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Species diversity and spatial distribution of amphibian fauna along the altitudinal gradients in Jigme Dorji National Park, western Bhutan

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Abstract: This paper presents the results of a study conducted on the diversity and spatial distribution of amphibian fauna in two different habitat types along the elevation gradients of Mochu River valley in Jigme Dorji National Park, Bhutan. The survey was conducted from November 2017 to February 2019. The study aimed to assess the diversity and distribution of amphibians using an opportunistic visual encounter survey technique. A total of 16 species of amphibians belonging to nine genera distributed among seven families were documented during the study period. The Shannon diversity index was relatively higher in the primary forest habitat than in the agro-ecosystem, however, there was no statistically significant difference of species abundance. The decreasing trend of diversity and abundance of amphibian fauna was noticed towards higher altitudinal zones. About 56% of species were recorded in the lower sampling sites (1200–1600m) indicating more favourable climatic conditions and habitat types for amphibian assemblages at lower elevations.

Keywords: Abundance, amphibian diversity, habitats, spatial distribution.

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Author contribution: BKK—field surveys, and data collection, and manuscript writing; KC—data analysis & editing; TP—manuscript writing and data analyses.

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INTRODUCTION

The current number of amphibian species is 8,043 as of 2019 (Frost, 2019). Of the 8,043 described amphibian species worldwide, 6,771 are evaluated and 2,157 of them are classified as threatened (IUCN 2019). More than 700 species of amphibians are known to exist in southern Asia (IUCN Red List 2009) and as per Global Amphibian Assessment (GAA) records 285 amphibian species are endemic to the region. The discovery of Himalayan Newts *Tylototriton verrucosus* (Anderson 1871), by Frost (1985) unfolds the very first chapter of history of Bhutan's amphibians. It was not until the 2000s, however, that the scientific documentation of amphibian fauna occurred when Das & Palden (2000) reported seven amphibians from three families (one megophryid, one bufonid, and five ranids) which were all new records for Bhutan (Wangyal 2014). According to Das & Palden (2000) and Ahmed et al. (2009), the total number of amphibian species so far recorded from Bhutan then was 13. Later, in 2012, five more species—*Duttaphrynus* cf. *stuarti* (Smith 1929), *Amolops* cf. *monticola* (Anderson, 1871), *Megophrys nankiangensis* (Liu & Hu, 1966), *Polypedates maculatus* (Gray, 1830), and an inadequately described species of *Nanorana* were added to the list of amphibians of Bhutan, pushing the number of species from Bhutan to 18 (Wangyal & Gurung 2012). According to the IUCN Red List (2013), seven families of amphibians (2 Bufonidae, 2 Dicroglossidae, 1 Megophryidae, 1 Rhacophoridae and 1 Salamandridae) are known from Bhutan, of which, five are Least Concern, one Vulnerable and one Data Deficient. After a comprehensive review of the Bhutanese herpetological records, today 58 species of amphibians (56 anurans, one caudata, and one caecilian) are known to occur in Bhutan (Wangyal 2014). With the latest discovery of *Leptobrachium bompu* byTenzin & Wangyal (2019), Bhutan now has 57 anurans. Some of these taxa, however, are identified only up to the genus level and many are yet to be described with proper taxonomic references.

Among vertebrates, amphibians are currently the most imperiled class, with about 41% of the more than 7,000 amphibian species on the planet threatened with extinction (Collins and Crump 2009; Hoffmann et al. 2010) making amphibians one of the most threatened groups of animals on earth (Hof et al. 2011). The GAA suggests that at least 158 amphibian species are believed to be extinct recently, nearly one in three remaining species is currently threatened with extinction, and one in four is inadequately known that they can only be called Data Deficient (IUCN et al. 2010).

Amphibians are perceived to be one of the most sensitive animal taxa and response very rapidly to substantial changes in their environments. Amphibians are sensitive to changes in thermal and hydric environments due to their unshelled eggs, highly permeable skin and unique biphasic life-cycles (Ochoa-Ochoa et al. 2012). For this reason, they are considered "an indicator species" the species that "indicate" the state of the health of their ecosystems (Saber et al. 2017). Amphibians can serve as food for predators in their community and they are voracious predators themselves. Amphibians are an important component of both terrestrial and aquatic ecosystems and play vital role in community ecology by serving as both predators and preys. With the Increasing human population and subsequent impact on the natural environment demand an urgent and immediate conservation intervention to save these least studied but ecologically significant taxa. Nevertheless, when Bhutan is striving forward to study and document its biodiversity, the field of herpetology has always received less priority. The current knowledge of amphibian fauna of Bhutan is scanty and fragmented. There has been no single study conducted on the amphibians in Jigme Dorji National Park (JDNP) despite the park being recognized as an important embodiment of the eastern Himalayan ecosystem. In the present study, an attempt has been made to document the diversity and spatial organization of amphibians in JDNP.

STUDY AREA

Jigme Dorji National Park, one of the 10 protected areas in Bhutan, was gazetted in the year 1995. It is located at (27.81927778 degree latitude and 89.73027778 degree longitude) and altitude ranges from 1200m to 7000m with corresponding vegetation types of warm broadleaved forest, cool broadleaved forest, mixed conifer, and alpine meadows that harbour rich repositories of biodiversity.

Majority of the study area is covered by primary forest (Image 1) with negligible human disturbance. The study area along Mochu River valleys, is characterized by patches of agricultural farmlands (Image 2), and the adjoining forests are used for timber, fire wood, non-wood forest products and livestock grazing.

The dry subtropical zone of the park experiences a hot summer with moderate rainfall, whereas in the warm temperate and cool temperate zones at higher altitudes, the climatic conditions are characterized by

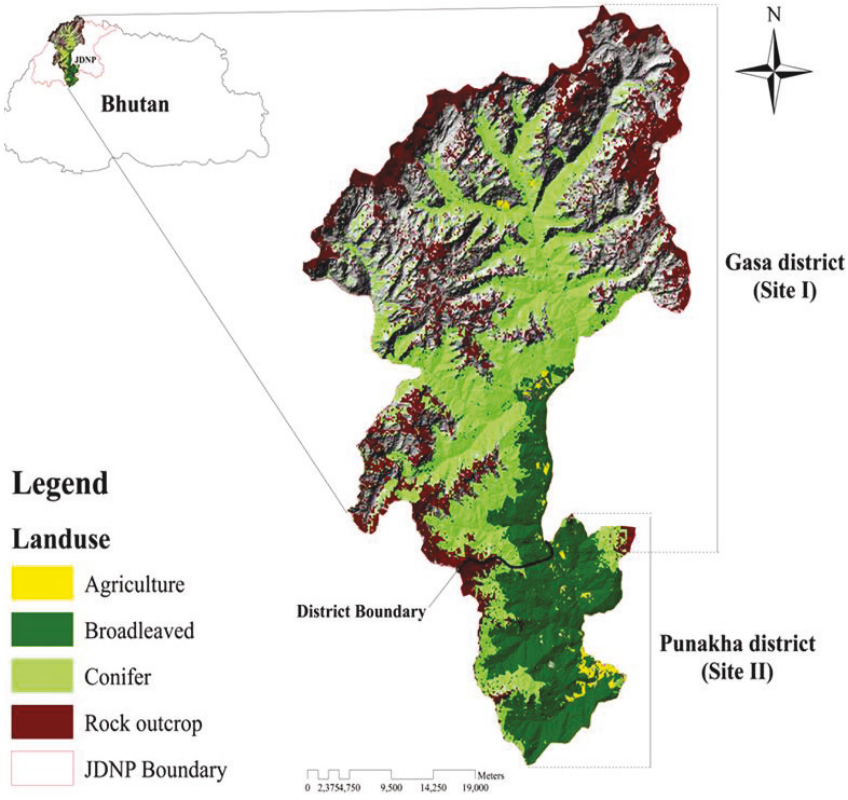

Figure. 1 Jigme Dorji National Park with landuse data.

Image 1. Primary forests habitat of JDNP.

Image 2. Agro-ecosystem habitat of amphibians in JDNP.

warm summers and cold winters. In 2017, the annual total rainfall of Punakha District (which falls under the dry subtropical zone) and Gasa (temperate zone) was 717.6mm and 1,848.1mm respectively (National Statistics Bureau 2018). The higher region of the park is covered by perennial snow; a home of glacial rivers which serves as an important source of water for household use, agriculture and hydropower generation in the downstream valleys. JDNP is a vital watershed covering almost half of northern Bhutan, and is an important natural conservatory of glaciers, alpine meadows and scrublands, sub-alpine and temperate conifer forests, warm and cool temperate broadleaved forests, major rivers and streams, and flora & fauna that inhabit these ecosystems.

METHODS

The survey was conducted from November 2017 to February 2019 in JDNP and adjoining areas in Bhutan. Visual encounter surveys (VES) were conducted by three people during each survey event. The survey was conducted at 08.00–11.00 h in the morning and at

14.00–18.00 h in the afternoon, in order to detect both diurnal and nocturnal species. Considering the rugged terrain with steep slopes and vast elevation range of the study area, specimens were recorded by VES as per Heyer et al. (1994) and Rödel & Ernest (2004), employing randomized walk, carried out along with active searches in day and night. Potential habitats of amphibian fauna, i.e., all microhabitats (rocks and boulders, dead and fallen logs, dense bushes and grass patches, rock and tree crevices, leaf litter, and water bodies) were thoroughly searched.

Standardized road searches were conducted at 14.00–18:00 h twice a month, covering all potential areas with the clearest ground visibility. Four man-hours were spent in each survey event. In total, 48 man-hours were spent to cover the entire study area searching for nocturnal species. The Punakha-Gasa secondary highway which is 85km long was surveyed using a motor vehicle traveling at 10 to 20 k/h.

Every individual specimen was noted and identified to the most specific taxonomic level possible. Identification of species was done using standard field guide books by Daniel (2002), Ahmed et al. (2009), and Vasudevan & Sondhi (2010). Digital photographs taken during the field survey were sent to experts to confirm species identity. Locality data were collected for all specimens that were encountered, live or dead. Whenever possible, the digital photographs were taken for specimens, elevation and geo-spatial location of each species was recorded using GPS. The Shannon diversity indices (H') were used to calculate the diversity, the Hutcheson statistical t-test was performed to compare the diversity index of the two different habitat types, and statistical software SPSS (Statistical Package for the Social Sciences) was used to compare the difference in species abundance of amphibians in two different habitat types.

RESULTS

Diversity

In total, six-day field trips or 300 man-hours were spent searching for amphibians across the various elevation gradients of JDNP, of which, 200 man-hours were spent searching in forests, 48 man-hours for standardized road surveys at night, and 50 man-hours for searching streams.

The amphibian diversity of Bhutan is represented by 58 species belonging to 18 genera distributed among eight families, of which, 16 species belonging to nine genera, distributed from seven families were documented during the field survey from Jigme Dorji National Park (Table 1). Of the observed species, three could not be identified to species level and so they were assigned to closely related species/genus level (e.g., *Amolops* sp., *Hyla* sp., and *Megophrys* cf. *major*).

Table 1. List of amphibian species documented in Jigme Dorji National Park in November 2017–February 2019 including percentage of relative abundance.

Family	Scientific name	Common name	Relative abundance (%)
Bufonidae	*Duttaphrynus himalayanus* (Günther, 1864)	Himalayan Toad	29.52
	Duttaphrynus melanostictus (Schneider, 1799)	Common Indian Toad	5.71
	Duttaphrynus stuarti	Stuart's Toad	8.57
Hyladae	*Hyla* sp.		2.38
Ranidae	*Amolops marmoratus* (Blyth, 1855)	Himalayan Torrent Frog	5.71
	Amolops mantzorum (David, 1872)	Sichuan Torrent Frog	2.85
	Amolops sp.		3.33
	Amolops monticola (Anderson, 1871)	Mountain Torrent Frog	1.90
Rhacophoridae	*Rhacophorus maximus* (Günther, 1858)	Giant Green Tree Frog	2.38
	Polypedates himalayensis (Gray, 1830).	Tree Frog	13.80
Dicroglossidae	*Nanorana liebigii* (Günther, 1860)	Liebigii's Paha	11.42
Megophryidae	*Scutiger sikkimmensis* (Blyth, 1855)	Sikkim Snow Toad	2.38
	Scutiger bhutanensis Delorme & Dubois, 2001	Bhutan Snow Toad	1.42
	Megophrys cf. *major* Boulenger, 1908	Major Horned Toad	3.33
	Megophrys parva Boulenger, 1893	Mountain Horn frog	2.38
Salamandridae	*Tylototriton verrucosus* Anderson, 1871	Himalayan Newt	2.85

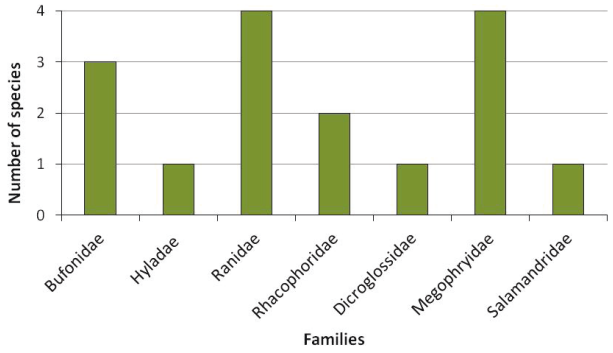

Figure 2. Species richness of amphibians with respect to families recorded during the study period in JDNP.

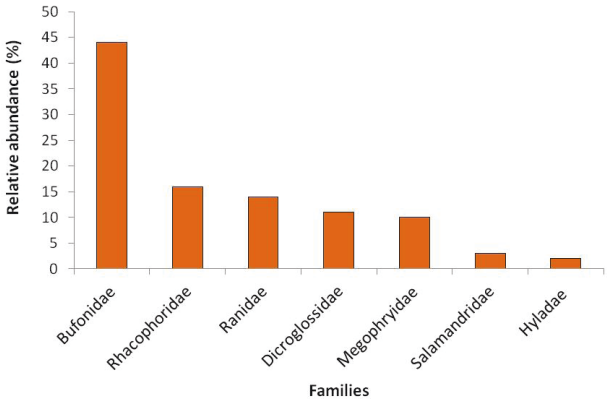

Figure 3. Relative abundance of amphibians with respect to families in JDNP in November 2017–February 2019.

Scutiger bhutanensis is the only species recorded as endemic to Bhutan.

The family-wise distribution of the amphibian fauna of JDNP is given in (Figure 2). Family Megophryidae and Ranidae dominated the amphibian fauna of JDNP each accounting for four species, belonging to three genera, followed by Bufonidae with three species belonging to one genus, Rhacophoridae with two species belonging to two genera. Dicroglossidae, Salamandridae and Hylidae together comprised three species belonging to three genera.

Abundance

At the species level, the abundance of amphibians varied from two to 62 individuals. A total of 210 individuals were recorded during the entire study period. Relative abundance data indicated that *Duttaphrynus himalayanus* was found to be the most common species (n=62, 29.52%) and *Scutiger bhutanensis* was found to be the least abundant species contributing (n=3, 1.42%) (Table 1).

At the family level, the relative abundance of amphibians varied from 2% to 44%. With reference to relative abundance (Figure 3), a deduction can be made that the family Bufonidae was found to be the most dominant family contributing (44%) of the total individuals. This was followed by Rhacophoridae (16%), Ranidae (14%), Dicroglossidae (11%), Megophryidae (10 %), Salamandridae (3%), and only 2% was contributed by Hylidae.

Species composition of amphibians in relation to habitat types

The present study found that amphibian species in Jigme Dorji National Park inhabit both primary forests and agro-ecosystems. Of the 16 species recorded, all 16 were found in primary forest habitats and 11 species were found in agro-ecosystems, however, there were more individuals in the agriculture farm land than in forest habitat. A total of (n=116, 55.24%) were recorded in the agro-ecosystem and a total of (n=94, 44.76%) in the forest habitat. All the four species belonging to the family Megophryidae such as *Scutiger sikkimmensis*, *S. bhutanensis*, *Megophrys* cf. *major*, and *M. parva*, including one species belonging to the family Ranidae such as *A. monticola*, were exclusively found in primary forests, while the remaining 11 species were found inhabiting both the habitats. Although more numbers of individuals were recorded in agro-ecosystems, there was no statistical significance in species abundance between the two different habitats. Mann-Whitney test indicated that the species abundance was not significantly greater for primary forest habitat (Mdn= 4) than for agro-ecosystem (Mdn=2), U=81, p= 0.07 (figure 4).

Most of the species have been found in both the habitat types; the Shannon diversity index (H') for

Table 2. Comparison of Shannon diversity index using the Hutcheson statistical t-test.

	Habitat types	
	Primary forest	Agro-ecosystem
Total individuals	94	116
Richness	16	11
H'	2.639874676	1.739164568
S^2_H	0.00389201	0.007581
t	8.409038716	
df	200	
Critical value	1.971896178	
CI	0.12477195	0.174137876

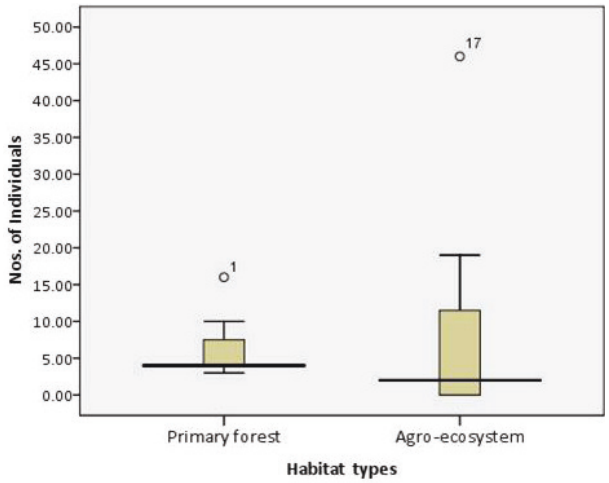

Figure 4. The box plots showing the median of species counts in two different habitat types

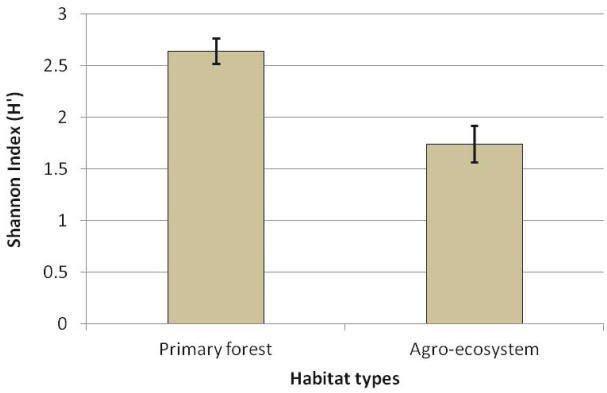

Figure 5. The Shannon diversity index of two different habitat types. Error bars are 95% confidence intervals.

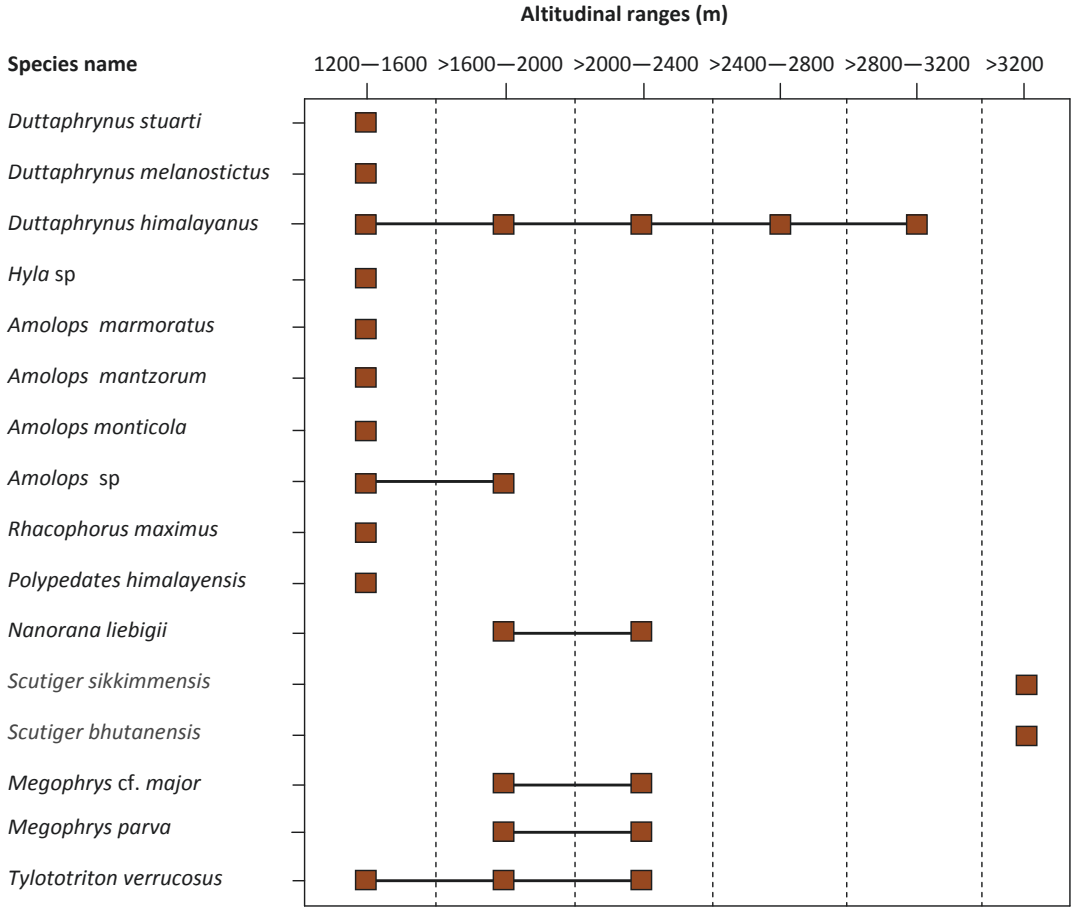

Figure 6. Elevational ranges of each amphibian species documented in JDNP. Brown boxes indicate altitudinal band where individuals were sighted

the primary forest habitat was (n=94, H'=2.63) and (n=116, H'=1.73) for the agro-ecosystem (Figure 5). The Hutcheson statistical t-test suggested that Shannon diversity index (H') between the two different habitats was statistically significant as calculated t-value exceeds the critical value (Table 2). It can, therefore, be deduced that primary forests serve as important habitats for amphibian assemblage in JDNP.

Geo-spatial distribution of amphibians

About 56% of amphibians showed a narrow distribution range and were concentrated in lower elevations, particularly between 1,200m and 1,600m. Both diversity and observed species abundance were found to be relatively higher in this altitudinal zone as compared to subsequent zones towards higher elevations. Of the total 16 recorded species, 11 species were recorded in a lower altitudinal band and nine of them demonstrated the distribution pattern by excluding elevation boundaries above 1,600m. Species such as *P. himalayensis* (Image 3), *A. monticola, A. marmoratus* (Image 4), *A. mantzorum* (Image 5), *Amolops sp* (Image 6), *R. maximus* (Image 7), *Hyla* sp. (Image 8), *D. stuarti*, and *D. melanostictus* (Image 9) were found below 1,600m. Whereas, species such as *N. liebigii* (Image 10) *M.* cf. *major*, *M. parva* (Image 11) were noticed between 1,700m and 2,400m. *Tylototriton verrucosus* (Image 12) was observed in the elevation ranges between 1,300m and 2,200m. *D. himalayanus* (Image 13) was the most widely distributed species across the altitudinal range up to 3,200m. Species such as *S. sikkimmensis* (Image 14) and *S. bhutannensis* were sighted only above 3,500m; both the species were recorded in an alpine ecosystem around 4,000m in JDNP (Figure 6).

DISCUSSION

Jigme Dorji National Park is one of the important conservation areas that encompasses a wide range of habitats from sub-tropical to alpine ecosystems of Bhutan. Considering the limited knowledge on diversity, distribution and natural history of the amphibian fauna of the region, the present study can be considered very significant. Of the eight families and 18 genera of amphibian fauna found in the country, seven families and nine genera were recorded in JDNP. This implies that JDNP is an important repository for amphibian conservation. The park representing a majority of amphibian fauna of the country may be due to the presence of largely-undisturbed forests, altitudinal variation with corresponding habitat types, and an organic farming system practised by park residents. Although most of the species (n=11, 68.78%) were found occupying both the habitat types, relatively more species diversity was observed in the primary forest. Many studies suggest that amphibian species are affected by the degree of canopy cover (Halverson et al. 2003; Skelly & Golon 2003). Most amphibian species tended to be either 'open canopy specialists' or 'canopy generalists' (Skelly et al. 2005). The present finding agrees with Krishnamurthy (2003) who reported that more than 50% of the amphibian species encountered during his study in the Western Ghats, India, were found exclusively in primary forests. Displaying such distribution pattern could be due to amphibians responding to moist condition, numerous perennial streams, marsh lands, and less human disturbance, thus providing potential habitats associated with primary forest. In some cases, however, composition of amphibian fauna might depend on specific ecological requirements of individual species, which is beyond the scope of this study.

The reduced species richness in the agro-ecosystem could be due to disturbances induced by frequent visits of people for farming activities, however, wide distribution of *Duttaphrynus himalayanus, D. melanostictus*, and *D. stuartii* across the human modified landscape and disturbed habitat could be due to the high adaptability of these species to such an environment. The present finding agrees with Ahmed et al. (2009) who claimed that the majority of amphibians and reptiles are sensitive to habitat quality, while only a few species are adaptable to such environments. The number of individuals that represents each species in the community might differ from place to place depending on the amount of rainfall, available habitats and human interference as the structure and diversity of an amphibian community is determined by the availability of food, moisture and microhabitat (Daniels 1992).

The present study has showed that observed species richness and species abundance were found to be comparatively higher in lower altitudinal regions particularly below 1,600m than subsequent zones in higher elevations. Higher species richness at lower elevation sites may be due to more favourable climatic conditions and habitat components for amphibian assemblages at lower elevations. This includes higher average temperatures, evapo-transpiration, productivity and precipitation, which are widely recognized as important for the spatial and temporal distribution pattern of amphibians (Buckley & Jetz 2007). For instance, species such as *S. sikkimmensis* and *S. bhutannensis*

Image 3. *Polypedates himalayensis*.

Image 4. *Amolops marmoratus*.

Image 5. *Amolops mantzorum*.

Image 6. *Amolops* sp.

Image 7. *Rhacophorus maximus.*

Image 8. *Hyla* sp.

Image 9. *Duttaphrynus melanostictus*.

Image 10. *Nanorana liebigii*.

Image 11. *Megophrys parva.*

Image 12. *Tylototriton verrucosus.*

Image 13. *Duttaphrynus himalayanus*.

Image 14. *Scutiger sikkimmensis*.

showing restriction in dispersion range boundary towards lower elevations could be due to their ability to withstand the prevailing cold temperatures of higher elevations and evolutionary adaptation. In contrast, the presence of relatively few amphibian species at higher elevation zones could be due to the inability of most amphibians to withstand cold temperature, limited precipitation, and inadequate food resource in higher elevational region.

This study is the first of its kind on the amphibians of Jigme Dorji National Park. It provides data that could be used to begin to understand local amphibian species' distribution and factors governing such distribution. This research was carried out with the intention of future continuation and long term data collection, to gain a thorough understanding of the species dynamics. Therefore, there is a good future scope to study on amphibian's habitat analyses with addition of more environmental parameters in order to comprehend the complex ecology of these vertebrates.

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