



Breeding Ecology of *Amolops himalayanus* (Amphibia: Anura: Ranidae) in Bodidrang Stream, Trashigang District, Bhutan

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Authors' contributions

This work was carried out in collaboration among all authors. Author ABL had designed the study, performed the statistical analysis, wrote the protocol and wrote the draft of the manuscript. All supporting authors contributed for the field works except author TN contributed in writing research methodology section. All authors read and approved the final manuscript.

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ABSTRACT

The breeding activity of Himalayan sucker frog (*Amolops himalayanus*), was studied under natural conditions at Bodidrang stream, Kanglung, Trashigang, Bhutan, for 12 months. We examined the correlation between meteorological factors (relative humidity, temperature and rainfall) and sightings of adult, juvenile, and tadpole/larvae individuals as well as egg masses from monthly surveys using Pearson Correlation in statistical software (Python 3.6). *A. himalayanus* was an explosive breeder influenced by the high seasonality in Bhutan. Adult, juvenile, and larvae individuals recorded had a positive Pearson correlation with monthly mean rainfall. There was a strong correlation between rainfall and adult sightings ($r=0.732$), however tadpoles sightings had a low correlation ($r=0.178$). We observed moderate positive correlations of temperature and relative humidity with adult ($r=0.536$; $r=0.442$) and juvenile ($r=0.398$; $r=0.252$) individuals, while tadpoles had low positive correlation for relative humidity ($r=0.048$) respectively. Brief note of amplexus position of *A. himalayanus* is discussed.

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Keywords: *Amolops himalayanus*; Bhutan; rainfall; relative humidity; temperature; tadpoles; juveniles.

1. INTRODUCTION

Research on the herpetological taxa of Bhutan is still very nascent, and much work remains to be done to document its species diversity and ecology [1]. Little research has been done on key aspects of the ecology of tropical frogs, including the basics of their breeding ecology. There are few studies of breeding ecology in this region. For instance [2-3] documented the breeding activities of *Duttaphrynus melanostictus* and *Fejervarya Cancrivora* in Jaba, Malaysia. [4] documented the breeding activities of *Hylarana erythraea* from Sarawak. [5] investigated the breeding patterns of seven species of Singaporean frogs, *D. melanostictus*, *Polypedates leucomystax*, *F. limnocharis*, *Leptobranchium nigrops*, *Kaloula pulchra*, *Microhyla butleri* and *M. heymonsi*. The breeding biology of *P. aspera*, *Limnonectes blythii*, *L. ibanorum*, *L. macrodon*, *Odorana hosii* and *Amolops jerboa* were studied [6]. Several studies highlight environmental factors, such as rainfall, can be an important correlate with breeding. For instance, [7] reported the reproductive mode of *Philautus glandulosus*. [8] reported the behavior and ecology of *P. leucomystax* from Northern Thailand. The breeding activities of *Ingerophrynus parvus* was investigated [9] in the tropical forest of Kedah and then the correlation between total rainfall and breeding parameters of white-lipped frog [10]. The breeding activity and larva development of *Amolops cremnobatus* was studied [11]. *Amolops* are randid frogs living in the torrent streams of mountains with only 49 described species globally and only four species reported from Bhutan [12-13] with little known of their breeding ecology. *Amolops himalayanus* except for report made [13] indicated breeding to occur in late May or early April. The aim of this study is to study their breeding pattern and correlate the breeding parameters of *Amolops himalayanus* with the environmental factors.

2. MATERIALS AND METHODS

2.1 Study Area

We conducted this study in Bodidrang stream which is located 4 km away from Sherubtse College. This area has a temperate climate with warm summer and cold winter. The vegetation surrounding the stream is dominated by *Alnus*

nepalensis, *Artemisia vulgaris*, *Aconogoum molle*, *Quercus griffithii*, *Pinus roxburgh*, *Rubus ellipticus*, *Rubia cordifolia*, *Musa* sp. *Erythrina* sp, *Myrica esculenta*, *Rhus chinensis*, *Ficus* sp. and *Stellaria* sp.

2.2 Sampling Approach

The nocturnal visual encountering survey [14] was conducted for 12 months in 2015 under natural conditions at Bodidrang stream (27° N & 91° E) Kanglung. The site was visited twice a month from 1800 to 2200 hours making 24 site visits in total. Specimens were caught by hand, identified, measured with slide Vernier caliper nearest to 0.1 mm, photographed by DSLR Nikon d3100 and released back to natural habitat unharmed. The *Amolops* tadpoles have an abdominal sucker which is the principal character [15] and has light brown color with long tail. In juvenile stage, the color changes to light green with some spots on its dorsal side and head and tail gets shorten. There will be formation of fore and hind limbs in later stage of juvenile.

The breeding parameters recorded were: presence of adult individual, tadpoles, juveniles, amplexing pairs and egg clutches. The environmental parameters were: rainfall (mm), temperature (°C) and relative humidity (%) and were obtained from the Meteorology Section, Hydromet Services Division, Department of Energy, MTI, Thimphu, Bhutan for the Kanglung station. We calculated the Pearson correlation (r) between rainfall, temperature and relative humidity against adult individual, juveniles and tadpoles in python 3.6 statistical software and the plots were made using pandas, matplotlib, SciPy and seaborn packages.

The sample specimen was anesthetized by chloroform (above 70%) then stored in 70% ethanol. The preserved specimens were labeled with tags [16] and subsequently deposited to the Sherubtse College Zoology museum.

2.3 Analysis

Overall, 62 adults, 9 juveniles, 48 tadpoles were captured across 12 months (Table 1). Data on rainfall, relative humidity and temperature highlight the seasonal climate variation on the study site (Table 2).

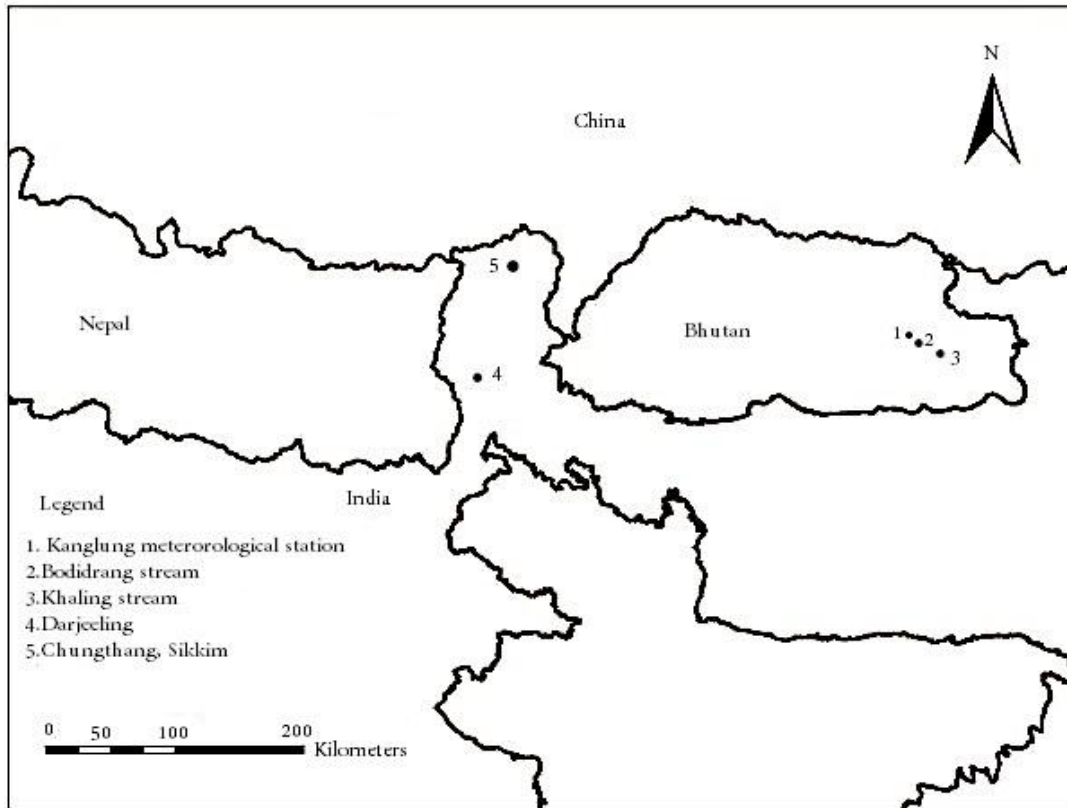


Fig. 1. Study area and the distribution of *Amolops himalayanus* in Bhutan and abroad. Map prepared by Kesang Gadhen

Environmental factors greatly affect the ecology of *A. himalayanus*. There was a strong correlation between the monthly mean rainfall and adults encountered during the survey ($r=0.732$) compared with juveniles ($r=0.458$) and tadpoles ($r=0.178$) (**Error! Reference source not found.**).

The monthly mean temperature also showed positive correlation to the adult ($r=0.536$), juvenile ($r=0.398$) and the tadpole ($r=0.270$) occurrence (**Error! Reference source not found.**). The monthly mean relative humidity also showed positive correlation to the occurrence of adult *A. himalayanus* ($r=0.442$), compared with juvenile ($r=0.252$). Tadpole occurrence was not well correlated with relative humidity ($r=0.048$). The highest number of adult ($n=16$) was spotted in May corresponding to the mean monthly rainfall of 8.35 ml, mean monthly temperature of 27.2°C and the monthly mean relative humidity (RH) of 80.2%. The highest number of juvenile ($n=3$) was spotted in the month of June corresponding to the mean monthly rainfall of

8.34 mm, temperature of 27.9°C and the RH of 88.3%. The highest number of tadpole ($n=19$) was spotted in the month of April corresponding to the mean monthly rainfall of 4.31 mm, temperature of 25.8°C and the RH of 72.3%.

During 5th May 2015, five egg clutches were encountered under two huge rocks in the stream. Tadpoles were observed from 3rd March till mid-June but during peak monsoon, due to floods, no tadpoles were observed. There was only one record of amplexus on the 10th April sampling and zero calling activities.

2.4 Reproductive Biology

There are color difference between the male and female of *A. himalayanus* suggesting that visual cues could be potentially important in sexual selection, especially in this nocturnal species. We observed strong size dimorphism with males ranging from (71.1 mm to 75.8 mm) and females ranging from (80.0 mm to 91.5 mm). Tactile cues are also important factor in the non-vocal species like *A. himalayanus* [17]. *A. himalayanus* is

explosive breeder influenced by the high seasonality in Bhutan. Amplectants were found either on the rock or dipping in the shallow water but oviposition must be happening by dipping in the shallow water since the eggs were found deposited under the rock surface with constant gentle freshwater current similar to the first record of *A. himalayanus* in Bhutan [13].

Amplexus is axillary type but unlike typical axillary position, the male forelimb grasps the female trunk, but the forelimb is under the armpit of female while in many other positions of forelimb is placed on the shoulder (Fig. 2B). This must be to compensate the size difference in maintaining the cloacae in the same line to favor synchronized oviposition and sperm release.



Fig. 2. A - Habitat in Bodidrang stream, B - Amplexus of *A. himalayanus*, C – *Philautus* cf. *annandali*, D – *Xenophrys parva*, E – *X. major*, F – *Xenophrys glandulosa*, G – juvenile of *A. himalayanus*, G – metamorphosing tadpole of *A. himalayanus*

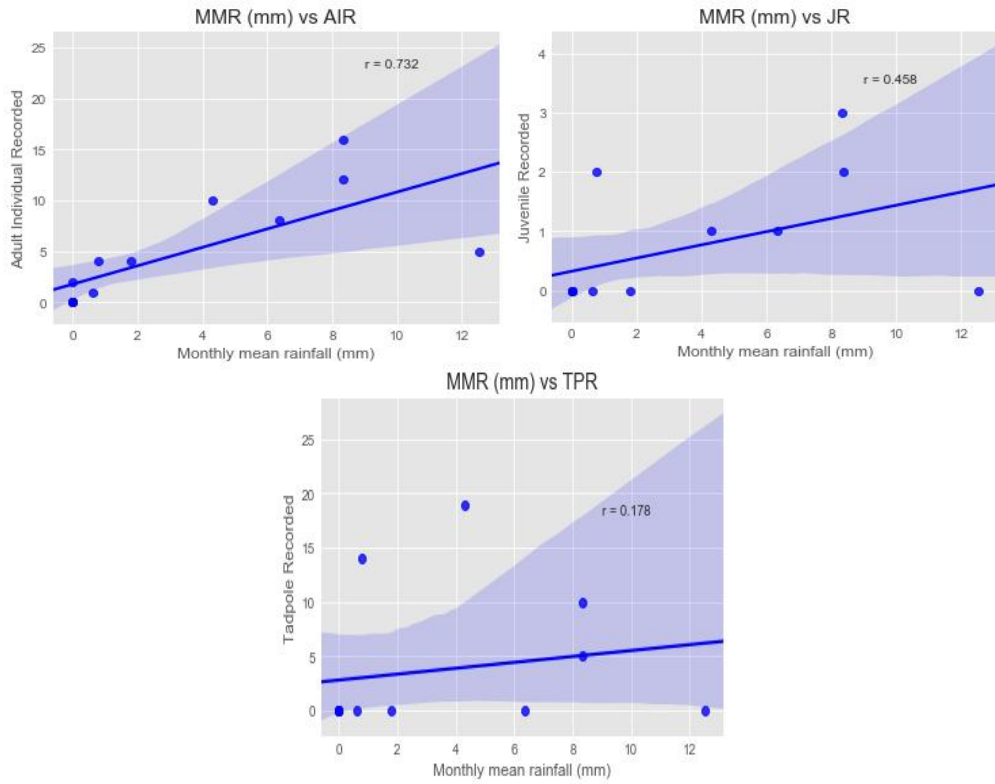


Fig. 3. The correlation between mean monthly rainfall (mm) and adult ($r=0.732$), juvenile ($r=0.458$) and tadpole ($r=0.178$) individuals recorded. The shaded area indicates 95% confidence intervals

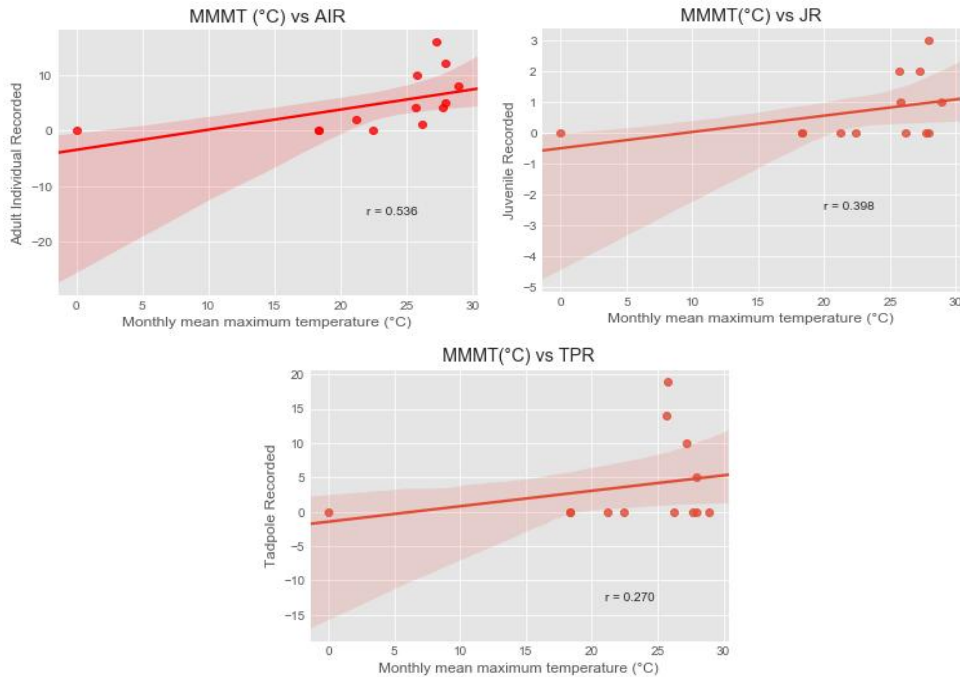


Fig. 4. The correlation between monthly mean maximum temperature and adult individual ($r=0.536$), juvenile ($r=0.398$), and tadpole ($r=0.270$) recorded

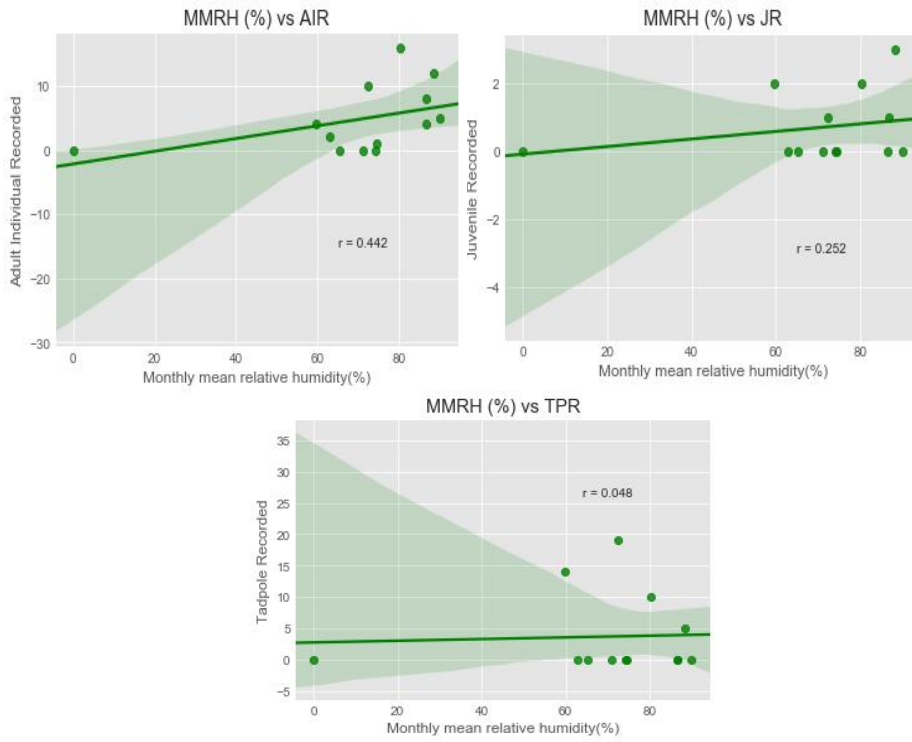


Fig. 5. The correlation between monthly mean relative humidity (%) and adult individual ($r=0.442$), juvenile ($r=0.252$), and tadpole ($r=0.048$) recorded

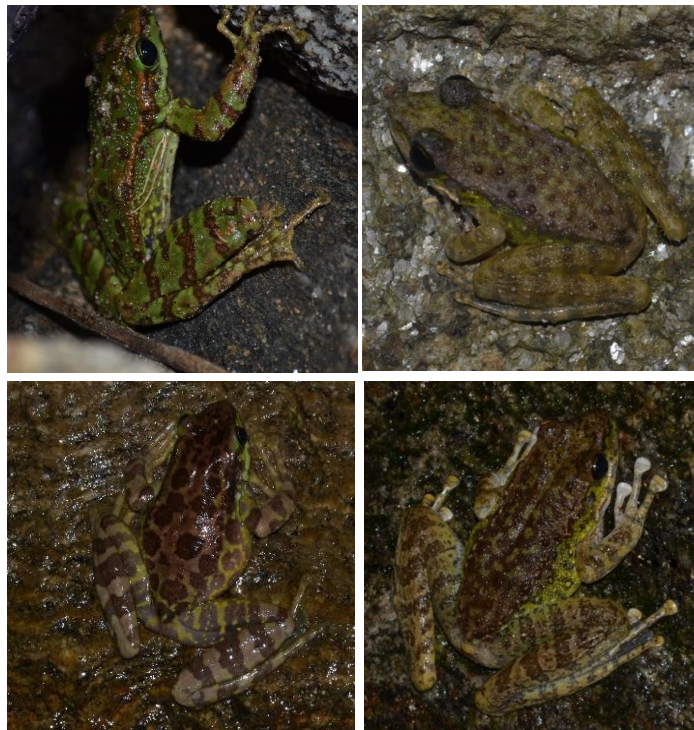


Fig. 6. The unknown sympatric species found in Bodidrang

3. DISCUSSION

The breeding of *A. himalayanus* was seasonal and explosive breeder breeding mainly from March to June. Breeding activity of *A. himalayanus* was generally correlated with periods of rain, temperature and relative humidity. No frogs of any life history stage (adult, juvenile or tadpoles) were observed from December to February. The developed tadpoles of *Amolops* spp. were found in early April [18], even [13] also noted that the breeding of *A. himalayanus* occurs in late March or early April. The torrent frogs like *A. himalayanus* breeds in moist conditions with higher relative humidity and temperature.

A. himalayanus is confirmed in two localities of Khaling-Kharungla Forest management Unit, Trashigang and with this study in Bodidrang stream. This study found several additional species at the study site including, *Philautus cf. annandali*, *Xenophrys parva*, *X. major*, and *X. glandulosa*. In Khaling-Kharungla 8 individual were recorded [13] and 62 individual adults were recorded during this study.

Besides species mentioned above in Fig. 2, there were also others sympatric species in the study site but due to lack of identification materials and resources, we could not confirm it. Besides *A. himalayanus*, there may be at least four other *Amolops* species in Bodidrang stream. The further research on the study site is require identifying the diversification of sympatric species. The Fig. 6 are unknown *Amolops* sympatric species found in Bodidrang.

4. CONCLUSION

The breeding ecology of *Amolops himalayanus* is the first ever extensive ecology research conducted in Bhutan in the field of herpetology besides brief ecology of *A. himalayanus* of Khaling-Kharungla Forest management Unit [13]. The torrent stream frogs, *A. himalayanus* was an explosive breeder influenced by high seasonality of Bhutan. Besides *A. himalayanus*, there are other sympatric species need to be identified and accordingly, needs conservational actions.

ETHICAL APPROVAL

Animal ethic Committee approval has been collected and preserved by the author.

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COMPETING INTERESTS

The authors have declared that no competing interests exist.

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APPENDIX

Table 1. Breeding parameters (AIR: Adult Individual Recorded; JR: Juvenile Recorded; TPR: Tadpole Recorded)

Months	AIR	JR	TPR
February, 2015	2	0	0
March, 2015	4	2	14
April, 2015	10	1	19
May, 2015	16	2	10
June, 2015	12	3	5
July, 2015	8	1	0
August, 2015	5	0	0
September, 2015	4	0	0
October, 2015	1	0	0
November, 2015	0	0	0
December, 2015	0	0	0
January, 2016	0	0	0

Table 2. Environmental parameters (MMR: Monthly mean rainfall; MMT: monthly mean maximum temperature; MMRH: monthly mean relative humidity)

Months	MMR (mm)	MMT (°C).	MMRH (%)
February, 2015	0	21.2	62.9
March, 2015	0.78	25.7	59.7
April, 2015	4.31	25.8	72.3
May, 2015	8.35	27.2	80.2
June, 2015	8.34	27.9	88.3
July, 2015	6.35	28.9	86.7

Months	MMR (mm)	MMMT (°C).	MMRH (%)
August, 2015	12.53	27.9	90
September, 2015	1.8	27.7	86.6
October, 2015	0.62	26.2	74.5
November, 2015	0	22.4	71
December, 2015	0	18.3	74.1
January, 2016	0	18.3	65.2

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