BJNRD (2017), 4(2): 29-38
Bhutan Journal of Natural Resources & Development

Article

www.bjnrd.org

Open Access

ISSN 2409–2797 (Print)
ISSN 2409–5273 (Online)

https://doi.org/10.17102/cnr.2017.07

Habitat Characteristics, Relative Abundance and Conservation Threats of Himalayan Bull Frogs (*Nanorana leibigii* Günther, 1860) in Primary Tributaries of Simkhar River, Bhutan

Jigme Tenzin[1] and Phub Dhendup[1]

Abstract

Nanorana leibigii Günther belongs to Dicroglossidae family. Its population in Bhutan is declining due to over collection for its medicinal values. This study documents the habitat characteristic and assesses the relative abundance and conservation threats within the six primary tributaries of Simkhar river under Sarpang District. Opportunistic visual encounter survey was used within the time span of 8:00–11:00 hours in the morning and 17:00–20:00 hours in the evening during the month of March-May of 2015. Systematic hand search within 5 m radial distance in potential habitats were conducted to categorize the niche habitats. Water samples were collected from each tributary and tested using Digital Hanna instrument and conservation threats assessed in their niche habitat. The results show that habitat of *N. leibigii* is characterized by permanent stream flows (28%), piled-up substrates (26%), moss-laden rock crevices (25%), and decaying vegetable matters (21%). Relative abundance (RA) was highest in Thortneykhola and Gurungkhola, and lowest in Darzhanikhola. RA was higher in habitat with 7.73 pH, 7.6 mg/L Dissolved Oxygen (DO), and 15 ppm Total Dissolved Solid (TDS). Overall, the study recorded intensive cattle grazing in the catchment areas as the highest conservation threat followed by lack of conservation awareness of nearby communities, and collection for medicine. Occurrence of *N. leibigii* was relatively higher in water with 7.73 pH, 7.6 mg/L DO, and 15 ppm TDS. Similar studies are recommended for an in-depth understanding of the frog's habitat ecology and conservation need.

Keywords: Habitat, *Nanorana leibigii*, relative abundance, water parameters, threats

Introduction

Although Bhutan is globally known for conservation stewardship, herpeto-faunal study is very new. Biswas (1976) was the pioneer researcher to study reptiles in Bhutan. Das and Palden (2000) explored amphibian fauna of Bhutan as well and conducted first ever herpeto-faunal collection workshop in the Royal Manas National Park (RMNP). Later, Wangyal (2014) had reviewed the work of Das and Palden (2000), Palden (2003), and Deuti (2010) and reported 56 species of amphibians belonging to 7 families including 35 confirmed and 21 expected species. Among 7 families, Dicroglossidae has 13 genera and 169 species worldwide (Frost, 2014), of which Bhutan has recorded only 8 genera and 17 species (Wangyal, 2014). Genus *Nanorana* has 28 species worldwide (Frost, 2014) and Bhutan has recorded only five species including one unconfirmed species and two expected species (Wangyal, 2014).

[1] Sarpang Forest Division, Dept. of Forest and Park Services
Corresponding author: jigmetenzin16@gmail.com
Received Aug., 2017, accepted Dec., 2017

Nanorana leibigii (Günther, 1860) is locally known as *Mon-paag,* which is crepuscular in nature, possessing small discs and entirely webbed toes (Daniel, 2002). Males have internal vocal sacs and arms of male get remarkably thickened with conspicuous black horny spines on the inner sides of the arm especially during breeding season (Daniel, 2002; Mathew and Sen, 2010). *N. leibigii* are mostly found in Oak and conifer forests within the elevation range of 1500–3000 masl (Schleich and Kastle, 2000; Wangyal and Gurung, 2012). This species is distributed throughout the midland and lowland mountain ranges of Nepal, Indian Himalayas, south China, and Bhutan (Molur, 2008; Wangyal and Gurung, 2012; IUCN Redlist, 2017). In India, *N. leibigii* is found in Arunachal Pradesh, Himachal Pradesh, Jammu and Kashmir, Sikkim, Utter Pradesh and West Bengal (Mathew and Sen, 2010). It is reported to be a common frog found especially in Eastern Himalayas (Daniel, 2002). In Bhutan, it is reported from the broadleaved forests of Samtse, Paro, Thimphu, and Punakha districts (Kuensel, 2012) and is considered by local people to cure wound, common cough, cold, fever, diarrhea and dysentery, contributing to over collection (Daniel, 2002; Kuensel, 2012; Wangchuk *et al*., 2017). In Bhutan, dried *N. leibigii* is claimed to cure piles, gastritis, gout and urinary tract infection (Kuensel, 2012; Gurung *et al*., 2012). Wangyal (2014) had also reported that *Hoplobatrachus tigerinus* (Doudin, 1803) is eaten in Jomotshangkha while a *Nanorana* species is eaten at Sakteng under Tashigang district. The frogs especially *Amolops* species are occasionally served as table menu by the Rai communities in Bhutan (Kuensel, 2012) and *Amolops* spp., *Nanorana* spp. and *Xenophrys* spp. are being collected by local communities of Sikkim in India (Chettri *et al.*, 2011).

Presence of *Nanorana leibigii* was firstly reported in Bhutan by Deuti (2010) from Susuna highway in Haa district at an elevation of 2350 masl. This species was identified based on the specimen collected in 1969 by Zoological Survey of India (Deuti, 2010; Wangyal and Gurung, 2012; Wangyal, 2014). D.B. Gurung and a team from District Forestry Sector, Samtse was the first Bhutanese researchers to report on *N. leibigii* from Mithun village in Dophuchen, Samtse district (Kuensel, 2012). First image of *N. leibigii* was also posted by Gurung in Bhutan Biodiversity Portal in 2012. In the same year, Wangyal and Gurung (2012) had also reported from Goemkha village in Teob, Punakha district at an elevation of 1900 masl. Later, Bhakta Bahadur Galley, a Forester from Jigme Kheser Strict Reserve (JKSR) had recorded *N. leibigii* from Haa Chu at an elevation of 2700 masl consecutively in May 2013 and February 2014. Nonetheless, conservation initiative study on *N. leibigii* was conducted by the first author in 2014 at Simkharthang in Jigmecholing under Sarpang district and Khorila (2016) from Gidakom under Thimphu district. The study of the frog in Mithun under Samtse district was further studied in-depth by collecting morphometric data in the same study area by District Forestry Sector (Wangchuk *et al*., 2017). Despite such efforts from various researchers, species-specific habitat study on amphibian fauna in Bhutan is just beginning and much of the study results are still unpublished. Therefore, this study aims to document the habitat characteristics, relative abundance, conservation threats, and the parental care exhibited by the male frogs of *N. leibigii*.

Methods and Materials

Study site

While the study was primarily conducted in six tributaries of Simkhar river in Jigmecholing gewog under Sarpang Dzongkhag (Figure 1), which are located within an elevation range of 1300-3000 m, other information like the parental care and species-specific habitat information were collected from sources outside the study areas. Topographically, the study area faces south-west aspect with the average slope gradient of 25-35 degree. The catchment area falls within the Biological Corridors (BC

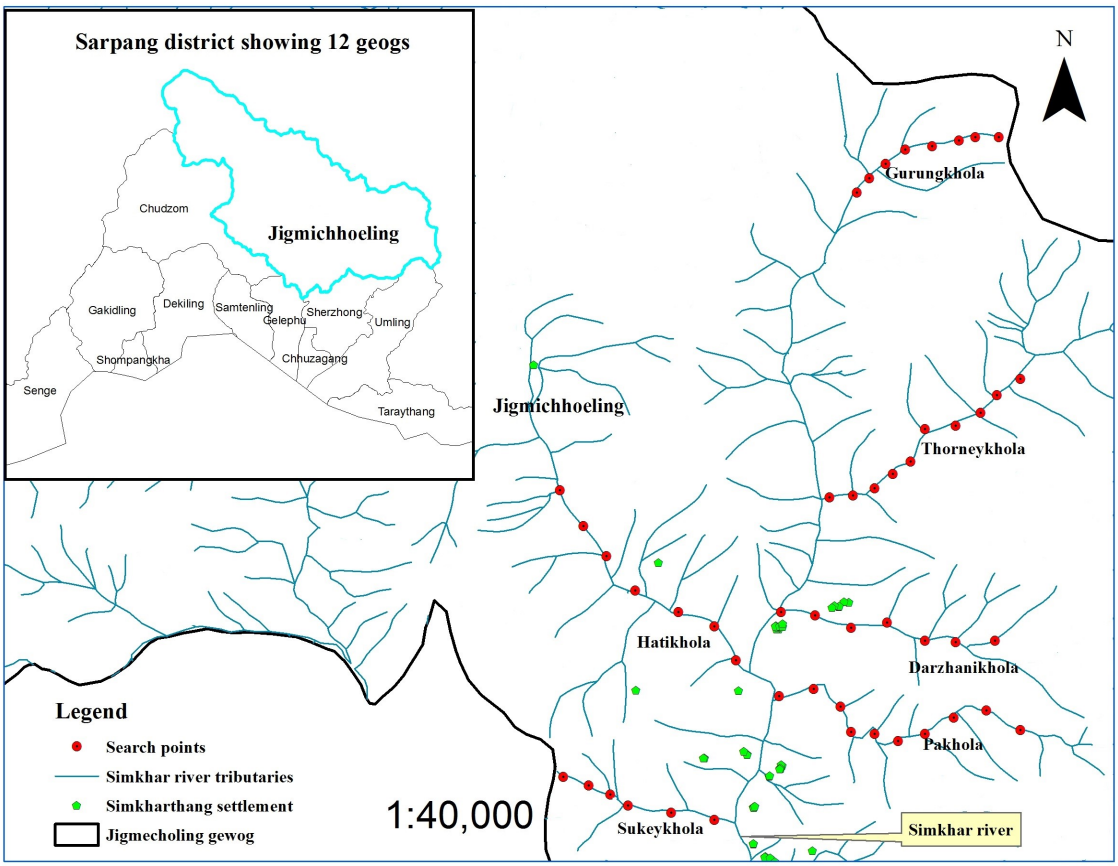

Figure 1: Map showing six primary tributaries of Simkhar river (study area)

No.3) which runs from Jigme Singye Wangchuck National Park (JSWNP) and Royal Manas National Park (RMNP) to Pibsoo Wildlife Sanctuary (PWS). The vegetation primarily comprises of *Castanopsis hystrix*, *Beilschmiedia gammieana, Quercus lamellosa, Quercus glauca, Lithocarpus elegans* and *Syzygium formusa.* Additionally, as undergrowth, *Chimonobambusa callosa, Cephalostachyum latifolium* and *Plectocomia himalayana* were abundant. All tributaries and the Simkhar river finally join Maokhola and drain to Brahmaputra in Assam.

Historically, whole catchment area was once a Tsamdro (registered grazing area) of Bumthap since 1969 until 2007. During those years, the area was heavily used by cattle herders both from within and outside the locality. This has resulted into huge forest degradation due to intense grazing pressures. However, with nationalization of registered Tsamdro area as per Land Act (2007), regular cattle herders have reduced significantly with reducing number of cattles/herder. This has not only reduced the grazing pressures especially within the riparian area, but also significantly contributed in improving the degraded forest condition and strengthen the catchment area. But on other hand, recent land excavation due to farm road construction (connecting Gonsekha and Gongduegang chiwogs from Gewog Centre) and felling of trees along power transmission corridors (electric line connecting from Gewog centre) have partially deteriorated the niche habitat of *N. leibigii*, especially in Pakhola area. Pakhola in Simkharthang was named due to the abundant presence of *N. leibigii* during early 1980s (D.M. Tamang, Pers. Comm. 20 August, 2014). However, proper habitat ecology, population status and pertinent conservation were not known. Therefore, this study has confirmed the presence of *N. leibigii* within Simkharthang catchment area and its conservation threats.

Field survey

Opportunistic visual encounter survey (Campbell and Christman, 1982; Corn and Bury, 1989, Browne and Paszkowski, 2009; Wangyal and Gurung, 2012; Johana *et al.,* 2016) was used from the base till the sources of six primary tributaries. Whenever individuals of species were encountered, or call detected along the stream courses, systematic hand search was done within 5 metre radial distance. Based on the frequency of sighting sites (niche habitat), habitat characteristics data were collected and categorised into four groups (Perennial streamflow; piled-up substrate; moss -laden rock crevices and availability of decaying vegetable matter). The survey was conducted from 08:00–11:00 hours in the morning and 17:00–20:00 hours in the evening for a period of three months (during breeding season: March, April and May) since breeding season provides higher detectability opportunity (Sutherland, 2006). Every individual species encountered were noted and identified using field guide books authored by Daniel (2002); Ahmed *et al*. (2009), and Mathew and Sen (2010). Geo-coordinates and elevation for every species captured were recorded using GPS (Garmin 62s). Wherever possible, digital photographs were taken using DSLR Camera (Sony) and voucher specimens were collected following standard protocol. Specimens were euthanized using 0.001 percent clove oil and treated in 10% formalin for fixation.

During the survey, water parameters such as pH, Total Dissolved Solid (TDS), Dissolved Oxygen (DO), Nitrate ($NO_{3)}$, and Phosphate (PO_4) were tested from water samples using Digital Hanna instrument. Only one sample each was collected from each sampling site (six tributaries). Water temperature and surrounding temperature were recorded. Frequency of threat signs (natural and anthropogenic) encountered within the surveyed area were recorded to infer the possible conservation threats

Results and Discussion

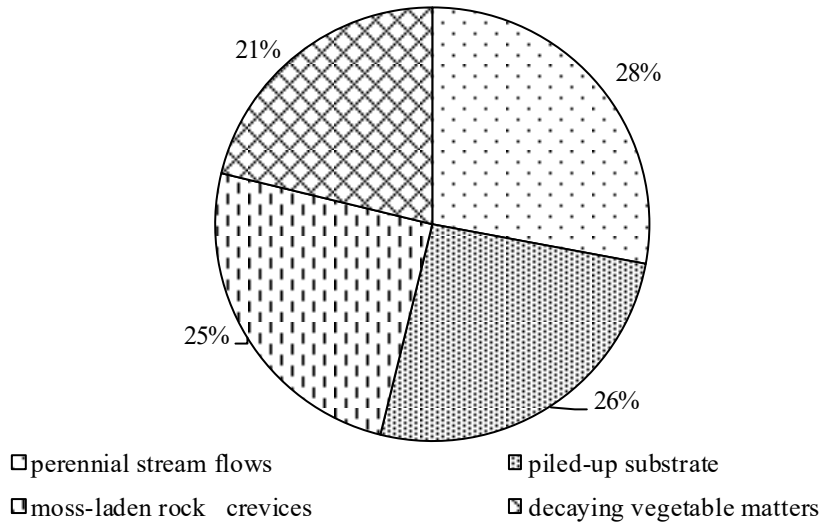

Figure 2: Primary constituents of niche habitat of *Nanorana leibigii*

Habitat characteristic

A total of 75 sample points were surveyed along the six perennial stream tributaries with an average of 15 search points per tributary. Among these, 47 individuals (N = 47) of *Nanorana leibigii* were recorded constituting 30 adults (Male : 12, Female : 18), 17 juveniles, 6 egg masses, and larvae from upper catchment pools. Calls were detected from five search points. Habitats mostly characterized of dense cool broadleaved forest species such as *Castanopsis hystrix, Syzygium formusa, Daphniphyllum chartaceum, Beilschmiedia gammieana* and *Quercus lamellosa.* The riparian area was mostly covered by bamboo thickets (*Chimonobambusa callosa* and *Cephalostachyum latifolium*), *Ligustrum confusum, Elatostema platyphyllum,* and *Acconogonon molle.* Since the area is located in leeward side, the slope was mostly humid favouring conducive

environment for the presence of amphibian fauna.

The niche habitat of *Nanorana leibigii* constituted perennial stream flows (28%), piled-up substrate (26%), moss-laden rock crevices (25%), and decaying vegetable matters (21%) (Figure 2). Presence of perennial clean-running stream is of utmost importance for *Nanorana leibigii* (Daniel, 2002). Abundant substrates and mosses laden rocks with free-flowing water passages underneath are pre-requisites for this frog for hiding. Rocks over water with water flowing spaces underneath provide refuge for hiding and for brooding especially during breeding season. According to D.B. Gurung (personal communication 2014), unlike other frogs, the eggs are attached underneath the rock surface. Eggs are laid in a single layered patch. The eggs are kept moist by the free-flowing water passing underneath the stone surface. After the mating is over mating is over, the male frog guards the eggs until the tadpoles' hatch. Kuensel (2012) and Gurung *et al.* (2012) also reported that the male frog continues to guard the young tadpoles until they are strong enough to feed and lead independent life. This characteristic parental care exhibited by the Himalayan Bull frog is not yet reported in detail. It is suspected that the male frog guarding the brood will not come out for feeding and guards the eggs and the young tadpoles 24 hours a day. During this time, the male frogs are vulnerable to collection by local people (Kuensel, 2012; Gurung *et al*., 2012). Abundant decaying vegetable matters in the pool streams are also preferred for hiding. Fast flowing and strong water currents are avoided for egg-laying. Breeding sites were noted mostly in densely montane forested streams that are clean and highly oxygenated. So, muddy and disturbed stream beds are not used by these frogs as habitats (Gurung *et al*., 2012; Wangchuk *et al*., 2017). To some extent, Vasudevan (1996) also suggested that rock covers and litter depth can also influence species richness and abundance of forest floor amphibian. Therefore, habitat preference of this frog species may warrant a separate in-depth study.

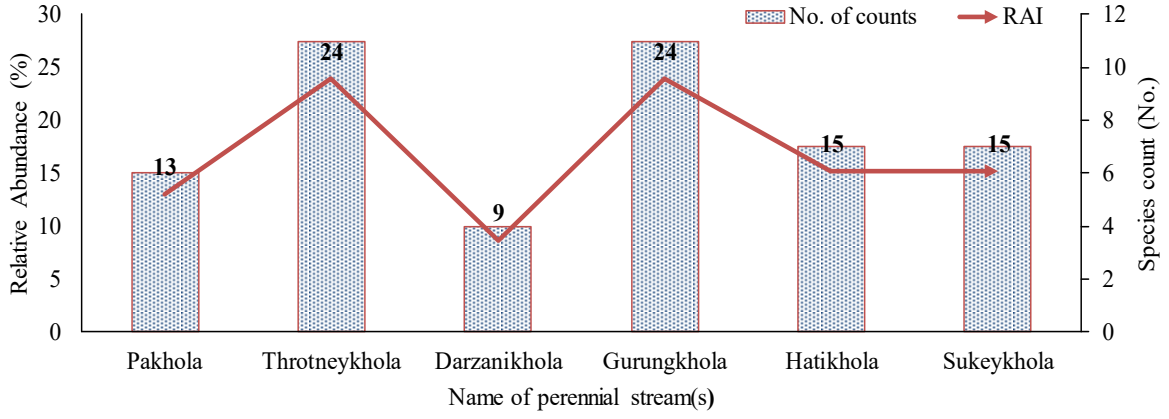

Figure 3: Relative abundance of *N. leibigii* in six different tributaries

Relative abundance within six perennial streams

Occurrence of *Nanorana leibigii* differed within the tributaries in the study site (Figure 3). The relative abundance of *N. leibigii* was highest (n = 24) in Thotneykhola and Gurungkhola, followed by Hatikhola and Sukeykhola (n = 15), Pakhola (n = 13) and lowest (n = 9) was in Darzanikhola.

Field survey indicated that Thotneykhola and Gurungkhola has undisturbed habitat which possess closed canopy forest covers with perennial, clean-running water, adequate mosses-laden rock over water courses and lots

of decaying vegetable matters in pools. Vasudevan (1996) also reported that presence of rock covers and abundant litter-depth influences the species richness and abundance of amphibians. Further, Purushotham *et al*. (2011) also suggested that stream running through undisturbed primary forests have higher species richness and abundance. This suggests that disturbance will have a significant effect on abundance of amphibians in any area.

In case of Darzanikhola which had the lowest relative abundance, the stream bed showed signs of frequent floods and stream banks erosion. Floods seem to wash down decaying vegetable matters and other aquatic insect communities such as that of macro-invertebrates. Gururaja (2002) and Purushotham *et al*. (2011) reported that even a slight modification of micro-habitat has adverse impact on amphibian as the disturbance would change the substrate availability leading to loss of shelter, prey, and egg laying sites.

Relationship between relative abundance and water parameters

Quality of water parameters such as the pH, DO, TDS, Electro-conductivity (EC), Salphide (SO_3), and water temperature (T) (Figure 4) do not seem to be the critical factors for the presence of amphibians in a stretch of river in the study area. These parameters along with the presence of undisturbed vegetation could affect the relative abundance of amphibians. Highest relative abundance was found in Thotneykhola and Gurungkhola where mean pH level was 7.73 which is slightly alkaline. Darzhanikhola had lowest water pH of 5.16 which is slightly acidic. However, *Nanorana leibigii* was found in both the sites where water pH ranged from 5.16–7.83. This indicates that *N. leibigii* can tolerate slightly acidic water as well. But, Mathew *et al*. (2014) suggested that extreme acidic condition was also found harmful for amphibian. Acidic environment affects the embryological stages (Pierce, 1993). Further, Farquharson *et al*. (2016) reported that chronic acidic exposure can decrease tadpole growth rates and increase abnormalities in tadpoles as well as adult frogs or can even cause mortalities. Pierce (1985) reported that amphibians can tolerate water with lower pH, since many amphibian species breeds during early spring when aquatic micro-habitat has low pH. However, Farquharson *et al.* (2016) suggested tadpole's size decrease and deformities increase with decreasing pH. Meanwhile, mortality is higher if water pH threshold crosses below 4 or exceeds 8 (Pierce, 1985; Pierce, 1987). Therefore, the normal range of pH for preferred habitat seems to range from 4–8 (Odum and Zip-

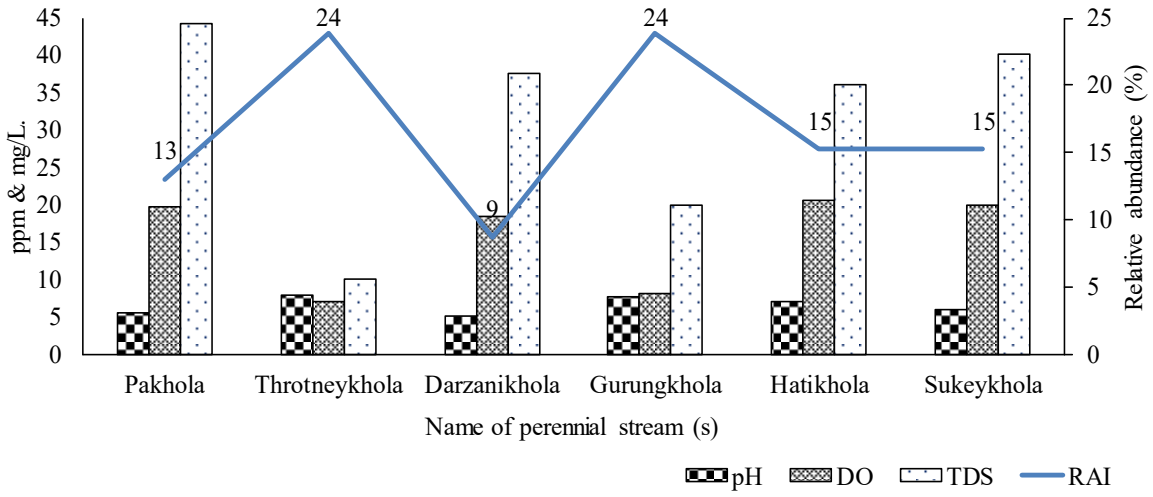

Figure 4: Relationship between water parameter and relative abundance

pel, 2011).

In case of dissolved oxygen, relative abundance (n = 24) was highest in mean DO of 7.6 mg/l and lowest (n = 9) in 7 mg/l. However, *Nanorana leibigii* occurred within the DO ranging from 7–20.54 mg/L in case of Simkharthang watershed. Odum and Zipple (2011) reported that normal range of DO for aquatic animals is 5–6 mg/l. The variance of DO between the threshold limits and incumbent results could be due to spatio-temporal change of water quality which could be attributed to different concentration of organic matters (Ngodhe *et al.,* 2014) and diurnal fluctuation due to daylights (Albarta Environment Protection, 1997). Odum and Zipple (2011) reported that if the threshold of DO recess below 5 mg/l, aquatic animals experience DO stress which will increase or decrease mortality rates. For instances, Adams and Saenz (2012) and Saenz *et al*. (2013) found that decreased dissolved oxygen levels in water can cause mortality in anuran larvae since it affects rate of air breathing in larval amphibians. Nonetheless, it can also change the behavior of amphibian larvae (Crowder *et al*., 1998). Thus, DO levels in surface water body indicate the ability to support aquatic life (Tiwari *et al*., 2016) including the amphibian fauna.

The relative abundance was found highest (n = 24) in mean TDS of 15 ppm and lowest (n = 9) in 37.7 ppm. However, Hoffmaster *et al*. (2007) reported that standard TDS value (healthy TDS) for amphibian survival is 50-250 ppm (parts per million) and anything below or above this range is unhealthy for amphibians. TDS lower than 50 ppm impedes an amphibian in finding sufficient nutrient as per Hoffmaster *et al*. (2007). The study sites was found with lower (10-44.4 ppm) TDS than the threshold limits (50-250 ppm) which could be the main reason for less abundance of *N. leibigii* within the study area. However, amphibian population is also affected if the TDS changes beyond 250 ppm since excess nutrient contains harmful toxins that can destroy the eggs and affect the population (Hoffmaster *et al*., 2007).

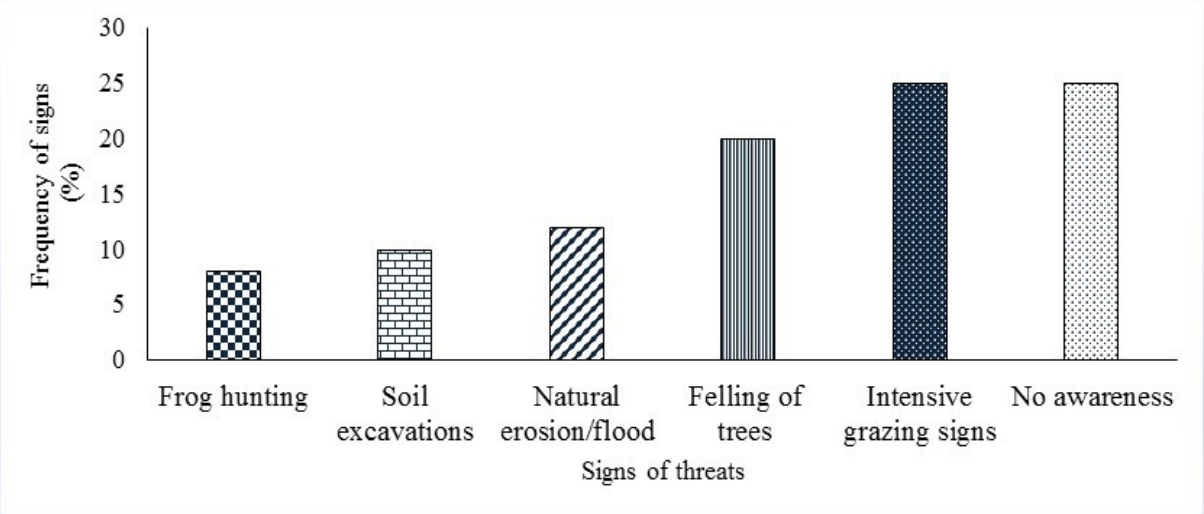

Figure 5: Signs of threats encountered within the niche habitat of *N. leibigii*

Conservation threats

Threats are activities that cause impacts to the niche habitat. Figure 5 show that signs of threats recorded in the niche habitat and as perceived by the local people dwelling within the proximity of the study area (Simkharthang village). The result shows that the study area has intensive grazing activity in the niche habitat areas of the *N. leibigii*, whereby there is lack of conservation awareness among the local people. This study found that the study area was once registered grazing land (Tsamdro) of Bumthap prior to nationalization of forest in 1969. Grazing in the area is common especial-

ly during winter along the river banks of six tributaries which is aggravated by local cattle herds. Hoffmaster *et al.* (2007) reported that cattle can increase soil erosion and create additional run-off, and lead to high levels of TDS that harm the amphibian. Nonetheless, grazing, lopping of fodder trees, collection of frogs, collection of rural house-building timbers are common practices within the study area. These activities modify or alter the niche habitat thereby affecting the presence of amphibian communities (Stuart *et al*., 2004; Gallant *et al.,* 2007; Jame *et al.,* 2009; Smith *et al*., 2009). Further, Chettri *et al*. (2011) reported that human intervention is one of the main drivers for amphibian decline. People in the study area were less aware on the frog conservation since the Simkharthang village is far from the gewog centre. So grazing regulation meetings and conservation awareness campaigns can be organized frequently to benefit the *Nanorana leibigii* conservation in the study area.

The frog collection practice was prevalent prior to 1980s when patients were treated for various ailments such as the chickenpox, leprosy and measles by eating the flesh of *Nanorana leibigii* (D.M. Tamang, Pers. Comm. 20 August, 2014). Now, the tradition of hunting frogs has declined due to availability of modern health facilities. Very few households continue to collect the frog for medical purposes especially for treating common cough, pneumonia, diarrhea and dysentery as is reported from Sikkim by Chettri *et al*. (2011). Further, drying up of existing streams, ponds and wetlands due to impact of climate change is also reported as a threat for declining amphibian populations worldwide (McMenamin *et al.,* 2008).

Conclusion

A study on the distribution and conservation threats of *Nanorana leibigii* was conducted using opportunistic visual encounter survey and systematic hand search method in the six tributaries of Simkharthang and the villages nearby. The frog prefers perennial streams with clean-running water with slightly alkaline pH and having abundant decaying vegetable matters in the stream beds. Mosses-laden rocks with adequate spaces underneath are preferred by the frogs for refuge as well as for breeding. There was no significant difference ($p > 0.05$) among the water parameters such as the conductivity in the six tributaries. Relative abundance was recorded higher in slightly alkaline pH water of undisturbed cool broadleaved forest streams. While the frog collection is relatively fewer these days, regular monitoring is required in the prime habitat and frog collection should be avoided during the breeding season. Conservation awareness campaigns could protect the frog from over collection during breeding season. Also, the medical efficacy of treating various ailments suing the frog flesh is still untested, which requires separate studies perhaps to dispel the myth in future. A comprehensive conservation plan for frogs can be prepared and implemented by engaging local people as beneficiaries. Further, studies on ecological and behavioral change are required to assess the population status, distribution, habitat ecology and vulnerability of crepuscular frogs due to climate change.

Acknowledgement

The authors are grateful to Rufford Small Grant Foundation based in UK for funding the study. Dr. L Scott Mills, Professor at Montana University, USA; Dr. D.B. Gurung, Professor at the College of Natural Resources (CNR), RUB and Mr. Sangay Wangchuk, UWICER are acknowledged for their invaluable assistance. The authors are also thankful to Mr. Singay Wangchuk, Range Officer, Gelephu and other forestry staff of Gelephu Range for their support during data collection. The authors are thankful to reviewers. The communities of Simkharthang village and Jigmecholing gewog, Sarpang district are thanked for their hospitality during the entire research period.

References

Adams, C.K. and Saenz, D. (2012). Leaf litter of the invasive Chinese tallow (*Triadica sebifera*) negatively affects hatching success of an aquatic breeding anuran, the southern leopard frog (*Lithobates shenocephalus*). *Canadian Journal of Zoology*, 90:991–998.

Ahmed, M.F., Das, A. and Dutta, S.K. (2009). *Amphibians and Reptiles of Northeast India-A Photographic Guide*. Bhabani Offset and Imaging Systems Pvt. Ltd, Assam, India.

Albarta Environment Protection. (1997). *Alberta water quality guideline for the protection of freshwater aquatic life.* Standards and Guidelines Branch, Environmental Assessment Division, Edmonton, Alberta.

Biswas, S. (1976). Reptilia from Bhutan with description of a new species of *Calotes rafinesque*. *Journal of Bombay Natural History Society,* 72:774-777.

Browne, C.L. and Paszkowski, C.A. (2009). The relationship of amphibian abundance to habitat features across spatial scales in the Boreal Plains. *Ecoscience,* 16(2):209-223. doi: https://doi.org/10.2980/16-2-3220.

Campbell, H.W. and Christman, S.P. (1982). Field techniques for herpeto-faunal community analysis, pp. 193 -200. In *Herpetological communities*, eds. N.J. Scott. Department of the Interior, Fish and Wildlife Service, U.S.

Chhetri, B., Acharya, B.K. and Bhupathy, S. (2011). *An overview of the herpeto-fauna of Sikkim with emphasis on the elevation distribution pattern and threats and conservation issues.* Retrieved on September 20, 2017.

Corn, P.S. and Bury, R.P. (1989). Logging in western Oregon: Responses of headwater habitats and stream amphibians. *Forest Ecology and Management,* 29:39-57.

Crowder, W.C., Nie, M. and Ultsch, G.R. (1998). Oxygen uptake in bullfrog tadpoles (*Rana catesbeiana*). *Journal of Experimental Zoology*, 280:121–134.

Daniel, J.C. (2002). *The book of Indian Reptiles and Amphibians*. Oxford University Press, India.

Das, I. and Palden, J. (2000). A herpetological collection from Bhutan with new country records. *Herpetological Review,* 31(4):256-258.

Deuti, K. (2010). *Nanorana leibigii* from Bhutan. *Herpetological Review,* 41(1):104.

Farquharson, C., Wepener, V. and Smit, N.J. (2016). Acute and chronic effects of acidic pH on four subtropical frog species. *Water SA*, 42(1). doi: http://dx.doi.org/10.4314/wsa.v42i1.07.

Frost, D.R. (1985). *Amphibian species of the world: A Taxonomic and geographic reference*. Allen Press, Lowrence.

Frost, D.R. (2014). *Dicroglossidae Anderson, 1871: Amphibian Species of the World.* Retrieved on July 20, 2017.

Gallant, A.L., Klaver, R.W., Casper, G.S. and Lannoo, M.J. (2007). Global rates of habitat loss and implications for amphibian conservation. *Copeia,* 2007(4):967–979.

Gurung, D.B., Ugyen, and Tshering, D. (2012). *Mon-paa Frog Survey Report from Mithun village (24th – 27th April 2012) under Dophuchen, Samtse district* (Unpublished technical report). District Forestry Sector, Samtse, Bhutan.

Gururaja, K.V. (2002). *Effect of habitat fragmentation on distribution and ecology of Anurans in Central Western Ghats*. Kuvempu University, Shankaraghatta.

Hoffmaster, C.B.C., Joyner, J.B.D., Leive, K.B.R., Pullen, K.C.J., Price, M.C.E., Ruiz, J.C.G., Solomon, E.E.A., Walls, J.H.T. and Yow, G.H.D. (2007). *A comparative study of amphibian populations in agricultural and non-agricultural aquatic ecosystems*. Retrieved on November 10, 2017.

IUCN Redlist. (2017). *Nanorana leibigii. Accessed from www.iucnredlist.org/details/58428/0,* Retrieved on August 9, 2017.

James, T.Y., Litvintseva, A.P., Vilgalys, R., Morgan, J.A.T., Taylor, J.W., Fisher, M.C., Berger, L., Weldon, C., Preez, L., Longcore, J.E. (2009). Rapid global expansion of the fungal disease chytridiomycosis into declining and healthy amphibian populations. *Plos Pathogens* 5. doi: https://doi.org/10.1371/journal.ppat.1000458.

Johana, J.N., Muzzneena, A.M, Grismer, L.L. and Norhayati, A. (2016). Species composition, diversity and relative abundance of amphibians in forests and non-forest habitats on Langkawi Island, Peninsular Malay-

sia. *AIP Conference Proceedings*, 1784(1). doi: https://doi.org/10.1063/1.4966873.
Kuensel. (2012). The forbidden frog. *Kuenselonline,* 2(30):1-2.
Mathew, R. and Sen, N. (2010). *Pictorial Guide to the Amphibians of North East India.* Power Printer, Kolkata, India.
Mathew, S., Barshy, B. and Hobday, D. (2014). *The basic truth: How pH level affect amphibian abundance*. Retrieved from web on November 10, 2017.
McMenamin, S.K., Hadly, E.A. and Wright, C.K. (2008). Climatic change and wetland desiccation cause amphibian decline in Yellowstone National Park**.** *PNAS*, 105(44):16988-16993. doi: https://doi.org/10.1073/pnas.0809090105.
Molur, S. (2008). South Asian amphibians: taxonomy, diversity and conservation status. *International Journal of Zoology,* 42:143–157. doi:10.1111/j.1748-1090.2008.00050.x.
Ngodhe, S.O., Raburu, P.O. and Achieng, A. (2014). The impact of water quality on species diversity and richness of macroinvertebrates in small water bodies in Lake Victoria Basin, Kenya. *Journal of Ecology and Natural Environment*, 6(1): 32-41. https://doi.org/10.5897/JENE2013.0403.
Odum, R.A. and Zippel, K. (2011). *Water quality*. Accessed from www.amphibianark.org. Retrieved on August 20, 2017.
Palden, J. (2003). New record of *Tylototriton verrucosus* Anderson, 1871 from Bhutan. *Hamadryard,* 27:286–287.
Pierce, B.A. (1992). The effect of acid precipitation on amphibians. *Journal of Ecotoxicology,* 2: 65-77.
Pierce, B.A. (1985). Acids tolerance in Amphibians. *Journal of Bioscience,* 35(4):239-243. doi: https://doi.org/10.2307/1310132.
Pierce, B.A. (1987) The effects of acid rain on amphibians. *The American Biology Teacher,* 49(6):342-347. doi: http://dx.doi.org/10.2307/4448545.
Purushotham, C.B., Daharmadhikari, K. and Vivek, R. (2011). A comparison of hill stream anuran diversity across two habitats in Kalakad-Mundanthurai Tiger Reserve: A pilot study. *Frog Leg,* 15:2-9.
Saenz, D., Fucik, E.M. and Kwiatkowski, M.A. (2013). Synergistic effects of the invasive Chinese tallow (*Tridica sebifera*) and climate change on amphibian survival. *Ecology and Evolution*, 3:4828–4840.
Schleich, H.H. and Kästle, W. (2002). Amphibians and Reptiles of Nepal. In *Biology, Systematics, Field Guide* R. G. Gantner, eds. pp.1211. Ruggell, Germany.
Smith, K.G., Lips, K.R., Chase, J.M. (2009). Selecting for extinction: nonrandom disease-associated extinction homogenizes amphibian biotas. *Ecol. Lett.*, 12:1069-1078. doi: 10.1111/j.1461-0248.2009.01363.
Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L. and Waller, R.W. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science*, 306:1783–1786.
Sutherland, W.J. (2006). *Ecological Census Techniques: A handbook*. Cambridge University Press, UK.
Tiwari, A., Dwivedi, A.C. and Mayank, P. (2016). Time scale changes in the water quality of the Ganga River, India and estimation of suitability for exotic and hardy fishes. *Hydrology Current Research,* 7(3): 1-8. doi: 10.4172/2157-7587.1000254.
Vasudevan, K. (1996). Effect of rainforest fragmentation on Western Ghats [WG] Amphibian. *Frog leg,* 1(2): 1–4.
Wangyal, J. and Gurung, D.B. (2012). Amphibians of Punakha-Wangdue Phodrang Valley, Bhutan. *Frog leg*, 18: 31-44.
Wangyal, J.T. (2014). The Status of herpitofauna of Bhutan. *Journal of Bhutan Ecological Society,* 1: 20-39.
Wangchuk, S., Choden, Y., Dawa, T., Dorji, T. and Kaka. (2017). *Morphometric study on Mon-paa Frog: A case study of Dophuchen, Dumtoe and Tendruk Gewog under Samtse District* (Unpublished Technical report). District Forestry Sector, Samtse, Bhutan.