



**Morphometric Variations and Factors Affecting Abundance
of Indian Bullfrog (*Hoplobatrachus tigerinus*) in Madhesh
Province, Nepal**

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A dissertation submitted

**In partial fulfillment of the requirements for the award of the degree
of Master of Science in Zoology with special paper Ecology and
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**Dissertation submitted in partial fulfilment of the requirements for the
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March 2024

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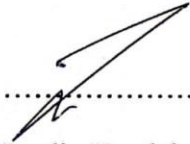
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Declaration

I hereby declare that the work presented in this dissertation “Morphometric variations and factors affecting abundance of Indian bullfrog (*Hoplobatrachus tigerinus*) in Madhesh Province, Nepal” has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the authors or institutions.



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Recommendation

This is to recommend that the dissertation entitled “Morphometric variations and factors affecting abundance of Indian bullfrog (*Hoplobatrachus tigerinus*) in Madhesh Province, Nepal” has been carried out by Pradip Kandel for the partial fulfilment of Master’s Degree of Science in Zoology with special paper Ecology and Environment. This is his original work and has been carried out under my supervision. To the best of my knowledge, this dissertation work has not been submitted for any other degree in any institutions.

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Letter of approval

On the recommendation of supervisor “Assistant Professor Dr. Bishnu Prasad Bhattarai” this dissertation submitted by Pradip Kandel entitled “Morphometric variations and factors affecting abundance of Indian bullfrog (*Hoplobatrachus tigerinus*) in Madhesh Province, Nepal” is approved for the examination in partial fulfillment of the requirements for Master’s Degree of Science in Zoology with special paper Ecology and Environment.

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Certificate of acceptance

This dissertation work submitted by Pradip Kandel entitled "Morphometric variations and factors affecting abundance of Indian bull frog (*Haplobatrachus tigerinus*) in Madhesh Province, Nepal" has been accepted as a partial fulfilment for the requirements of Master's Degree of Science in Zoology with special paper Ecology and Environment.

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Abstract

Indian bullfrogs (*Hoplobatrachus tigerinus*) serve a diverse array of ecosystem services, acting as pest controllers and bio-indicators. However, their numbers are declining due to various factors, including climate change, biological invasion, and habitat destruction. Despite the diverse habitat in Madhesh Province, comprehensive information about the Indian bullfrog is little known. This study examined the morphometry of the species and factors influencing their abundance. For this study, 56 grids having size of $5 \times 5 \text{ km}^2$ were laid in seven districts (8 grids in each district) of Madhesh Province. In each grid at least three transects of 100 m length were surveyed based on available habitat types using visual encounter survey method. The species was searched for 30 minutes by two observers. All encountered individual were captured, sexually identified and morphometric characters were measured. Furthermore, factors affecting abundance such as distance to road, forest, farmland, settlement, water bodies, humidity, temperature and time of survey were measured in each transects. This study revealed that there was no significant morphometric difference between sexes of this species. The abundance of Indian bullfrog was affected by nearest distance to road and temperature. Distance to road had positive effects (0.17 ± 0.08 ; $p = 0.04$), whereas temperature had negative effects (-0.22 ± 0.1 ; $p = 0.03$). This study suggests conducting research spanning multiple phases in the monsoon season to obtain a comprehensive understanding of the fluctuations in morphological traits and factor affection on their abundance.

शोध सार

सिके पाहा भ्यागुताले किराहरुको नियन्त्रण तथा जैविक सुचकको रूपमा विविध पारिस्थितिक सेवाहरु प्रदान गर्दछ । यद्यपि, जलवायु परिवर्तन, मिचाहा प्रजाति र वासस्थानको विनाशका कारण तिनीहरुको संख्या घट्दै गईरहेको छ । मधेश प्रदेशमा विविध उपयुक्त वासस्थानका बावजुत पनि सिके पाहा सम्बन्धि यस अघि न्युन जानकारी रहेको थियो । यस अध्ययनले उक्त प्रजातिको मोर्फोमेट्रिक र तिनीहरुको संख्यालाई प्रभाव पार्ने कारकहरुको खोजि गरेको थियो । त्यसका लागि मधेश प्रदेशका सात जिल्लामा ५६ वटा ५×५ वर्ग किलोमिटर को ग्रिडहरु निर्माण गरियो । प्रत्येक ग्रिडमा उपलब्ध वासस्थानको आधारमा कमिमा ३ वटा १०० मिटरका ट्रान्जेक्टहरुमा भिजुअल इन्काउन्टर विधिबाट सिके पाहाको खोजि गरियो । प्रत्येक ट्रान्जेक्टमा दुई व्यक्तिद्वारा ३० मिनेट सम्म भ्यागुताको खोजि गरि भेटिएका पाहाहरुलाई समातेर तिनीहरुको शारीरिक नाप लिनुका साथै तिनीहरुको लिङ्ग पनि पहिचान गरियो । यस अध्ययनले लिंगहरु बीच कुनै महत्वपूर्ण भिन्नता देखाएको छैन । सिके पाहा भ्यागुताको संख्यामा सडकको दूरीले सकारात्मक र तापक्रमले नकारात्मक प्रभाव पारेको थियो । भ्यागुताको संख्या मनसुनको विभिन्न समयमा फरक पर्न सक्ने हुनाले मनसुनको सम्पूर्ण समयावधिमा अध्ययन गर्न सिफारिस गरिन्छ ।

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1. Introduction

1.1 Background

Amphibians have successfully colonized a diverse range of habitats playing a crucial role in food chain and effective regulation of agricultural pests (Pokhrel & Thakuri, 2016). It provides a diverse array of ecosystem services as it acts as pest controllers (Hocking and Babbitt, 2014; Khatiwada et al., 2016) and bio indicators of ecosystem health (Saber et al., 2017) since it is sensitive to small changes in aquatic and terrestrial habitats (Becker et al., 2010). In Nepal, 57 species of amphibians belonging to 22 genera, 8 families, and 3 orders are found (Rai et al., 2022) and this diversity is due to its diverse geography (Kästle et al., 2013). Amphibians exhibit a wide range of distribution patterns and are distributed across a wide altitudinal range from 80m to 5,490m in Nepal (Shah & Tiwari, 2004). However, globally there is decline in amphibian populations due to climate change, invasion, and habitat degradation (Hamer and McDonnell, 2008; Blaustein et al., 2011; Buckley et al., 2012; Pilliod et al., 2012). In addition, increasing urbanization causes a reduction in the density of anurans (Bowles et al., 2006; Gagne & Fahrig, 2007; Miller et al., 2007) placing them at the forefront of the ongoing biodiversity crisis (Bishop et al., 2013).

The Indian bullfrog (*Hoplobatrachus tigerinus*) is an amphibian of order Anura that exhibits green and brown coloration on its dorsal body with distinct dark patches, featuring fore and hind limbs with dark bars or blotches, and thighs often marbled posteriorly, typically in black and occasionally in yellow, while ventral part is whitish in color (Tagar et al., 2019). The head is pointed, longer than broad and from the snout to the vent there runs a light yellowish or white mid dorsal line (Nepali, 2023). Their nostrils are located closer to the mouth than the eye, and they have narrow inter-orbital space with a distinct tympanic membrane and short fingers (Tagar et al., 2019).

The Indian bullfrog has the distribution across the South Asia from Myanmar to Afghanistan including Nepal (Padhye et al., 2008). It resides near majority of the wetland areas from low land to 2,000 m elevation in Nepal (Khatiwada & Haugaasen, 2015). The species inhabits in holes and bushes near permanent water bodies, and forages terrestrially at water margins (Dutta, 1990). It consumes a wide range of prey including arthropods, annelids, mollusks (Freed, 1982) with higher percentage of Coleoptera and

Hymenoptera (Sapkota, 2022; Sapkota et al., 2022). Frogs are amazing creatures that are sometimes excellent hunters (Jancowski & Orchard, 2013) as Indian bullfrog was found consuming small blind snake (Sapkota, 2022).

Various factors including climatic, biological, geographical, and anthropogenic activities, contribute to the amphibians abundance (Rahbek, 1995; Khatiwada and Hugaasen, 2015; Khatiwada et al., 2019; Aryal et al., 2020; Nepali and Singh, 2021). Studies have established that factors such as water quality (Bank & Beebee, 1987) urbanization (Baillie et al., 2004; Beebee & Griffiths, 2005; A. J. Hamer & McDonnell, 2008; Measey & Tolley, 2011), vegetation structure (Bosch and Inigo, 2003; Bousbouras and Ioannidis, 1997), type of aquatic habitat and substrate (Bousbouras & Ioannidis, 1997) influence distribution and abundance of amphibians.

Morphometric characteristics of amphibian play a vital role in identification of species, conducting phylogenetic analyses, and comprehending evolutionary shifts in physical attributes. Morphology of frogs reveals adaptations for specific ecological roles and behaviors. However, there is a notable lack of consistency in both measurements and descriptions across different taxonomic groups, and even within them (Dubois, 2010). Arbour and Brown (2014) emphasized the importance of precise measurement of amphibian morphometrics in taxonomy. Morphometry is a quantitative analytical approach, which encompasses both size and shape considerations (Singleton et al., 2011). In the case of frogs, between 12 to 16 morphometric traits are typically utilized to differentiate species (Watters et al., 2016).

Overall, this research on Indian bullfrog is crucial for addressing the knowledge gaps about the morphometry and factors that affect the abundance of Indian bullfrog in Madhesh Province.

1.2 Statement of problem

The limited ability of Indian bullfrog for dispersion increases threats as of ongoing global climate change. Even small fluctuations in water levels in their breeding ponds can result in reproductive failure, causing a significant decline in the population size (Thuiller & Pearson, 2006). If these changes persist, it can ultimately lead to the extinctions. In addition, most of the amphibian studies in Nepal are focused to identify the diversity and distribution of amphibians. As a result there remains a knowledge gap in the species

specific study of amphibians in Nepal. Furthermore, the existing knowledge lacks detailed information about the morphometry and factors influencing Indian bullfrog abundance in Madhesh Province. Addressing these knowledge gaps will contribute significantly to the effective conservation and management of Indian bullfrog in this region.

1.3 Objectives

1.3.1 General objective

The general objective of this study was to examine the morphometric variations and the factors affecting abundance of Indian bullfrog in Madhesh Province, Nepal.

1.3.2 Specific objectives

- i. To examine the morphometric variations between sexes of Indian bullfrog in Madhesh Province, Nepal
- ii. To assess the factors affecting abundance of Indian bullfrog in Madhesh Province, Nepal

1.4 Research questions

- i. Is there morphometric difference in between sexes of Indian bullfrog in Madhesh Province?
- ii. What are the significant factors that influence the abundance of Indian bullfrog in Madhesh Province?

1.5 Significance of the study

Indian bullfrog has significant role in the ecosystem and contributes significantly to public health by consuming the larvae of dengue vectors (Murugan et al., 2015). Understanding and documenting the factors that affect the abundance of this ecologically and medically important species is crucial.

Madhesh Province is an important habitat for Indian bullfrog, having different habitats that provide suitable environment. However, little information is known about the factors that affect the abundance of Indian bullfrog and it is important as this species is listed in CITES Appendix II (CITES, 2023). This study provides information on the morphometric

as well as the factors that affect the abundance of this species. This study helps to provide detail knowledge on the abundance of Indian bullfrog.

1.6 Limitations of the study

Primarily, the study was conducted during the late monsoon season, representing only one season of data collection. Consequently, variations in factors influencing the abundance of the Indian bullfrog across different seasons were not fully explored. Secondly, the selection of sampling grids was influenced by the availability of accommodations, leading to a clustering of grids. Moreover, the selection of transects within these grids was based on available habitat types, which could introduce bias if certain habitats are disproportionately represented. Furthermore, our survey was confined to lower elevations of Madhesh Province due to challenges in accessing the Chure region. These constraints may have influenced the comprehensiveness and representativeness of our findings.

2. Literature review

2.1 Morphometric study of Indian bullfrog

Morphometric characters within populations is used for the investigation of the impacts of biotic and abiotic factors on biological populations over time (Ballinger, 1977). In addition to that morphological characteristics have been utilized both in distinguishing between species and in the identification of new species. For example, after conducting molecular studies followed by morphometric measurements, researchers identified a new species within genus *Hoplobatrachus* (Thongproh et al., 2022).

Sexual dimorphism in body size is influenced by foraging competition (Shine, 1989), age differences (Liao et al., 2011; Monnet and Cherry, 2002; Young, 2005), survival strategy (Vargas-salinas, 2006), elevation (Ashton & Feldman, 2003), local climate (Zhang et al., 2014), altitude and longitude (Schäuble, 2004). However study conducted by Amor et al. (2009) in Tunisia found no significant difference between sexes of *Rana saharica*. Similarly, Amor et al. (2010) also found no difference in *Pelophylax saharicus* species. The external morphology of *Pelophylax bedriagae* was found no statistical sexual difference in northwestern, western and southwestern of Iran (Pesarakloo et al., 2018). In Pakistan, Indian bullfrogs had a snout-vent length of 108.5 ± 10.2 mm, snout length of 18.8 ± 1.8 mm, head length of 35.7 ± 1.9 mm, and eye diameter of 9.4 ± 0.8 mm (Majid et al., 2023). However in Nepal Indian bullfrogs were found to have a snout-vent length ranging from 85.0 to 115.0 mm, head length from 29.0 to 37.0 mm, head width from 34.0 to 41.0 mm, forelimb length from 48.0 to 55.0 mm, and hind limb length from 95.0 to 117.0 mm (Nepali, 2023). In the morphometric assessment of both male and female of Indian bullfrog, body length measured 135.8 ± 3.0 in males and 112.5 ± 10.5 in females (Tagar et al., 2019). In contrast to that, Khatiwada et al. (2021) found that the snout-vent length of female Indian bullfrogs (55–113 mm) was higher compared to males (49–135 mm).

Hillman et al. (2008) and Moen et al. (2013) found that frog species inhabiting the similar habitat displayed both similar morphology and performance, irrespective of their geographic location. Likewise, the study from different geographical region of central Europe did not find any notable morphometric difference between sexes, suggesting that the sexual dimorphism in *Rana arvalis* may not be pronounced in terms of external

morphological characteristics (Babik & Rafiński, 2000) which may be due to similar climatic factors. Significant morphometric differences in morphometric character were observed in between surveyed geographic location in Tunisia and Algeria where total four morphotypes of *Pelophylax saharicus* were exist (Amor et al., 2010). The morphological and morphometric variations of water frog populations (*Pelophylax*) from different localities of Iran were studied (Mohaymeni et al., 2022).

Most of the previous studies on frogs have focused on their diversity and distribution and limited research has been conducted on morphology of frog (Khatiwada et al., 2019; Khatiwada et al., 2021; Sapkota et al., 2022; Shrestha et al., 2022; Nepali, 2023) in Nepal.

2.2 Factors affecting abundance of Indian bullfrog

Frogs are found in various habitats, despite that, there are multiple factors associated with the abundance of frogs. Presence and abundance of frogs are likely to be influenced by a number of abiotic factors such as temperature (Pope et al., 2000), hydroperiod (Watson et al., 2003), water quality (Bank & Beebee, 1987; Akram et al., 2015) and biotic factors such as vegetation structure in their habitat (Bosch and Inigo, 2003; Akram et al., 2015). In addition, predation and invasion also alters the structure of amphibian communities (Hamer & Parris, 2013). Even low densities of invasive fish species may have detrimental effects on native amphibians (Bronmark & Edenhamn, 1994; Pilliod et al., 2012). Heavy metals, pesticides, salinity, temperature, disease, competition from introduced species and human collection and human influenced habitat changes are also responsible decline in number of frogs (Ferraro & Burgin, 1993).

Gillespie et al. (2004) reported that *Limnonectes cf. grunniens* and *Limnonectes cf. modestus* showed association with the rocks and ground and Ra et al. (2010) found high abundance of gold-spotted pond frog (*Rana chosenica*) from sites with greater coverage of shallow vegetated water. Similarly, the occurrence of carpenter frogs was significantly correlated with wetland, pH, hydroperiod, wetland size, and terrestrial forest cover. The frequency of carpenter frog occurrence showed a positive relationship with forest cover, wetland size and a negative relationship with pH (Otto et al., 2007). The abundance of *Phyzelaphryne miriamae* was negatively associated with precipitation, and a positive relationship with deeper water, suggesting that this species tends to be found in places that do not flood in drier areas (Moreno et al., 2022).

Indian bullfrogs abundance is associated with multiple factors such as, elevation, vegetation type, water quality, resource availability, distance to road (IUCN, 2023). The structure of the amphibian community consistently displayed a reduction in species richness as elevation increases (Malonza and Veith, 2012; Khatiwada and Haugaasen, 2015; Khatiwada et al., 2017, 2019). At lower elevations, Indian bullfrog emerged as one of the predominant species (Mishara, 2019).

Amphibian diversity was observed to be greater near water sources, with the presence of water bodies showing a statistically significant positive impact on amphibian diversity (Zancoffi et al., 2014; Gautam, 2018). Similarly, Malonza and Veith (2012) found that amphibian species richness increased with habitat disturbance, transitioning from forests to streams and dams.

Ramesh et al. (2013) observed high diversity of frogs within forest habitats in southern India. Thapa (2016) reported the highest diversity of amphibia in agricultural land and the lowest in forests of Palpa. Similarly, Nepali and Singh (2021) recorded that amphibian richness was higher in agricultural fields compared to forests. In case of abundance of amphibian, high abundance was recorded from wetland followed by agriculture, grassland, forest, barren and least at settlement (Gibbons, 2003). Settlement areas exhibited a negative significance with amphibian richness (Gautam, 2018).

Aryal et al. (2020) found a negative association between frog occurrence and soil condition, while observing a positive association with increased distance from roads. Additional research has recognized the impact of environmental factors, including land use (Marsh et al., 2017), human population (McKinney, 2002), urban development (Shu et al., 2016), and linear infrastructure (Vos & Chardon, 1998), on the presence of frogs. Understanding distribution of frogs with relation to different variables is necessary for conservation.

3. Materials and methods

3.1 Study area

This study was carried out within the seven districts (Saptari, Siraha, Dhanusha, Mahottari, Sarlahi, Rautahat and Bara) of Madhesh province (Figure 1). Madhesh province ($84^{\circ}27'$ to $86^{\circ}54'30''$ E and $26^{\circ}23'38''$ to $27^{\circ}28'17''$ N) is situated in the southeastern region of Nepal, covering area of $9,661 \text{ km}^2$ (MPPPC, 2020) in physical contact to the borders of Koshi province to the east, Bagmati Province to the north, and India to the south (LGIS, 2023). The climate of Madhesh Province experiences variation owing due to its diverse topography and altitude, leading to distinct dry and wet monsoon periods. During hot and sunny days, temperatures soar to $38\text{-}40^{\circ}\text{C}$, whereas in winter, plummets to $5\text{-}10^{\circ}\text{C}$ (PPPC, 2023). The province typically receives an average rainfall ranging from 1100 to 2100 mm (Outlook Nepal, 2024).

About 48.19% of the Chure-Tarai Madhesh landscape is used for agriculture and settlement, while approximately 47.16% consists of forest, shrub-land, and grassland. The remaining 4.65% is occupied by rivers and their beds (GoN-RTCM, 2017).

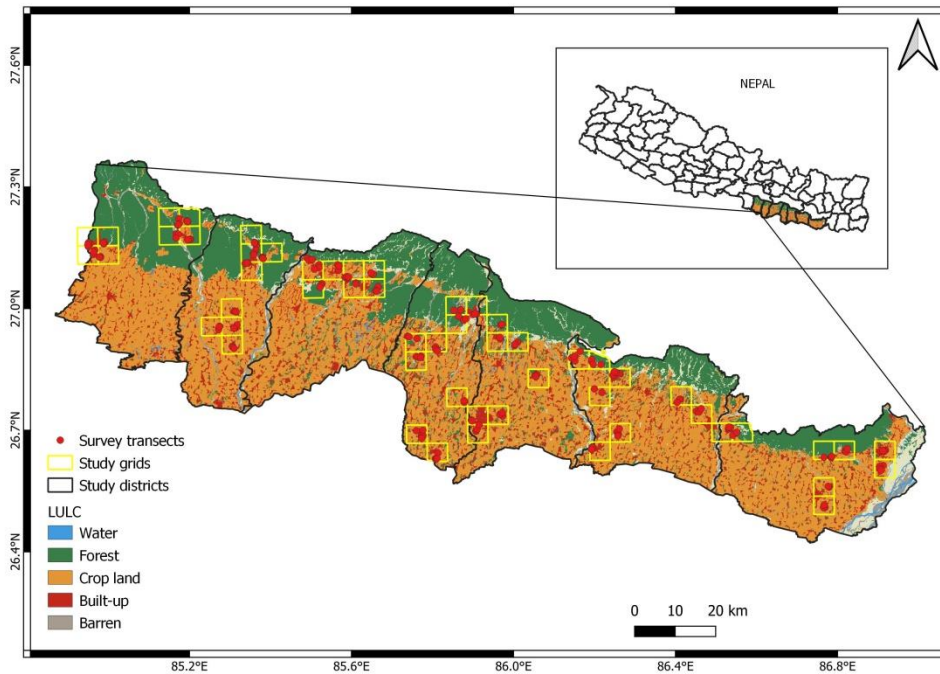


Figure 1. Study grids and transects of Indian bullfrog in Madhesh province, Nepal

Madhesh province harbors many community forests, religious forests, and corridor forests with great ecological value (Nagendra, 2002; Timilsina and Heinen, 2008; Dhakal and Masuda, 2009; DFRS/MoFSC, 2015; Chaudhary et al., 2016). The forest is tropical type with dominated by sal (*Shorea robusta*) with other vegetation like saaj (*Terminalia alata*), kusum (*Cleistocalyx operculata*), North Indian rosewood (*Dalbergia sissoo*), chir pine (*Pinus roxburghii*), toon tree (*Toona ciliate*), uttis (*Alnus nepalensis*), bot dhangero (*Lagerstroemia parviflora*), sindhure (*Mallotus philippensis*), jamun (*Syzygiumcumini*), chilaune (*Schima wallichii*), kapok (*Bombax ceiba*) (Chaudhary & Subedi, 2019). Major faunal species include the Bengal tiger (*Panthera tigris*), leopard (*P. pardus*), Asian elephant (*Elephas maximus*), gaur (*Bos gaurus*), striped hyena (*Hyaena hyaena*), Bengal fox (*Vulpes bengalensis*), golden jackal (*Canis aureus*), jungle cat (*Felis chaus*), large Indian civet (*Viverra zibetha*), smooth-coated otter (*Lutrogaleper spicillata*), and gharial crocodile (*Gavialis gangeticus*) (Jnawali et al., 2011; Chaudhary and Subedi, 2019). Amphibian species like Asian common toad (*Bufo melanostictus*), Indian marbled toad (*Bufo stomatics*), Ornate narrow-mouthed frog (*Microhyla ornate*), Malaysian Narrowmouth Toad (*Kaloula pulchra*), Sri Lankan Bullfrog (*Uperodon taprobanica*), Indian globular frog (*Uperodon globulosus*), Skipper Frog (*Euphlyctis cyanophlyctis*), Jerdon`s bullfrog (*Hoplobatrachus crassus*), Indian bullfrog (*Hoplobatrachus tigerinus*), Madhese kithre bhyaguto (*Minivarya teraiensis*), Spotted Tree Frog (*Polypedates maculatus*), Syhadra frog (*Minivarya syhadrensis*) were documented in this region (Shah & Tiwari, 2004).

3.2 Methods

The study was carried out from August 13 to September 22, 2023 on the predesigned grids of vertebrate fauna survey with each grid of 5 × 5 sq. km. For the survey of Indian bullfrog 56 grids were selected in seven districts using QGIS 3.22.13 with eight grids in each district. In each grid, 3 to 4 transects were selected based on available habitat types within the grid. Transects covered different habitats like agricultural land, forest, grassland, road, human habitat and water bodies.

Visual encounter survey was carried out using capture measure and release method (Heyer et al., 1994). Two observers walked through 180 transects for 30 minutes covering 100 m length and 5 m on the either side of the transects. Further, within transect frogs were also be searched in riverbank, sandy soil, under big boulder, stones and decaying

logs. The search was conducted mostly during evening (18:00 to 22:00) hours and occasionally in day time. As the study was conducted in the night time a torch beam was used to locate the frogs.

The morphometric measurements such as snout vent length (SVL), head length (HL), head width (HW), snout length (SL), eye diameter (ED), tympanum diameter (TD), fore limb length (FLL), length of first finger (L1F), hind limb length (HLL), length of first toe (L1T) and body weight (BWt) were measured using the digital caliper of 0.01 mm accuracy (Khatiwada et al., 2021). The body weight of frogs was measured using digital weighing machine (0.1 gm accuracy) and was released without any harm after measurements and being photographed for reference.

Table 1. Morphological measurement and their descriptions

Morphology	Measurement description
Snout vent length (SVL)	Total length from tip of snout to edge of vent
Head length (HL)	Total length from angle of jaws and snout-tip
Head width (HW)	Distance between posterior angle of jaws
Snout length (SL)	Total length from snout to anterior corner of eye
Eye diameter (ED)	Horizontal diameter of eye
Tympanum diameter (TD)	Largest tympanum diameter
Fore limb length (FLL)	Total length from shoulder to tip of longest finger
Length of first finger (L1F)	Total length from base of first finger to tip of first finger
Hind limb length (HLL)	Total length from hip bone to tip of longest toe
Length of first toe (L1T)	Total length from base of first toe to tip of first toe
Body weight (BWt)	Weight of frog's body

Male and female were identified by observing the vocal sacs as male had colored vocal sac in the breeding season. Individuals which have less than 70 mm of SVL were excluded for the morphometric analysis.

The geographical coordinates of starting and ending points of each transects was recorded using global positioning system (GPS). Similarly, start and end time of the survey was also recorded. In addition, humidity and temperature were measured with the help of digital indoor/outdoor thermo-hygrometer.

Midpoint of transect was measured and was used to calculate the distance from transect to the nearest water, forest, settlement, farmland and road using “NNjoin” package in QGIS. To calculate the distance from water body, permanent water bodies like ponds and the rivers was used. Vector files for water bodies, areas of farmland and grassland, and major roads were extracted from Open Street Map (OpenStreetMap Contributors, 2023), and the vector layer of human settlements was extracted from the website of the Humanitarian Data Exchange (Humanitarian Data Exchange, 2015). Time of the survey was divided into three category as day (before 18:00), evening (18:00–20:00) and night (20:00–22:00).

3.3 Data analysis

The dataset was loaded, and filtered, arranged and summarized using the packages dplyr and tidyverse in R Program. Subsequently, normality of the data was tested using Shapiro Wilk test. For the morphological analysis, student t- test or Mann-Whitney U test was performed based on the normality to know whether there was any morphological difference between male and female Indian bullfrog.

The Generalized Linear Model (GLM) with “poisson” family was used to assess the factor affecting the abundance of Indian bullfrog. Eight factors including distance to road, forest, farmland, settlement, water bodies, humidity, temperature and time of survey were used as explanatory variables. All the continuous variables were standardized with the help of “Wiqid” package (Meredith, 2017). Correlation analysis was performed to identify high correlation $|r| > 0.7$ between explanatory variables as no high correlation was found among variables so all variables were used for the analysis.

For a GLM with a poisson family and log link function, the model equation can be written as:

$$g(\mu) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_n X_n$$

Where,

$g(\mu) = \log(\mu)$ is the log link function, which relates the linear predictor to the expected abundance of the Indian bullfrog.

μ is the expected likelihood of the Indian bullfrog abundance.

$\beta_0, \beta_1, \beta_2, \dots, \beta_n$ are the coefficient associates with intercepts and each explanatory variables.

$X_0, X_1, X_2, \dots, X_n$ are the explanatory variables.

Global model with all explanatory variable was developed using glm function in R Studio. In addition, models with all possible combinations of explanatory variables were generated using dredge function of MuMIn package (Barton, 2023). Given the absence of a dominant model (with distinct Akaike weight), model selection uncertainty was addressed by employing model averaging based on their $\Delta AICc$ (Burnham & Anderson, 2002). The top sets of models with $\Delta AICc \leq 2$ were selected and model averaging were employed to ascertain the effect size (parameter estimates) of each variable across the top set of models (Burnham & Anderson, 2002). All analysis was performed in R program (R Core Team, 2023).

4. Results

4.1 Morphometric variation in Indian bullfrog

During the field survey total of 145 Indian bullfrogs were captured and measured. Out of which 12 were adult male, 13 were adult female and others were juvenile. 11 different morphological characters were measured in all individuals. No significant difference was observed in morphological characters between adult male and female (Table 2).

The average snout-vent length of Indian bull frog was [male: 98.49 ± 12.50 mm (range; 72.88 – 125.10 mm); female: 100.67 ± 14.93 mm (range; 81.39 – 126.80 mm)], average head length was [male: 36.28 ± 5.01 mm (range; 25.56 – 44.90 mm); female: 36.51 ± 4.73 mm (range; 29.25 – 47.21 mm)], average head width was [male: 34.66 ± 5.75 mm (range; 21.28 – 46.23 mm); female: 33.00 ± 5.80 mm (range; 25.11 – 46.07 mm)], average snout length was [male: 15.95 ± 1.97 mm (range; 13.12 – 20.48 mm); female: 16.36 ± 2.18 mm (range; 11.59 – 20.01 mm)], average eye diameter was [male: 10.92 ± 1.39 mm (range; 7.31 – 12.44 mm); female: 10.22 ± 1.67 mm (range; 8.03 – 13.48 mm)], average tympanum diameter was [male: 8.06 ± 0.97 mm (range; 5.41 – 8.98 mm); female: 7.52 ± 1.74 mm (range; 5.18 – 11.45 mm)], average fore limb length was [male: 47.74 ± 9.48 mm (range; 27.86 – 62.99 mm); female: 43.93 ± 8.81 mm (range; 32.22 – 60.96 mm)], average first finger length was [male: 15.68 ± 2.28 mm (range; 9.34 – 18.66 mm); female: 14.87 ± 3.13 mm (range; 9.49 – 20.27 mm)], average hind limb length was [male: 158.12 ± 17.45 mm (range; 114.66 – 193.28 mm); female: 148.21 ± 22.25 mm (range; 110.0 – 180.86 mm)], average first toe length was [male: 16.18 ± 2.96 mm (range; 8.80 – 19.30 mm); female: 14.87 ± 3.89 mm (range; 7.45 – 20.98 mm)] and average body weight was [male: 121.02 ± 45.18 mm (range; 24.50 – 213.90 mm); female: 108.72 ± 61.51 (range; 38.80 – 218.10 mm)]. These measurements didn't show statistically significant difference between male and female of Indian bullfrog (Table 2).

Table 2. Illustration of statistical analysis of the morphometric characterization between sexes of Indian bullfrog in Madhesh province.

Measurements	Male (n=12)		Female (n=13)		P
	Mean \pm SD	Range	Mean \pm SD	Range	
Snout vent length (mm)	98.49 \pm 12.50	72.88 - 125.10	100.67 \pm 14.93	81.39 - 126.80	0.69
Head length (mm)	36.28 \pm 5.01	25.56 - 44.90	36.51 \pm 4.73	29.25 - 47.21	0.91
Head width (mm)	34.66 \pm 5.75	21.28 - 46.23	33.00 \pm 5.80	25.11 - 46.07	0.48
Snout length (mm)	15.95 \pm 1.97	13.12 - 20.48	16.36 \pm 2.18	11.59 - 20.01	0.63
Eye diameter (mm)	10.92 \pm 1.39	7.31 - 12.44	10.22 \pm 1.67	8.03 - 13.48	0.23
Tympanum diameter (mm)	8.06 \pm 0.97	5.41 - 8.98	7.52 \pm 1.74	5.18 - 11.45	0.18
Fore limb length (mm)	47.74 \pm 9.48	27.86 - 62.99	43.93 \pm 8.81	32.22 - 60.96	0.31
First finger length (mm)	15.68 \pm 2.28	9.34 - 18.66	14.87 \pm 3.13	9.49 - 20.27	0.45
Hind limb length (mm)	158.12 \pm 17.45	114.66 - 193.28	148.21 \pm 22.25	110.0 - 180.86	0.57
First toe length (mm)	16.18 \pm 2.96	8.80 - 19.30	14.87 \pm 3.89	7.45 - 20.98	0.35
Body weight (gm)	121.02 \pm 45.18	24.50 - 213.90	108.72 \pm 61.51	38.80 - 218.10	0.57

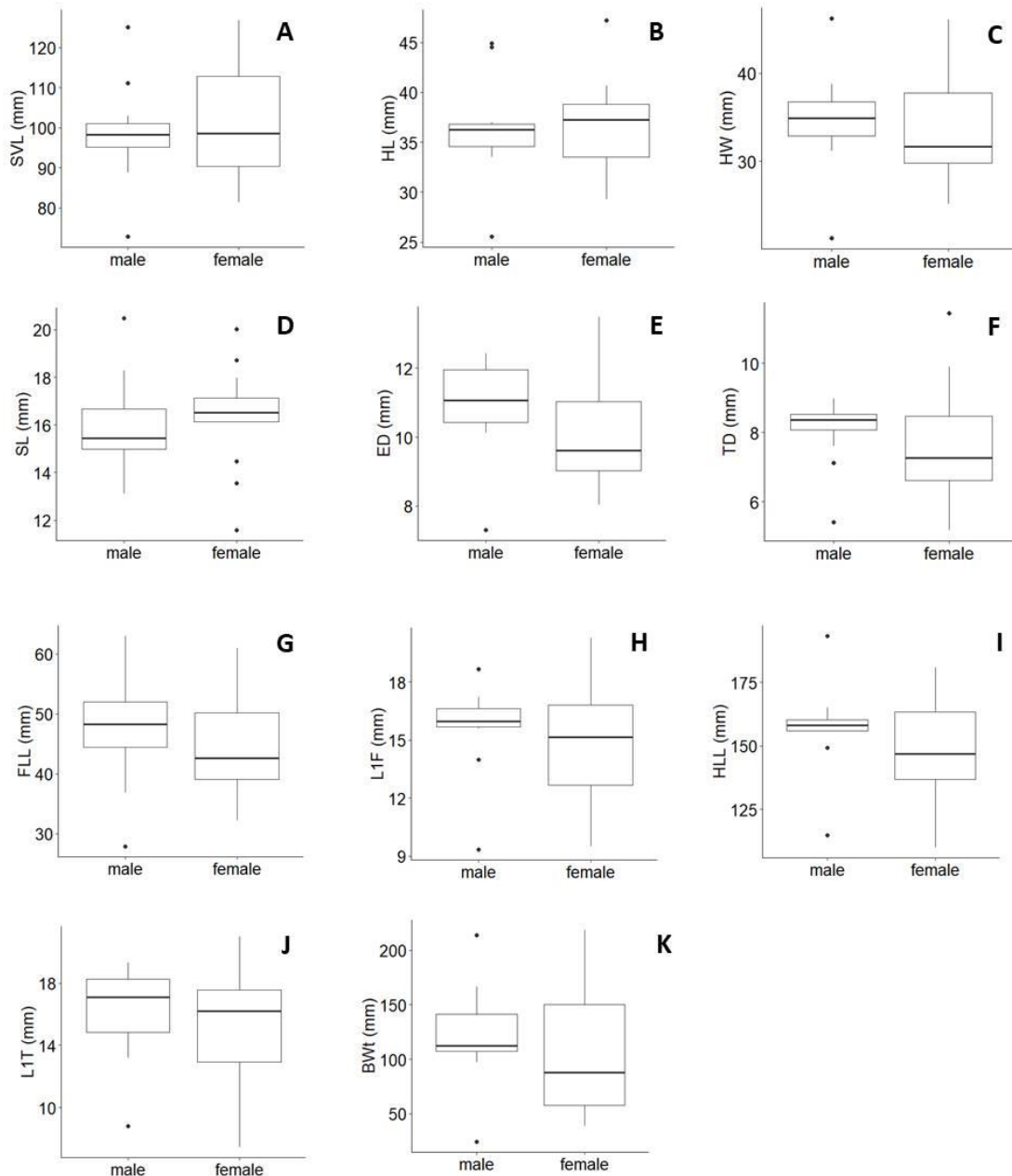


Figure 2. Morphological difference between male and female of Indian bullfrog

4.2 Factors affecting abundance of bull frogs

The average distance from transect to road was (64.42 ± 86.73 m), mean distance to settlement was (246.38 ± 307.67 m), mean distance to water was (236.17 ± 298.54 m), mean distance to forest was (268.91 ± 334.82 m), average distance from farmland was (57.76 ± 140.58 m), average temperature was (27.51 ± 1.48 °C) and mean humidity was (94.08 ± 5.54 %).

Distance to the road and temperature significantly influenced the abundance of Indian bullfrog (Table 3). Predicted Indian bullfrog abundance showed an increase with distance to road and decrease with increase in temperature. Although not statistically significant, Indian bullfrog abundance exhibited a positive association with the distance to water and humidity and negative association with the distance to settlement, evening and night time of the survey, distance to farmland and distance to forest (Table 3).

Table 3. Model-averaged parameters and their Lower Confidence Interval (LCI) and Upper Confidence Interval (UCI) (95%) confidence limits describing factors affecting on the abundance of Indian bullfrog. Abundance of Indian bullfrog was the response variable whereas distance to road (m), distance to settlement (m), distance to farmland (m), distance to forest (m), distance to water (m), temperature (°C), humidity (%) and time of survey was predictive variables. Significance effects are in bold.

Parameters	Estimates	SE	LCI	UCI	Z	P
(Intercept)	-0.03	0.39	-0.79	0.73	0.08	0.94
Distance to road	0.17	0.08	0.01	0.34	2.04	0.04
Distance to settlement	-0.19	0.11	-0.40	0.03	1.69	0.09
Temperature	-0.22	0.1	-0.43	-0.02	2.16	0.03
Time (Evening)	-0.11	0.44	-0.98	0.76	0.25	0.8
Time (Night)	-0.55	0.47	-1.48	0.38	1.16	0.25
Distance to water	0.08	0.07	-0.06	0.23	1.1	0.27
Distance to farmland	-0.09	0.11	-0.30	0.11	0.89	0.37
Distance to forest	-0.06	0.09	-0.24	0.11	0.72	0.47
Humidity	0.03	0.1	-0.17	0.23	0.29	0.78

5. Discussion

5.1 Morphometric variation of Indian bullfrog

Female Indian bull frogs are larger in size than the male, however the difference was not significant. Similar result was found in the study of Tagar et al. (2019) where the body length of female was higher than the male. Sexual size dimorphism (SSD) is common in anurans with nearly 90% of the species studied known to exhibit SSD with larger female size (Shine, 1979; Monnet and Cherry, 2002; Liao et al., 2013). At natural breeding sites, males tend to be smaller than females (Dutta and Mohanty-Hejmadi, 1981) this may be due to males were become sexual mature faster than the female (Gramapurohit et al., 2004). However, in this study by comparing the mean t- test or Mann-Whitney U test based on the normality of the data between sexes did not show statistical significances for all the characters. Similar to this result no significant difference was observed in the study of Amor et al. (2009; 2010) and Pesarakloo et al. (2018).

5.2 Factors affecting abundance of Indian bullfrogs

This study found that the distance from road was positively associated with the abundance of Indian bullfrog. This relation revealed that the abundance of Indian bullfrog decreases as close to the road and suggest that road is one of the disturbance factors that prohibit abundance of this species. Roads are associated with human and vehicle mobility that negatively impact the amphibians (Pellet et al., 2004). Habitat loss is the most important cause of local declines in amphibian populations and that habitat modification directly impacts in amphibians (Goosem, 2007; Hayes et al., 2010).

Linear infrastructures such as roads lead to alterations and loss of wetlands, resulting in decreased breeding habitats and restricted access for adult amphibians to suitable breeding grounds (Vos and Chardon, 1998; Marsh et al., 2017). Similarly, Rytwinski and Fahrig (2015) suggest amphibians are highly vulnerable to negative impacts from roads, including increased mortality rates due to vehicle collisions and habitat fragmentation caused by road infrastructure. In addition, traffic noise associated with roads lowers the abundance and disrupt the breeding ecology of amphibian species (Lukanov et al., 2014). The acoustic signals are employed mainly for communication, to organize the chorus of males and also helpful to advertise specific location to attract receptive females (Rand, 1985). However, noise and vibrations from passing vehicles can disrupt Indian bullfrog

behavior, reducing reproductive success. That may be the reason the number of Indian bullfrog decreases with proximity to the road. Roads are source of pollution, runoff and other sorts of drainages (Hwang et al., 2016), which can leach into nearby wetlands and water bodies where Indian bullfrog resides. Exposure to chemicals from pollutants can negatively impact the health and survival of individual frogs and reduce the population size. Similar results were found by the following studies (Vos and Chardon, 1998; Li et al., 2016; Marsh et al., 2017).

The negative influence between the abundance of Indian bullfrog and temperature in Madhesh Province indicates that it become less active or remain submerged in the water during high temperature. Presence and abundance of amphibian are likely to be influenced by abiotic factor such as temperature (Pope et al., 2000). Increase in temperature predicted to have many consequences on amphibians (Blaustein & Kiesecke, 2002). It possess a thin, sensitive skin responsible for respiration and which is extremely susceptible to moisture loss (Beebee & Griffiths, 2005; Halliday, 2008; Wyman, 1990). A dehydrating frog at high temperatures may be faced with conflicting physiological demands. By increasing dermal mucous secretion, a North American bullfrog (*Rana catesbeiana*) keeps its body temperature below dangerous levels through evaporative cooling, but at the same time accelerates water loss (Lillywhite, 1971). To avoid such type of extreme condition they might be submerged in the water so low abundance was observed during the high temperature.

6. Conclusions and recommendations

6.1 Conclusions

This study examined morphological variation and factors affecting abundance of Indian bullfrog in Madhesh Province, Nepal. The result showed no morphological difference between sexes of the Indian bullfrog. The abundance of this species of frog was positively affected by distance to road and negatively affected by temperature. Understanding these interactions is crucial for maintaining healthy ecosystem.

6.2 Recommendations

This study was conducted in single phase of monsoon season, therefore recommends a multi-phase study as different factors might affect species on different phase. This research underscores the importance of adopting road construction practices that prioritize the preservation of natural habitats and water bodies for frog conservation.

7. References

- Akram, A., Rais, M., Asadi, M. A., Jilani, M. J., Balouch, S., Anwar, M., & Saleem, A. (2015). Do habitat variables correlate anuran abundance in arid terrain of Rawalpindi-Islamabad Areas, Pakistan? *Journal of King Saud University - Science*, 27(3), 278–283. <https://doi.org/10.1016/j.jksus.2015.02.001>
- Amor, N., Farjallah, S., Ben-yacoub, S., Merella, P., & Said, K. (2010). Karyological and morphometric variation of the North African green frog *Pelophylax saharicus* (Anura) in north-eastern Africa. *Current Zoology*, 56(6), 678–686.
- Amor, N., Farjallah, S., & Said, K. (2009). Morphometric variation in the Tunisian green frog, *Rana saharica* Anura: Ranidae. *African Zoology*, 44(2), 194–203. <https://doi.org/10.3377/004.044.0207>
- Arbour, J. H., & Brown, C. M. (2014). Incomplete specimens in geometric morphometric analyses. *Methods in Ecology and Evolution*, 5, 16–26. <https://doi.org/10.1111/2041-210X.12128>
- Aryal, P. C., Aryal, C., Neupane, S., Sharma, B., Dhamala, M. K., Khadka, D., Kharel, S. C., Rajbanshi, P., & Neupane, D. (2020). Soil moisture & roads influence the occurrence of frogs in Kathmandu Valley, Nepal. *Global Ecology and Conservation*, 23, e01197. <https://doi.org/10.1016/j.gecco.2020.e01197>
- Ashton, K. G., & Feldman, C. R. (2003). Bergmann's rule in nonavian reptiles: turtles follow it, lizards and snake reverse it. *Evolution*, 57(5), 1151–1163.
- Babik, W., & Rafiński, J. (2000). Morphometric differentiation of the moor frog (*Rana arvalis* Nilss.) in Central Europe. *Journal of Zoological Systematics and Evolutionary Research*, 38(4), 239–247. <https://doi.org/10.1046/j.1439-0469.2000.384148.x>
- Baillie, J. E. M., Hilton-taylor, C., & Stuart, S. N. (Eds.). (2004). *2004 IUCN red list of threatened species. A global species assessment*. IUCN, Gland, Switzerland and Cambridge, UK.
- Ballinger, R. E. (1977). Reproductive Strategies: Food Availability as a Source of Proximal Variation in a Lizard. *Ecology*, 58(3), 628–635. <https://doi.org/https://doi.org/10.2307/1939012>

- Bank, B., & Beebee, I. J. C. (1987). Factors influencing breeding site choice by the pioneering amphibian *Bufo caiamita*. *Holarctic Ecology*, *10*, 14–21.
- Barton, K. (2023). "MuMIn": Multi-Model Inference. Version 1.47.5 <https://CRAN.R-project.org/package=MuMIn>
- Becker, C. G., Fonseca, C. R., Haddad, C. F. B., & Prado, P. I. (2010). Habitat Split as a Cause of Local Population Declines of Amphibians with Aquatic Larvae. *Conservation Biology*, *24*(1), 287–294. <https://doi.org/10.1111/j.1523-1739.2009.01324.x>
- Beebee, T. J. C., & Griffiths, R. A. (2005). The amphibian decline crisis : A watershed for conservation biology ? *Biological Conservation*, *125*, 271–285. <https://doi.org/10.1016/j.biocon.2005.04.009>
- Bishop, P. J., Angulo, A., Lewis, J. P., Moore, R. D., Rabb, G. B., & Moreno, G. (2013). The Amphibian Extinction Crisis - what will it take to put the action into the Amphibian Conservation Action Plan? *Sapiens [Online]* *5.2*, 5(August), 1–16. <http://sapiens.revues.org/1406>
- Blaustein, A. R., & Kiesecke, J. M. (2002). Complexity in conservation : lessons from the global decline of amphibian populations. *Ecology Letters*, *5*, 597–608.
- Bosch, J., & Inigo, S. (2003). Factors Influencing Occupancy of Breeding Ponds in a Montane Amphibian Assemblage. *South American Journal of Herpetology*, *37*(2), 410–413. [https://doi.org/10.1670/0022-1511\(2003\)037](https://doi.org/10.1670/0022-1511(2003)037)
- Bousbouras, D., & Ioannidis, Y. (1997). The distribution and habitat preferences of the amphibians of Prespa National Park. *Hydrobiology*, *351*, 127–133. <https://doi.org/10.1023/A:1003072709927>
- Bowles, B. D., Sanders, M. S., & Hansen, R. S. (2006). Ecology of the Jollyville Plateau salamander (*Eurycea tonkawae* : Plethodontidae) with an assessment of the potential effects of urbanization. *Hydrobiologia*, 111–120. <https://doi.org/10.1007/s10750-005-5440-0>
- Bronmark, C., & Edenhamn, P. (1994). Does the Presence of Fish Affect the Distribution of Tree Frogs (*Hyla arborea*)? *Society for Conservation Biology*, *8*(3), 841–845. <https://doi.org/10.1046/j.1523-1739.1994.08030841.x>

- Buckley, L. B., Hurlbert, A. H., & Jetz, W. (2012). Broad-scale ecological implications of ectothermy and endothermy in changing environment. *Global Ecology and Biogeography*, 873–885. <https://doi.org/10.1111/j.1466-8238.2011.00737.x>
- Burnham, K. P., & Anderson, D. R. (2002). *Model Selection and Inference: A Practical Information-Theoretic Approach* (Second). Springer-Verlag. <https://doi.org/10.2307/3803117>
- Chaudhary, R. P., & Subedi, C. K. (2019). Chure-terai madhesh landscape, Nepal from biodiversity research perspective. *Plant Archives*, 19, 351–359.
- Chaudhary, R. P., Uprety, Y., & Rimal, S. K. (2016). Deforestation in Nepal: Causes, Consequences, and Responses. In *Biological and Environmental Hazards, Risks, and Disasters*. <https://doi.org/10.1016/B978-0-12-394847-2.00020-6>
- CITES. (2023). Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendices I, II and III. In *CITES and UNEP*. <https://doi.org/10.5040/9798400656385.0078>
- DFRS/MoFSC. (2015). *State of Nepal's forests*. Department of forest research and survey.
- Dhakal, M., & Masuda, M. (2009). Local pricing system of forest products and its relations to equitable benefit sharing and livelihood improvement in the lowland community forestry program in Nepal. *Forest Policy and Economics*, 11(4), 221–229. <https://doi.org/10.1016/j.forpol.2009.02.004>
- Dubois, A. (2010). Describing new species. *Taprobonica*, 02(01), 6–24.
- Dutta, S. ., & Mohanty-Hejmadi, P. (1981). Sex ratio and size correlation of Indian bull frog *Rana tigerina* Daudin. *Pranikee. Pranikee Journal of Zoological Society Orissa India*, 2, 26–36.
- Dutta, S. K. (1990). Ecology, natural history and conservation of herpetofauna of Orissa, India. *Tigerpaper*, 20–28.
- Ferraro, T. john, & Burgin, S. (1993). Review of environmental factors influencing the decline of Australian frogs. *Herpetology in Australia*, 205–218. <https://doi.org/10.7882/RZSNSW.1993.031>
- Freed, A. N. (1982). A treefrog's menu: selection for an evening's meal. *Oecologia*, 53,

20–26.

- Gagne, S. A., & Fahrig, L. (2007). Effect of landscape context on anuran communities in breeding ponds in the National Capital Region , Canada. *Landscape Ecology*, 205–215. <https://doi.org/10.1007/s10980-006-9012-3>
- Gautam, B. (2018). *Diversity and distribution of herpetofauna in Ghandruk region of Annapurna Conservation Area, Nepal*. 53.
- Gibbons, J. W. (2003). Terrestrial habitat: A vital component for herpetofauna of isolated wetlands. *WETLANDS*, 23(3), 630–635.
- Gillespie, G. R., Lockie, D., Scroggie, M. P., & Iskandar, D. T. (2004). Habitat use by stream-breeding frogs in south-east Sulawesi , with some preliminary observations on community organization. *Journal of Tropical Ecology*, 20, 439–448. <https://doi.org/10.1017/S0266467404001361>
- GoN-RTCM. (2017). *President Chure-Tarai Madhesh Conservation and Management Master Plan*.
- Goosem, M. (2007). Fragmentation impacts caused by roads through rainforests. *Current Science*, 93, 1587–1595.
- Gramapurohit, N. P., Shanbhag, B. A., & Saidapur, S. K. (2004). Growth , Sexual Maturation and Body Size Dimorphism in the Indian Bullfrog , *Hoplobatrachus tigerinus* (Daud). *Herpetologica*, 60(4), 414–419.
- Halliday, T. R. (2008). Why amphibians are important. *International Zoo Yearbook*, 42(1), 7–14. <https://doi.org/10.1111/j.1748-1090.2007.00037.x>
- Hamer, A. J., & McDonnell, M. J. (2008). Amphibian ecology and conservation in the urbanising world: A review. *Biological Conservation*, 141(10), 2432–2449. <https://doi.org/10.1016/j.biocon.2008.07.020>
- Hamer, A., & Parris, K. (2013). Predation modifies larval amphibian communities in urban wetlands. *Wetlands*, 33(4), 641–652. <https://doi.org/10.1007/s13157-013-0420-2>.
- Hayes, T. B., Falso, P., Gallipeau, S., & Stice, M. (2010). The cause of global amphibian declines: a developmental endocrinologist 's perspective. *The Journal of*

- Experimental Biology*, 213, 921–933. <https://doi.org/10.1242/jeb.040865>
- Heyer, W., Donnelly, M., McDiarmid, R., Hayek, L., & Foster, M. (1994). Measuring, and monitoring biological diversity: standard methods for amphibians. *Smithsonian Institution Press. Washington, DC, USA.*, 364p.
- Hillman, S. S., Withers, P. C., Drewes, R. C., & Hillyard, S. D. (2008). *Ecological and environmental physiology of amphibians*. Oxford University Press.
- Hocking, D. J., & Babbitt, K. J. (2014). University of New Hampshire Scholars ' Repository Amphibian Contributions to Ecosystem Services. *Herpetological Conservation and Biology*, 9(1), 1–17. http://www.herpconbio.org/Volume_9/Issue_1/Hocking_Babbitt_2014.pdf
- Humanitarian Data Exchange. (2015). *Settlements in Nepal*.
- Hwang, H., Fiala, M. J., Park, D., Wade, T. L., Fiala, M. J., Park, D., & Wade, T. L. (2016). Review of pollutants in urban road dust and stormwater runoff: part 1 . Heavy metals released from vehicles. *International Journal of Urban Sciences ISSN:*, 20(3), 334–360. <https://doi.org/10.1080/12265934.2016.1193041>
- IUCN. (2023). *Hoplobatrachus tigerinus* (Indian Bullfrog). *The IUCN Red List of Threatened Species 2023*, 8235, e.T58301A53729148.
- Jancowski, K., & Orchard, S. A. (2013). Stomach contents from invasive American bullfrogs *Rana catesbeiana* (= *Lithobates catesbeianus*) on southern Vancouver Island, British Columbia, Canada. *NeoBiota*, 16, 17–37. <https://doi.org/10.3897/neobiota.16.3806>
- Jnawali, S. ., Baral, H. ., Lee, S., Acharya, K. ., Upadhyay, G. ., Pandey, M., ... & Amin, R. (2011). *The Status of Nepal ' s Mammals : The National Red List Series*.
- Kästle, W., Rai, K. R., & Schleich, H. H. (2013). *Field Guide to Amphibians and Reptiles of Nepal*. ARCO- Nepal.
- Khatriwada, J. R., Ghimire, S., Paudel Khatriwada, S., Paudel, B., Bischof, R., Jiang, J., & Haugaasen, T. (2016). Frogs as potential biological control agents in the rice fields of Chitwan, Nepal. *Agriculture, Ecosystems and Environment*, 230, 307–314. <https://doi.org/10.1016/j.agee.2016.06.025>

- Khatiwada, J. R., & Haugaasen, T. (2015). Anuran species richness and abundance along an elevational gradient in Chitwan, Nepal. *Zoology and Ecology*, 25(2), 110–119. <https://doi.org/10.1080/21658005.2015.1016775>
- Khatiwada, J. R., Shu, G. C., Wang, S. H., Thapa, A., & Wang, B. (2017). A new species of the genus *Microhyla* (Anura: Microhylidae) from Eastern Nepal. *Zootaxa*, 4254(2), 221–239.
- Khatiwada, J. R., Shu, G., Subedi, T. R., Wang, B., Canatella, D. C., Xie, F., & Jiang, J. (2019). A New Species of Megophryid Frog of the Genus *Scutigera* from Kangchenjunga Conservation Area, Eastern Nepal. *Asian Herpetological Research*, 10(9), 139–157. <https://doi.org/10.16373/j.cnki.ahr.180076>
- Khatiwada, J. R., Wang, B., Zhao, T., Xie, F., & Jiang, J. (2021). An integrative taxonomy of amphibians of Nepal: An updated status and distribution. *Asian Herpetological Research*, 12(1), 1–35. <https://doi.org/10.16373/j.cnki.ahr.200050>
- Khatiwada, J. R., Zhao, T., Chen, Y., Wang, B., Xie, F., Cannatella, D. C., & Jiang, J. (2019). Amphibian community structure along elevation gradients in eastern Nepal Himalaya. *BMC Ecology*, 19(1), 1–11. <https://doi.org/10.1186/s12898-019-0234-z>
- LGIS. (2023). *Province 2 Nepal • LGIS Nepal • Local Governance Information System Nepal*.
- Li, B., Zhang, W., Shu, X., Pei, E., Yuan, X., Sun, Y., Wang, T., & Wang, Z. (2016). The Impacts of Urbanization on the Distribution and Body Condition of the Rice-paddy Frog (*Fejervarya multistriata*) and Gold-striped Pond Frog (*Pelophylax plancyi*) in Shanghai, China. *Asian Herpetological Research*, 7(3), 200–209. <https://doi.org/10.16373/j.cnki.ahr.150061>
- Liao, W. B., Lu, X., Shen, Y. W., & Hu, J. C. (2011). Age structure and body size of two populations of the rice frog *Rana limnocharis* from different altitudes. *Italian Journal of Zoology*, 78(2), 215–221. <https://doi.org/10.1080/11250001003639590>
- Liao, W. B., Zeng, Y., Zhou, C. Q., & Jehle, R. (2013). Sexual size dimorphism in anurans fails to obey Rensch's rule. *Frontiers in Zoology*, 10(10), 1–7.
- Lillywhite, B. H. (1971). Thermal Modulation of Cutaneous Mucus Discharge as a Determinant of Evaporative Water Loss in the Frog, *Rana catesbeiana*. *Zeitschrift*

Vergleichende Physiologie, 73, 84–104.

- Lukanov, S., Simeonovska-nikolova, D., & Tzankov, N. (2014). Effects of traffic noise on the locomotion activity and vocalization of the Marsh Frog , *Pelophylax ridibundus*. *North-Western Journal of Zoology*, 10(2), 359–364.
- Majid, A., Azim, S., & Nawaz, K. (2023). Diversity , Abundance , and Distribution of Anurans in Khyber Pakhtunkhwa , Pakistan. *Asian Journal of Research in Zoology*, 6(4), 51–70. <https://doi.org/10.9734/AJRIZ/2023/v6i4122>
- Malonza, P. K., & Veith, M. (2012). Amphibian community along elevational and habitat disturbance gradients in the taita hills, Kenya. *Herprtotropicos*, 7(1–2), 07–16.
- Marsh, D. M., Cosentino, B. J., Jones, K. S., Joseph, J., Beard, K. H., Bell, J. M., ...& Colleges, W. S. (2017). Effects of roads and land use on frog distributions across spatial scales and regions in the Eastern and Central United States. *Diversity and Distributions*, 23, 158–170. <https://doi.org/10.1111/ddi.12516>
- McKinney, M. L. (2002). Urbanization, Biodiversity, and Conservation. *BioScience*, 52(10).
- Measey, G. J., & Tolley, K. A. (2011). Investigating the cause of the disjunct distribution of *Amietophrynus pantherinus* , the Endangered South African western leopard toad. *Conservation Genetics*, 12, 61–70. <https://doi.org/10.1007/s10592-009-9989-7>
- Meredith, M. (2017). *Package 'wiqid.'* CRAN Repository.
- Miller, J. E., Hess, G. R., & Moorman, C. E. (2007). Southern two-lined salamanders in urbanizing watersheds. *Urban Ecosystem*, 73–85. <https://doi.org/10.1007/s11252-006-0012-5>
- Mishara, M. raj. (2019). *Diversity and distribution pattern of amphibians in Mahakali river basin, Far-Western Nepal*. Tribhuvan University.
- Moen, D. S., Irschick, D. J., & Wiens, J. J. (2013). Evolutionary conservatism and convergence both lead to striking similarity in ecology, morphology and performance across continents in Frogs. *Proceedings of the Royal Society B: Biological Sciences*, 280(1773). <https://doi.org/10.1098/rspb.2013.2156>
- Mohaymeni, F., Nikcheh, K., Kami, H. G., & Mohammadi, Z. (2022). Morphological and

- morphometric variations of the water frogs (genus *Pelophylax*) in Iran. *Journal of Wildlife and Biodiversity*, 6(3), 54–71. <https://doi.org/https://doi.org/10.5281/zenodo.6826803>
- Monnet, J., & Cherry, M. I. (2002). Sexual size dimorphism in anurans. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 269(1507), 2301–2307. <https://doi.org/10.1098/rspb.2002.2170>
- Moreno, E., Pequeno, P., Sergio, S. S., Ferrão, M., Lima, P. A., & William, M. (2022). How environmental factors affect the abundance and distribution of two congeneric species of Amazonian frogs. *Biotropica*. <https://doi.org/10.1111/btp.13272>
- MPPPC. (2020). *Province profile of province 2. Madhesh Province Policy and Planning Commission*. https://pc.p2.gov.np/public/images/service/Province_Profile_Province_2.pdf
- Murugan, K., Priyanka, V., Dinesh, D., Nicoletti, M., Alarfaj, A. A., Higuchi, A., & Munusamy, M. A. (2015). Predation by Asian bullfrog tadpoles, *Hoplobatrachus tigerinus*, against the dengue vector, *Aedes aegypti*, in an aquatic environment treated with mosquitocidal nanoparticles. *Parasitol Res*. <https://doi.org/10.1007/s00436-015-4582-0>
- Nagendra, H. (2002). Tenure and forest conditions: Community forestry in the Nepal Terai. *Environmental Conservation*, 29(4), 530–539. <https://doi.org/10.1017/S0376892902000383>
- Nepali, P. B. (2023). *Secies diversity and habitat preference of herpetofauna in Lumbini region, Nepal*. Tribhuvan University.
- Nepali, P. B., & Singh, N. B. (2021). Documentation of Documentation of Herpetofaunal Diversity in Nawalparasi District , Nepal. *Uttar Pradesh Journal of Zoology*, 41(24), 56–70.
- OpenStreetMap Contributors. (2023). *Planet dump [Data file from 2023]*. <https://doi.org/https://planet.openstreetmap.org>
- Otto, C. R. V., Forester, D. C., & Snodgrass, J. W. (2007). Influence of wetland and landscape characteristics on the distribution of carpenter frogs. *Wetlands*, 27(2), 261–269. [https://doi.org/10.1672/0277-5212\(2007\)27](https://doi.org/10.1672/0277-5212(2007)27)

- Outlook Nepal. (2024). *Madhesh Province - Nepal Outlook / Believe in Data*.
- Padhye, A., Manamendra-Arachchi, K., Silva, A. de, Dutta, S., Shrestha, K., Bordoloi, S., Papenfuss, T., Anderson, S., Kuzmin, S., & Khan, M. S. (2008). *Hoplobatrachus tigerinus*. *The International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species 2008*.
- Pellet, J., Hoehn, S., & Perrin, N. (2004). Multiscale determinants of tree frog (*Hyla arborea* L.) calling ponds in western Switzerland. *Biodiversity Conservation*, *13*, 2227–2235.
- Pesarakloo, A., Najibzadeh, M., Rastegar-pouyani, N., & Rastegar-pouyani, E. (2018). Taxonomic survey of water frog populations of *Pelophylax bedriagae* (Anura: Ranidae) in western Iran: a morphometric and bioacoustic approach. *Biologia*, *73*, 673–681. <https://doi.org/10.2478/s11756-018-0077-7>
- Pilliod, David S., Griffiths, R. A., & Kuzmin, S. L. (2012). *Ecological Impacts of Non-Native Species* (pp. 3343–3382). Surrey Beatty and Sons, Australia.
- Pokhrel, G. K., & Thakuri, S. (2016). Herpetofaunal diversity in Manaslu Conservation Area, Nepal. *Our Nature*, *14*(1), 99–106. <https://doi.org/10.3126/on.v14i1.16448>
- Pope, Shealagh E., Fahrig, L., & Merriam, Gray H. (2000). Landscape complementation and metapopulation effects on leopard frog populations. *Ecology*, *81*(9), 2498–2508.
- PPPC. (2023). *Madhesh Province: A Data Overview*.
- Ra, N.-Y., Park, D., Cheong, S., Kim, N.-S., & Sung, H.-C. (2010). Habitat Associations of the Endangered Gold-Spotted Pond Frog (*Rana chosonica*). *Zoological Science*, *27*, 396–401.
- Rahbek, C. (1995). The elevational gradient of species richness: a uniform pattern? *Ecography*, 200–205.
- Rai, T. P., Adhikari, S., & Antón, P. G. (2022). An Updated Checklist of Amphibians and Reptiles of Nepal. *Arco-Nepal Newsletter*.
- Ramesh, T., Hussain, K. J., Satpathy, K. K., & Selvanayagam, M. (2013). Community composition and distribution of herpetofauna at Kalpakkam Nuclear campus, Southern India. *Herpetology Notes*, *6*, 343–351.

- Rand, A. S. (1985). Tradeoffs in the evolution of frog calls. *Proceedings of the Indian Academy of Sciences*, 94(6), 623–637.
- Rytwinski, T., & Fahrig, L. (2015). The Impacts of Roads and Traffic on Terrestrial Animal Populations. *Handbook of Road Ecology*, 237–246. <https://doi.org/10.1002/9781118568170.ch28>
- Saber, S., Tito, W., & Said, R. (2017). Amphibians as Bioindicators of the Health of Some Wetlands in Ethiopia. *The Egyptian Journal of Hospital Medicine*, 66, 66–73. <https://doi.org/10.12816/0034635>
- Sapkota, S. (2022). *Diet composition and niche overlap of sympatric anurans in cropland and forest of western side of Barandabhar corridor forest, Nepal*. Tribhuvan University, Kirtipur, Kathmandu.
- Sapkota, S., Bhattarai, B. P., Mishra, M. R., Adhikari, J. N., & Khatiwada, J. R. (2022). Diet composition and overlap of sympatric amphibians in paddy fields of Nepal. *Herpetological Conservation and Biology*, 17(1), 155–164.
- Schäuble, C. S. (2004). Variation in body size and sexual dimorphism across geographical and environmental space in the frogs *Limnodynastes tasmaniensis* and *L. peronii*. *Biological Journal of the Linnean Society*, 82(1), 39–56.
- Shah, K. B., & Tiwari, S. (2004). Herpetofauna of Nepal: A conservation Companion. *IUCN- The World Conservation Union, Nepal*, viii+237 pp.
- Shine, R. (1979). Sexual Selection and Sexual Dimorphism in the Amphibia. *Copeia*, 2, 297–306. <https://doi.org/10.2307/1443418>
- Shine, R. (1989). Ecological causes for the evolution of sexual dimorphism: a review of the evidence. *The Quarterly Review of Biology*, 64(4), 419–461.
- Shrestha, B., Suwal, S., Pandey, B., Joshi, J., Manandhar, P., Karmacharya, D., Ohler, A., Dubois, A., & O'connell, K. A. (2022). Molecular and morphological identification of frog species collected at Rara Lake in Rara National Park, Nepal. *Zootaxa*, 5168(2), 222–236. <https://doi.org/10.11646/zootaxa.5168.2.8>
- Shu, X., Zhang, W., Li, B., Pei, E., Yuan, X., Wang, T., & Wang, Z. (2016). Major factors affecting the distribution of Anuran communities in the Urban, Suburban and Rural areas of Shanghai, China. *Asian Herpetological Research*, 7(4), 287–294.

<https://doi.org/10.16373/j.cnki.ahr.150069>

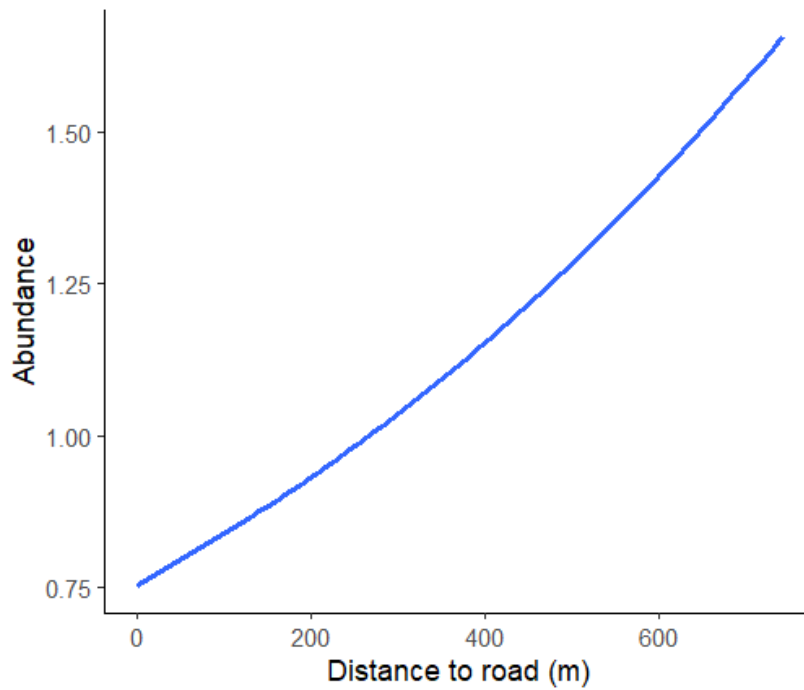
- Singleton, M., Rosenberger, A. L., Robinson, C., & O'Neill, R. (2011). Allometric and Metameric Shape Variation in Pan Mandibular Molars : A Digital Morphometric Analysis. *THE ANATOMICAL RECORD* 294:322–334 (2011) *Allometric*, 294, 322–334. <https://doi.org/10.1002/ar.21315>
- Tagar, I., Shaikh, K., Gachal, G. S., Arain, I., Arain, M., & Sahindi, B. abida. (2019). Assessment of anurans (frogs and toads) inhabiting district Naushahro Feroze , Sindh , Pakistan. *Journal of Entomology and Zoology Studies*, 7(1), 1419–1422.
- Team, R. core. (2023). R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria*. [//www.r-project.org/](http://www.r-project.org/)
- Thapa, K. B. (2016). *Herpetofaunal diversity of Palpa district, Nepal*. Tribhuvan University.
- Thongproh, P., Chunskul, J., Sringurngam, Y., Waiprom, L., Makchai, S., Cota, M., Duengkae, P., Duangjai, S., & Hasan, M. (2022). A new species of the genus *Hoplobatrachus* Peters, 1863 (Anura, Dicroglossidae) from northwestern Thailand. *Agriculture and Natural Resources*, 56, 1135–1152. <https://doi.org/10.34044/j.anres.2022.56.6.08>
- Thuiller, W., & Pearson, R. G. (2006). *Climate warming and the decline of amphibians and reptiles in Europe*. 1712–1728. <https://doi.org/10.1111/j.1365-2699.2006.01482.x>
- Timilsina, N., & Heinen, J. T. (2008). Forest structure under different management regimes in the Western Lowlands of Nepal. *Journal of Sustainable Forestry*, 26(2), 112–131. <https://doi.org/10.1080/10549810701879628>
- Vargas-salinas, F. (2006). Sexual size dimorphism in the Cuban treefrog *Osteopilus septentrionalis*. *Amphibia-Reptilia*, 27(3), 419–426.
- Vos, C. C., & Chardon, J. P. (1998). Effects of habitat fragmentation and road density on the distribution pattern of the moor frog *Rana arvalis*. *Journal of Applied Ecology*, 35(1), 44–56. <https://doi.org/10.1046/j.1365-2664.1998.00284.x>
- Watson, J. W., Mcallister, K. R., & Pierce, J. D. (2003). Home Ranges , Movements , and Habitat Selection of Oregon Spotted Frogs (*Rana pretiosa*) Home Ranges,

- Movements, and Habitat Selection of Oregon Spotted Frogs (*Rana pretiosa*). *Journal of Herpetology*, 37(2), 292–300. [https://doi.org/10.1670/00221511\(2003\)037](https://doi.org/10.1670/00221511(2003)037)
- Watters, J. L., Cummings, S. T., Flanagan, R. L., & Siler, C. D. (2016). Review of morphometric measurements used in anuran species descriptions and recommendations for a standardized approach. *Zootaxa*, 4072(4), 477–495. <https://doi.org/10.11646/ZOOTAXA.4072.4.6>
- Wyman, R. (1990). What's happening to the amphibians? *Conservation Biology*, 4(4), 350–352.
- Young, K. A. (2005). Life-history variation and allometry for sexual size dimorphism in Pacific salmon and trout. *Proceedings of the Royal Society B*, 272(1559), 167–172. <https://doi.org/10.1098/rspb.2004.2931>
- Zancolli, G., Steffan-Dewenter, I., & Rödel, M. O. (2014). Amphibian diversity on the roof of Africa: Unveiling the effects of habitat degradation, altitude and biogeography. *Diversity and Distributions*, 20(3), 297–308. <https://doi.org/10.1111/ddi.12161>
- Zhang, X., Xiong, J. L., Lv, Y. Y., Zhang, L., & Sun, Y. Y. (2014). Sexual size and shape dimorphism in the Wushan salamander, *Liaxia shihi* (Liu, 1950) (Urodela: Hynobiidae). *Italian Journal of Zoology*, 81(3), 368–373. <https://doi.org/10.1080/11250003.2014.920927>

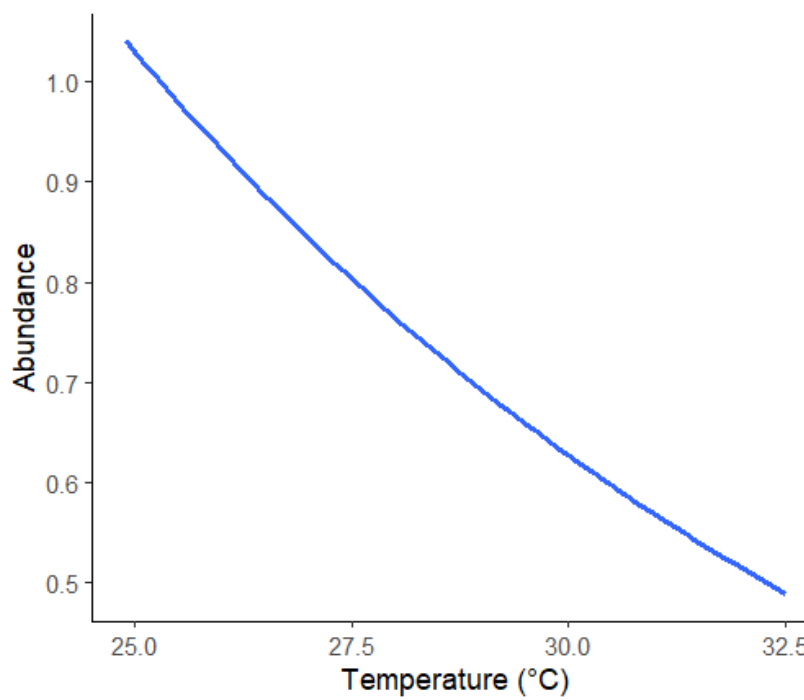
Appendices

Appendix Table 1. Generalized linear model describing the abundance of Indian bullfrog, ranked according to the Akaike Information Criterion (AICc). Model parameters include distance to road (m), distance to settlement (m), distance to water bodies (m), distance to farmland (m), distance to farmland (m), temperature (°C) and humidity (%). The number of parameters is df, ΔAIC_c is the difference between the AICc value of the best-supported model and successive models, and w_i is the Akaike model weight.

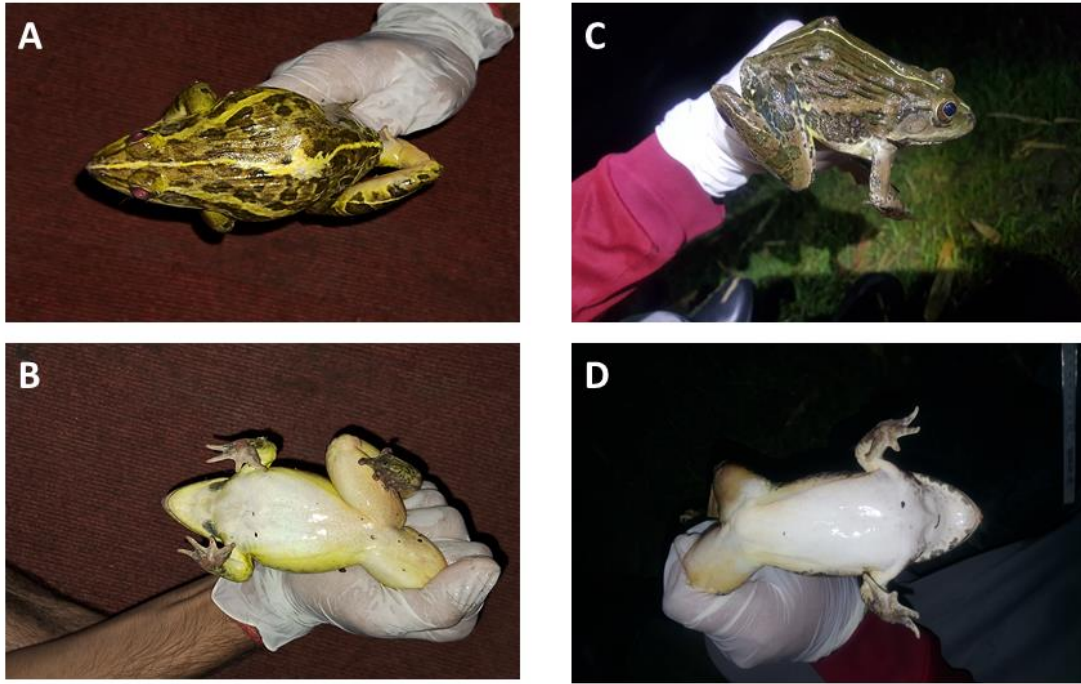
S.N.	Covariates	df	AICc	ΔAIC	w_i
1	Distance to road + temperature + distance to settlement+ survey time	6	428.24	0	0.06
2	Distance to road + temperature + distance to settlement+ survey time + distance to water bodies	7	429.31	1.07	0.03
3	Distance to road + temperature + distance to settlement+ survey time + distance to farmland	7	429.82	1.58	0.02
4	Distance to road + temperature + distance to settlement+ survey time + distance to forest	7	429.89	1.65	0.02
5	Distance to road + temperature + distance to settlement	4	429.97	1.73	0.02
6	Distance to road + temperature + distance to settlement+ survey time + humidity	7	430.23	1.99	0.02



Appendix Figure 1. Variation in abundance of Indian bullfrog with distance to road



Appendix Figure 2. Variation in abundance of Indian bullfrog with temperature



Appendix Figure 3. Photographs of Indian bullfrog (A) Dorsal side of male Indian bullfrog, (B) Ventral side of male Indian bullfrog, (C) Lateral side of female Indian bullfrog, (D) Ventral side of female Indian bullfrog