to contribute the understanding of the diets of three *Duttaphrynus* species, viz., *himalayanus*, *melanostictus*, and *stomaticus* from Uttarakhand, the western Himalaya, India. Gut content analysis of three bufonids revealed acceptance of a wide range of terrestrial insects and other invertebrates as their food. The index of relative importance indicated that the most important preys were Formicidae, Coleoptera and Orthoptera. *Duttaphrynus melanostictus* had the broadest dietary niche breadth, followed by *D. himalaynus* and *D. stomaticus*. The wide prey spectrum well indicates that these species are the generalist and opportunist invertebrate feeder. Information pertaining to the food spectrum analysis contributes to understanding the ecological roles and used as a baseline data for future successful amphibian conservation and management programs in the Himalayan ecosystem.

Keywords: Bufonid, importance of relative index, Levin's measure, stomach flushing, western Himalaya.

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UTTARANCHAL UNIVERSITY

INTRODUCTION

The family Bufonidae (Gray 1830) is one of the most species-rich families of anurans belonging to the class Amphibia. It is a large and geographically widespread taxon of neobatrachian frogs (Reig 1958; Lynch 1973; Duellman & Trueb 1986). It comprises more than 550 species in ca. 50 recognized genera geographically ubiquitous, only two of the remaining 32 genera have more than 10 species and all have relatively restricted geographic ranges (Frost 1985, 2011). Bufonidae comprises the true toad: they are best known for their thick, warty skin appearances and have prominent skin glands especially a pair of parotoid glands on the back of their heads. In the context of Uttarakhand, western Himalayan anuran fauna comprises three species of the family Bufonidae, namely, *Duttaphyrnus himalayanus* (Günther, 1864), *D. melanostictus* (Schneider, 1799), and *D. stomaticus* (Lutken, 1864).

Food is an important item for any living organism. The body requires the range of nutrition in organism's diet to keep all organs alive and in the correct balance. Diet is a also crucial part of the natural history of an animal, because not only does it reveal the source of the animal's energy for growth, reproduction and survival (Zug et al. 2001; Norval et al. 2014), but it also indicates part of the ecological roles such as food webs, resource portioning and ecological energetic. Anurans are thought to be opportunistic predators with their diets just reflecting the availability of food of appropriate size. Different studies suggest that food is a vital factor that explains the structure of anuran communities in different parts of the world (Duellman 1967; Inger & Colwell 1977; Duellman & Toft 1979; Toft 1980; Clcek & Mermer 2007). The stomach contents of many Bufonidae species have been examined in the past to determine their role in an ecosystem (Yu & Guo 2012; Sulieman et al. 2016).

Although the Uttarakhand region of the western Himalayan ecosystem embraces all types of amphibians on account of its varied climate, topographical, altitudinal and vegetational conditions, information about diets of amphibians is very scarce and the biology of most amphibians is poorly known from this region (Ray 1995; Bahuguna & Bhutia 2010). Therefore, the present work on a food spectrum analysis of three toad species fills the lacuna that would be helpful in understanding their feeding habitat and ecological role in Uttarakhand, the western Himalaya. Our analysis was aimed at (1) identifying and determining small invertebrate prey, (2) examining importance of the relative index of three toad species, (3) comparing the food spectrum and niche breadth among three toad species from its natural range.

MATERIALS AND METHODS

For the present study fieldwork was carried out in several localities, viz., Dayara (S1) (2,800m), Triyuginarayan (S2) (2,300m), Badhani tal (S3) (2,089m), Joshimath (S4) (2,240m), and Sem Mukhem (S5) (2,200m) (Fig. 1). Samples were studied in breeding seasons, i.e.,

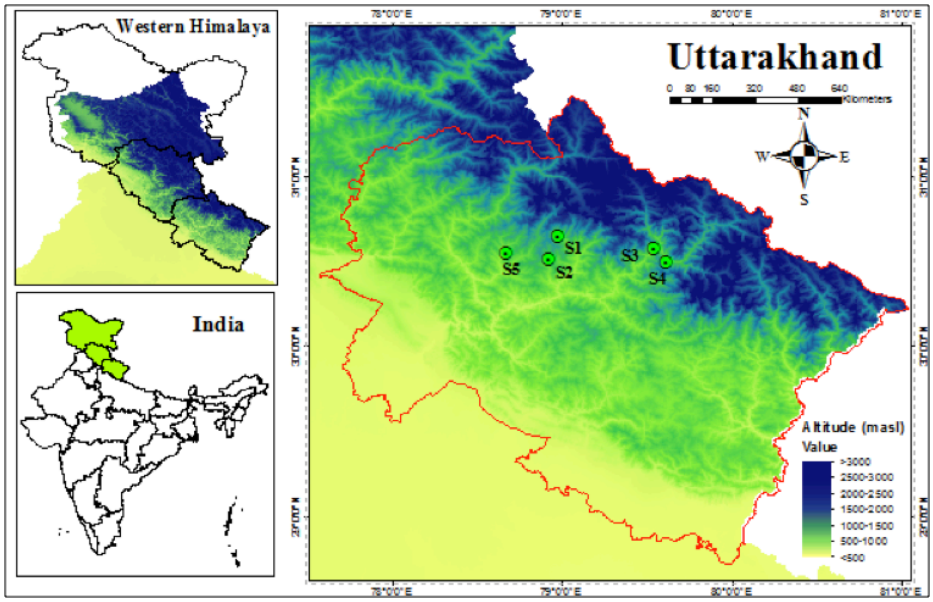

Figure 1. Location map of study area.

March–September from 2014–2017 at evening hours (18.00–23.00 h) in their natural habitats such as pools, ponds and in the vicinity of shaded mountain streams and so on. It was based on nocturnal visual encounter survey (Heyer et al. 2014).

Toads were collected manually in their habitats and stomach flushing was carried out immediately. Flushing was applied as soon as possible after capturing anurans, in order to precede digestion (Secor & Faulkner 2002; Sole et al. 2005). The subsequent immediate release of all specimens into their habitats ensured that the current activity of the treated specimens was not essentially disturbed by the stomach-flushing. The stomach contents were picked up with forceps and fixed in 70% ethanol in a vial. All contents were analyzed under a stereomicroscope (Olympus SZX 7). Identifications of food items were possible up to the order level with the exception of Hymenoptera, which was classified as Formicidae and non Formicidae and the rest of the items have been categorized as 'miscellaneous' (for broken materials) or unidentified (Gibb & Oseto 2006; Chowdhary et al. 2016). The food contents were then identified with the aid of keys provided by Ward & Whipple (1959). The food preferences of the three toad species were analyzed in terms of number, volume and frequency of occurrence. Prey's length and width were evaluated with a digital vernier caliper (Aerospace) to the nearest 0.1mm accuracy. Preserved items were measured and their volume (in mm^3) was calculated using the formula for ellipsoid bodies (Griffiths & Mylotte 1987).

$$V = \frac{4}{3}\pi\left(\frac{L}{2}\right)\left(\frac{W}{2}\right)$$

where, L=prey length, W=prey width

We obtained the frequency of occurrence of each prey categories in the diet dividing the number of stomachs which contained that category by the total number of stomach analyzed, with the exception of empty ones.

The index of relative importance (IRI) was employed as a measure that reduces bias in the description data of animal dietary items (Pinkas et al. 1971).

$$IRI = (N\% + V\%)\, F\%$$

Where N%=numeric percentage, V%=volumetric percentage, F=frequency of percentage

In order to compare the habitat trophic niche breadth the standardized Shannon-Weaver entropy index J' was used (Shannon & Weaver 1949).

$$J' = H'/\ln(n)$$

whereby,

$$H' = -\Sigma pi \ln(pi)$$

pi is the relative abundance of each prey categories, calculated as the proportion of prey items of given categories to the total number of prey items (n) in all compared species. To make H' index number more biological sense, it was converted into the effective number of species (ENS), which is the real biodiversity and allows to compare the biodiversity with the other community containing equally-common species of exp(H'), the ENS.

The niche breadth was obtained by Levins' standardized index (Krebs 1999), in which the value of Levins' measure (B) was first obtained by the following equation

$$B = 1/\Sigma pi^2$$

where, pi =fraction of item i in the diet

Levins' measure was then standardized on a scale of 0-1.0 by the following equation:

$$B_A = (B-1)/(n-1)$$

where, B_A corresponding to Levins' standardized niche breadth ranges from 0 (narrowest amplitude), when there is exclusive use of a single resource categories, to 1 (broadest amplitude), when all categories are equally used (Krebs 1999); the species is considered to have a wide niche breadth when $B_A \geq 0.5$.

RESULTS

The anurans used in this study, consisted of 84 specimens of three toad species. We recorded 1,222 prey items from 27 invertebrate categories (Table 1). Because toad samples were stomach-flushed within three hours after capture, few of the food materials were totally intact, most were partially digested. Parts with heavily sclerotised cuticle remained undigested so that heads, thorax, abdominal segments and single wings of arthropods allowed an identification of the item, at least to order level. Identified diet items belonging to the order Hymenoptera were categorized into Formicidae and non Formicidae. Mostly male *Bufo* specimens seem to stop feeding during courtship so some of them had an empty stomach (Table 1).

The most numerous prey taxon on the basis of number percent in the diet was Formicidae in all three toad species. The predominant food in terms of volume was Orthoptera in *D. himalayanus* and *D. melanostictus* while it was Lepidoptera in *D. stomaticus*. The index of relative importance (IRI) was maximum for Formicidae in the three toad species (Table 2; Fig. 2). Based on

Table 1. Prey details for all three bufonid species in studied sites of Uttarakhand, western Himalaya.

Total sample size	*Duttaphrynus himalayanus*	*Duttaphrynus melanostictus*	*Duttaphrynus stomaticus*
Individual with empty stomach	7	7	8
Total prey taxa present	24	25	19
Total no. of prey	376	322	524
Average no. of prey items/sample	22	13	20
Maximum no. of prey/sample	26	19	25
Terrestrial preys (%)	95.73	96.89	94.46
Aquatic preys (%)	4.26	3.10	5.53
Maximum length of prey items (mm)	26	26	22
Minimum length of prey item (mm)	9	4	2

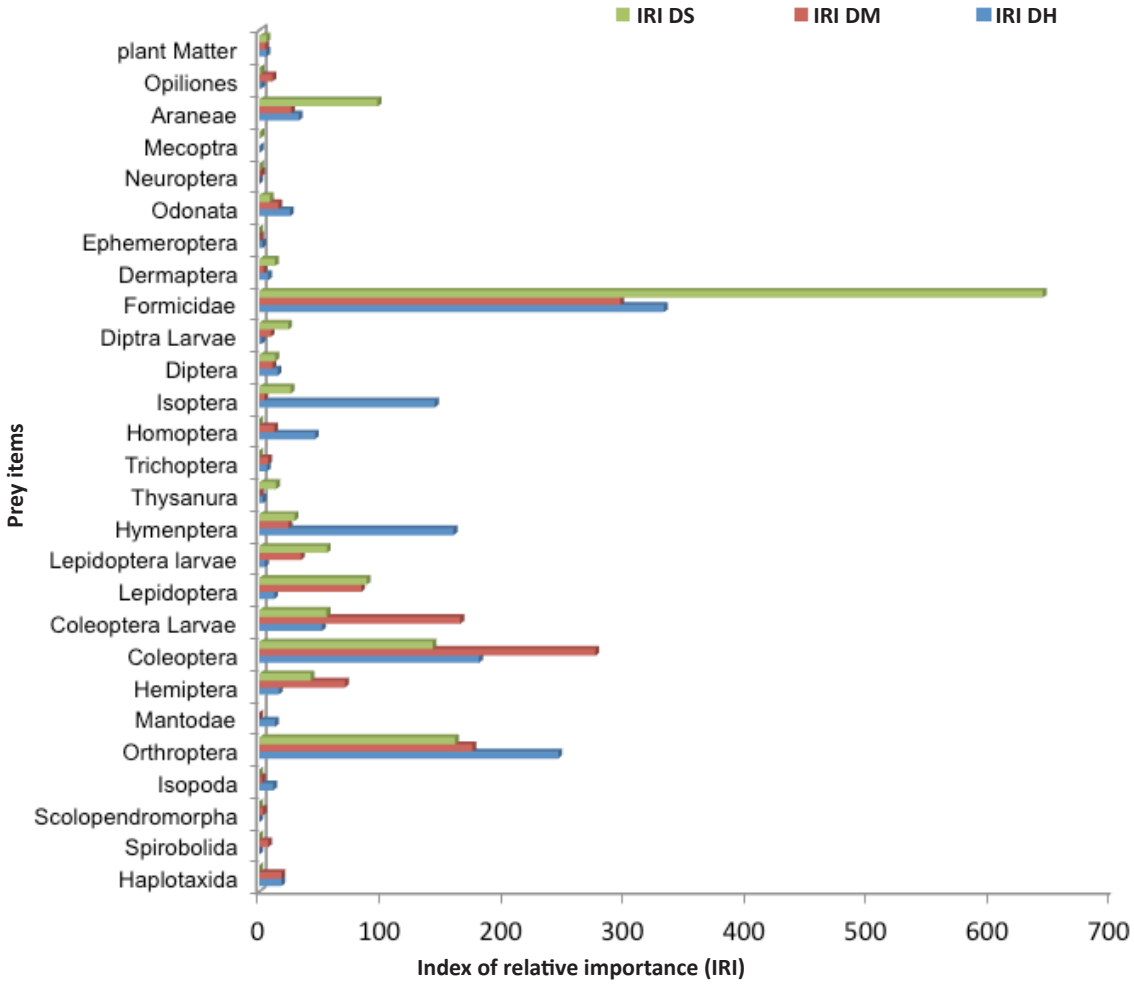

Figure 2. Index of relative importance (IRI) for prey items based on total diet contents of *D. himalayanus* (D.H.), *D. melanostictus* (D.M.) and *D. stomaticus* (D.S.) in Uttarakhand.

the Shannon-Wiener function, *D. melanostictus* had the highest prey diversity followed by *D. himalayanus* and *D. stomaticus* (Table 3). As for the niche breadth, *Duttaphrynus melanostictus* also had the broadest dietary niche breadth, followed by, *D. himalayanus* and *D. stomaticus*, in that order (Table 3).

Table 2. Shannon-Wiener function of niche breadth (H'), evenness measure (J'), Levin's measure of niche breath (B'), and standardized Levin's measure of niche breath (B_A) of prey items of studied bufonid species in Uttarakhand.

Species	Shannon-Wiener function		Levin's measure	
	H'(*)	J'	B	B_A
D. himalayanus	2.37 (10.69)	0.757	7.60	0.300
D. melanostictus	2.76 (15.79)	0.859	11.52	0.438
D. stomaticus	2.20 (9.02)	0.748	4.86	0.214

DISCUSSION

D. himalayanus is a large toad distributed in the high altitudinal region of the Himalaya, while *D. melanostictus* and *D. stomaticus* are found up to 2,500m but prefer lowland plains and agricultural as well as urban areas in Uttarakhand (Husain 2015). The inter-locality variations and similarities in the diets of these three toad species suggest that these are generalist predators that lack an apparent food preference, and that their diets are most likely dependent on what type of prey is available in inhabited areas, but prey diversity may vary among regions. As a result, *D. melanostictus* can be expected to have access to a greater variety of prey types. *D. melanostictus* was the only species that preyed upon all about the prey orders recorded and shown rich prey species biodiversity index by Shannon-Wiener measure of niche breadth (H'=2.76). In spite of this, due to the dominance of Formicidae in its diet, *D. stomaticus* has a lower prey diversity index (H'=2.20) than other toad species. *D. himalayanus* has intermediate value of prey diversity (H'=2.37) (Table 3). Toft (1980, 1981) stated that many species from the family Bufonidae are specialists, characterized by the preference of some arthropods (often Formicidae). Levins' measure of niche breadth does not allow for the possibility that resources vary in abundance. In many cases, ecologists should allow for the fact that some resources are very abundant and common, and other resources are uncommon or rare. Levin's measure of niche breadth (B_A) calculated for the three species of toads are less than 0.5 in our study which shows the opportunistic feeding behavior of the studied toad species. Study of Levin's measure of niche breadth (B_A) in *D. melanostictus* from southwestern Taiwan also showed resemblance (Norval et al. 2014).

Toad feeds exclusively on the ground on a wide variety of terrestrial food in which arthropods are dominant (Mercy 1999; Hirai & Matsui 2000; Kidera et al. 2008; Menin et al. 2015). Our study showed that arthropods and invertebrates including other prey groups are the main constituents of the diet. This study revealed consistency in the presence of a few dominant taxonomic groups of prey in these species, but differences in diversity of the occurrence of other prey items. This may be due to the fact that the diets of these toads are defined by prey availability more than by active choice. Previously, it had been reported that a higher frequency of prey and presence of different prey sizes in the stomachs of some toad species were due to the availability of prey in the habitat of the predator (Guix 1993; Sulieman et al. 2016).

Toads might be classified as an ant specialist and wide forager, this classification is justified by having slow moving locomotion, possessing toxins in the parotid glands, prefer small preys, and high frequency of ants founds per stomach (Ferreira & Teixeira 2009). Ants and several beetle groups are unpalatable to many predators due to formic acids and quinones, respectively (Zug & Zug 1979). Therefore, specialization on those preys might confer certain advantages. Predators specialized in eating unpalatable preys decrease food competition with other predators. In our study, Formicidae was the most common prey category consumed maximum in comparison to other prey categories. This is due to their abundance and wide range of habitats. Zug et al. (2001) and Damasceno (2005) also reported that ants are common and the basic food content of toads with low energy value due to a large amount of exoskeleton when compared to other insects such as larvae of some insects (e.g., caterpillars); however, the studied toad species readily feeds on arthropods, such as ants, beetles, millipedes and centipedes that contain noxious chemicals. Toads actually incorporate the noxious chemicals produced by such type of arthropods into their own defensive mechanisms (Daly 2007). Therefore, the kind of food spectrum is very important for the composition of the toad poison and its defensive activity also.

Observations of stomach content analysis of adult toads revealed that the diet composed of insects of the orders Coleoptera, Hymenoptera, Isoptera, Lepidoptera, Orthoptera, Hemiptera, and Diptera. Some of these are major pests of an agricultural crop of this region. Toads feed on these harmful pests and help in controlling them. Apart from insects, the diet also includes annelids, crustaceans and some plant materials. Plant matter such as stem of Doab Grass *Cynodon dactylon* was observed in the diet of *D. himalayanus* and plant seeds in *D. melanostictus* and *D. stomaticus*. Similar

Table 3. Dietary items of the D. himalayanus, D. melanostictus, and D. stomaticus with their respective absolute values and relative abundance (N and N%), frequency (F and F%), volume (V and V%) and Importance of relative index (IRI).

	Duttaphrynus himalayanus				*Duttaphrynus melanostictus*				*Duttaphrynus stomaticus*			
Prey Taxa	**N (%)**	**V (%)**	**F (%)**	**IRI**	**N (%)**	**V (%)**	**F (%)**	**IRI**	**N (%)**	**V (%)**	**F (%)**	**IRI**
Class: Clitellata												
Haplotaxida	4 (1.06)	209.34 (5.5)	3 (2.65)	17.42	9 (2.8)	226.08 (3.6)	4 (2.7)	17.43	0	0	0	0
Class: Diplopoda												
Spirobolida	0	0	0	0	5 (1.55)	117.75 (1.9)	3 (2.03)	6.99	0	0	0	0
Class: Chilopoda												
Scolopendromorpha	0	0	0	0	1 (0.31)	242.82 (3.9)	1 (0.68)	2.86	0	0	0	0
Class: Malacostraca												
Isopoda	9 (2.39)	66.98 (1.76)	3 (2.65)	11.02	3 (0.93)	32.15 (0.5)	2 (1.35)	1.96	0	0	0	0
Class: Insecta												
Orthoptera	10 (2.66)	1360 (35.74)	7 (6.19)	237.87	19 (5.9)	1360.67 (22)	9 (6.08)	169.38	18 (3.44)	736.85 (22.85)	11 (6.11)	160.61
Mantodea	5 (1.33)	84.78 (2.23)	4 (3.54)	12.59	0	0	0	0	0	0	0	0
Hemiptera	10 (2.66)	65.41 (1.72)	4 (3.54)	15.5	13 (4.04)	445.2 (7.2)	9 (6.08)	68.25	21 (4.01)	58.61 (1.82)	13 (7.22)	42.07
Coleoptera	48 (12.77)	267.94 (7.04)	10 (8.85)	175.32	46 (14.29)	183.16 (3)	23 (15.54)	268	43 (8.21)	169.56 (5.26)	19 (10.56)	142.11
Coleoptera larvae	21 (5.59)	94.2 (2.48)	7 (6.19)	49.96	31 (9.63)	267.94 (4.3)	17 (11.49)	160.27	29 (5.53)	50.24 (1.56)	14 (7.78)	55.16
Lepidoptera	6 (1.6)	66.98 (1.76)	4 (3.54)	11.89	17 (5.28)	602.88 (9.7)	8 (5.41)	81.12	13 (2.48)	942 (29.21)	5 (2.78)	88.02
Lepidoptera larvae	3 (0.8)	66.98 (1.76)	2 (1.77)	4.53	9 (2.8)	435.41 (7)	5 (3.38)	33.19	11 (2.1)	468.9 (14.54)	6 (3.33)	55.46
Hymenoptera	29 (7.71)	538.51 (14.15)	8 (7.08)	154.77	12 (3.73)	37.68 (0.6)	8 (5.41)	23.45	17 (3.24)	104.66 (3.25)	8 (4.44)	28.84
(Non Formicidae)												
Formicidae	101 (26.86)	47.1 (1.24)	13 (11.5)	323.25	61 (18.94)	83.73 (1.4)	21 (14.19)	287.91	221 (42.18)	75.36 (2.34)	26 (14.44)	642.95
Thysanura	4 (1.06)	18.84 (0.5)	2 (1.77)	2.75	1 (0.31)	37.68 (0.6)	1 (0.68)	0.62	9 (1.72)	32.94 (1.02)	9 (5)	13.69
Trichoptera	3 (0.8)	100.48 (2.64)	2 (1.77)	6.09	3 (0.93)	263.76 (4.3)	2 (1.35)	7.01	0	0	0	0
Homoptera	11 (2.93)	205.14 (5.39)	6 (5.31)	44.18	9 (2.8)	183.16 (3)	3 (2.03)	11.67	0	0	0	0
Isoptera	62 (16.49)	125.6 (3.3)	8 (7.08)	140.11	9 (2.8)	10.46 (0.2)	2 (1.35)	4.01	33 (6.3)	10.46 (0.32)	7 (3.89)	25.75
Diptera	9 (2.39)	32.96 (0.87)	5 (4.42)	14.41	11 (3.42)	28.26 (0.5)	4 (2.7)	10.48	14 (2.67)	8.63 (0.27)	8 (4.44)	13.06
Diptera larvae	2 (0.53)	18.84 (0.5)	2 (1.77)	1.81	14 (4.35)	6.28 (0.1)	3 (2.03)	9.02	22 (4.2)	2.09 (0.06)	10 (5.56)	23.68

	Duttaphrynus himalayanus				*Duttaphrynus melanostictus*				*Duttaphrynus stomaticus*			
Prey Taxa	**N (%)**	**V (%)**	**F (%)**	**IRI**	**N (%)**	**V (%)**	**F (%)**	**IRI**	**N (%)**	**V (%)**	**F (%)**	**IRI**
Dermaptera	4 (1.06)	61.23 (1.61)	3 (2.65)	7.09	13 (4.04)	56.52 (0.9)	1 (0.68)	3.35	13 (2.48)	14.13 (0.44)	8 (4.44)	12.97
Ephemeroptera	3 (0.8)	20.93 (0.55)	2 (1.77)	2.39	2 (0.62)	25.12 (0.4)	1 (0.68)	0.69	0	0	0	0
Odonata	6 (1.6)	200.96 (5.28)	4 (3.54)	24.36	1 (0.31)	1356 (21.9)	1 (0.68)	14.99	3 (0.57)	235.2 (7.29)	2 (1.11)	8.74
Neuroptera	0	0	0	0	2 (0.62)	14.13 (0.2)	2 (1.35)	1.15	2 (0.38)	18.84 (0.58)	1 (0.56)	0.54
Mecoptera	1 (0.27)	18.84 (0.5)	1 (0.88)	0.68	0	0	0	0	1 (0.19)	42.39 (1.31)	1 (0.56)	0.84
Class: Arachnida												
Araneae	19 (5.05)	32.37 (0.85)	6 (5.31)	31.33	16 (4.97)	23.55 (0.4)	7 (4.73)	25.3	37 (7.06)	28.26 (0.88)	22 (12.22)	97.01
Opiliones	1 (0.27)	58.61 (1.54)	1 (0.88)	1.6	7 (2.17)	58.61 (0.9)	5 (3.38)	10.53	4 (0.76)	5.23 (0.16)	2 (1.11)	1.03
Unidentified	0	14.13 (0.37)	3 (2.65)			33.49 (0.5)	4 (2.7)			216.66 (6.72)	4 (2.22)	
Plant matter	5 (1.33)	28.26 (0.74)	3 (2.65)	5.5	8 (2.48)	65.41 (1.1)	2 (1.35)	4.78	13 (2.48)	4.18 (0.13)	4 (2.22)	5.8
Total	376 (100)	3805.41 (100)	113 (100)	1296.423	322 (100)	6197.9 (100)	148 (100)	1224.4	524 (100)	3225.19 (100)	180 (100)	1418.36

observations for the intake of plant matter in Bufonidae were also made by Winston (1955) and Tyler (1958) as they had recorded the ingestion of the calyces of *Morinda lucida* by *D. regularis* and presence of the flowers of *Polygonum amphibium* and grass in the stomachs of *Rana esculanta*, respectively. Although the immediate most used explanation would imply accidental ingestion of vegetation while foraging for invertebrate preys, the idea that anurans may actually select plant matters as food items must be considered. According to Anderson et al. (1999) and Santos et al. (2004), plant contents may help in the elimination of intestinal parasites; provide roughage to assist in grinding up arthropod exoskeletons, and an additional source of water and nutrients.

CONCLUSION

The present findings indicate a high percentage of terrestrial food items found in three Bufonids reaffirms that *D. himalayanus, D. melanostictus*, and *D. stomaticus* are natural predator of various insect pests especially those which are considered as serious crop pests in this region. Diverse food items found in the bufonids' stomachs illustrate the ability to utilize a wide variety of prey taxa in the high altitude region of the western Himalaya also. Thus, they play a very important role in ecological balance as well as the economy of nature. This is the first unique report on feeding of these toads

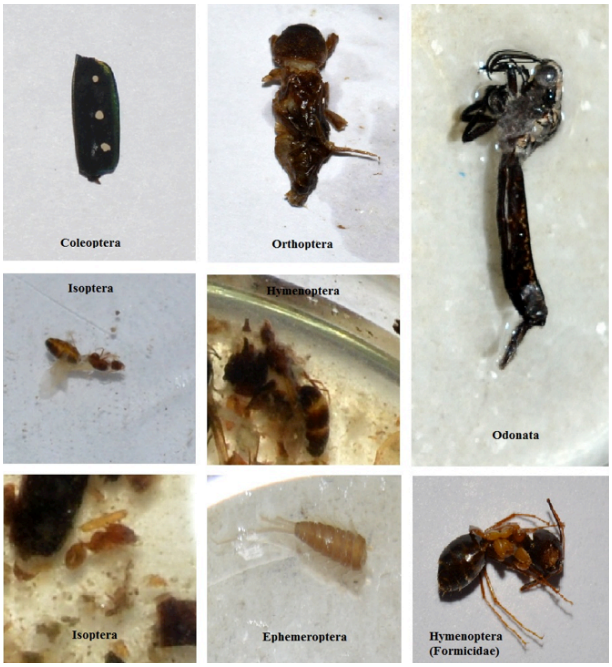

Image 1. Some major diet items of *Duttaphrynus himalayanus*.
© Vivekanand Bahuguna.

Image 2. Some major diet items of *Duttaphrynus melanostictus.* © Vivekanand Bahuguna.

Image 3. Some major diet items of *Duttaphrynus stomaticus.* © Vivekanand Bahuguna.

from Uttarakhand region of the western Himalaya. Information pertaining to the food spectrum analysis contributes to understanding the ecological roles in the ecosystem and used as a baseline data for future successful amphibian conservation and management programs in the Himalayan ecosystem.

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