Original Research Article

Distribution pattern and conservation threats of Himalayan Newt (*Tylototrinusverrucosus*Anderson, 1871) in different habitats of Thinleygang, Punakha District, Bhutan.

ABSTRACT

Aims: To report the distribution of *Tylototritonverrucosus* in different habitats and its conservation threat in the locality.

Study design: The study aims to study the distribution and conservation threats of the Himalayan Newt (*Tylototritonverrucosus)* in the Thinleygang locality under the Punakha district, Bhutan. Opportunistic collection and measurements in diverse habitats (forest, stream, paddy fields, and roads) were gathered in the post-monsoon seasons. A semi-structured questionnaire survey was employed to gather localities' perceptions and awareness. A 3200m^2 (10m X 10m)study site was surveyed by dividing it into 32 plots for sampling (8 plots per habitat type).

Place and duration of study: The study area is located in Thinleygang (27.66°N: 89.83°E), Toebisageog under Punakha district covering an area of 12.62 sq. kilometers with an elevation ranging from 1709m to 3200 meters asl.

Methodology: A field study in Thinleygang, Bhutan, assessed Himalayan newt distribution and conservation perception. Opportunistic sampling across diverse habitats (forest, stream, paddy field, roads) recorded 70 newt individuals. Questionnaire surveys (n=30) gathered local perceptions. Data on habitat distribution, threats, and awareness were analyzed to inform conservation insights.

Results: The study investigated *Tylototritonverrucosus* in Thinleygang, Bhutan. A population of 70 newts was observed. Female dominance was noted (1:1.45). Morphological traits showed minimal sexual dimorphism. The highest density occurred in paddy fields (31.05/100 m²). People were aware of newts, but habitat changes, pollution, and habitat loss were perceived threats. Altitude and slope showed no significant correlation with newt presence. Conservation was deemed important due to cultural beliefs and habitat deterioration.

Conclusion: The study revealed notable insights into the distribution and ecology of *Tylototritonverrucosus* in Thinleygang, Bhutan. Newts were prevalent in various habitats, with the highest occurrence in paddy fields (52%), followed by forests (24%), streams (22%), and roadsides (2%). Their activity was influenced by weather conditions, being more frequent during rainy periods and specific times of day. Habitat preferences and behavior varied during the breeding and non-breeding seasons. Conservation concerns were evident, with perceptions of population decline attributed to human-induced threats like land use change, pollution, and

habitat loss. Preserving existing habitats, creating new aquatic environments, and promoting traditional agricultural practices emerged as potential conservation strategies.

Keywords: Bhutan, Conservation, Distribution, Habitat, Himalayan Newt, Threats

1. INTRODUCTION

The Himalayan newt (Tylototritonverrucosus Anderson, 1871) belongs to the family Salamandriadae of the order:Urodela(1). The species is reported from the mountainous regions of west China, North Vietnam, Bhutan, North-East India, and East Nepal(2). According to(3) the Himalayan newt is the only species recorded of the order Caudata in the Indian subcontinent. Amongst the most primitive species of living salamanders, the Himalayan newt is the only recorded salamander species in Bhutan(4). The first report on the occurrence of Himalayan newt in Bhutan was reported without a specific description of locality(5), and later the species occurrence in the localities ofThinleygang and Kabjisa was confirmed (6). Further studies on the distribution of the Himalayan newt were conductedin Punakha-Wangdue valley to enumerate the habitat and morphological measurements(4). Apart from these studies on Himalayan newt

in Bhutan, no advanced studies on its ecology and conservation threats have been conducted in the country.

The amphibian populations have been experiencing a widespread decline and extinctions as early as the 1990s (7). Several factors are responsible for the decline in the population of the salamanders in the locality including land-use pattern, pollution, water quality, limited suitable habitat, and anthropogenic activities (3). *T. verrucosus*is listed as the Least Concern (LC) in the International Union for Conservation of Nature (IUCN) and as a protected species in the Wildlife Preservation and Protection Act in Thailand (8). Due to the scanty of information on Himalayan newt in Bhutan, the taxon is not categorized under the Forest and Nature Conservation Rules of Bhutan. Without precise information on the distribution and ecology of the species, no long-term conservation activities can be implemented or even planned.

Amphibians are abundant and integral parts of diverse ecosystems. Considered the major carnivore feeding mainly on invertebrates, especially insects, a widespread decline in the populations of amphibians would have consequences and might lead to local extinction of the species leading to fragmentation in its distribution(9). The study on the distribution pattern and conservation threats of *T. verrucosus* in Thinleygang locality aims to enumerate the environmental parameters and determine its distribution in different habitat types. Due to the lack of in-depth study of the Himalayan newt, its future survival and threat have not been addressed. Survey and morphological measurements during the course of the study will help build baseline data for the species enabling its conservation status and threats. Objectives of the study:

1. To assess the distribution of Himalayan Newt (HN) in different habitats.

2. To identify the habitat criterion influencing the distribution of Himalayan Newt.

3. To report the local people's perception of the occurrence of Himalayan Newt.

2. MATERIALS AND METHOD

2.1. Study Area

The study area is located in Thinleygang (27.66 °N: 89.83 °E), Toebisageog under Punakha district covering an area of 12.62 sq. km with an elevation ranging from 1709m to 3200 masl (Figure 1). Thinleygang is a developing area with settlements near the roads. Thinleygang Valley has a subtropical climate with cool winters. The annual rainfall ranges from 500mm to 1500mm. The vegetation in the area includes Cool-broadleaved species and Chirpine forest.

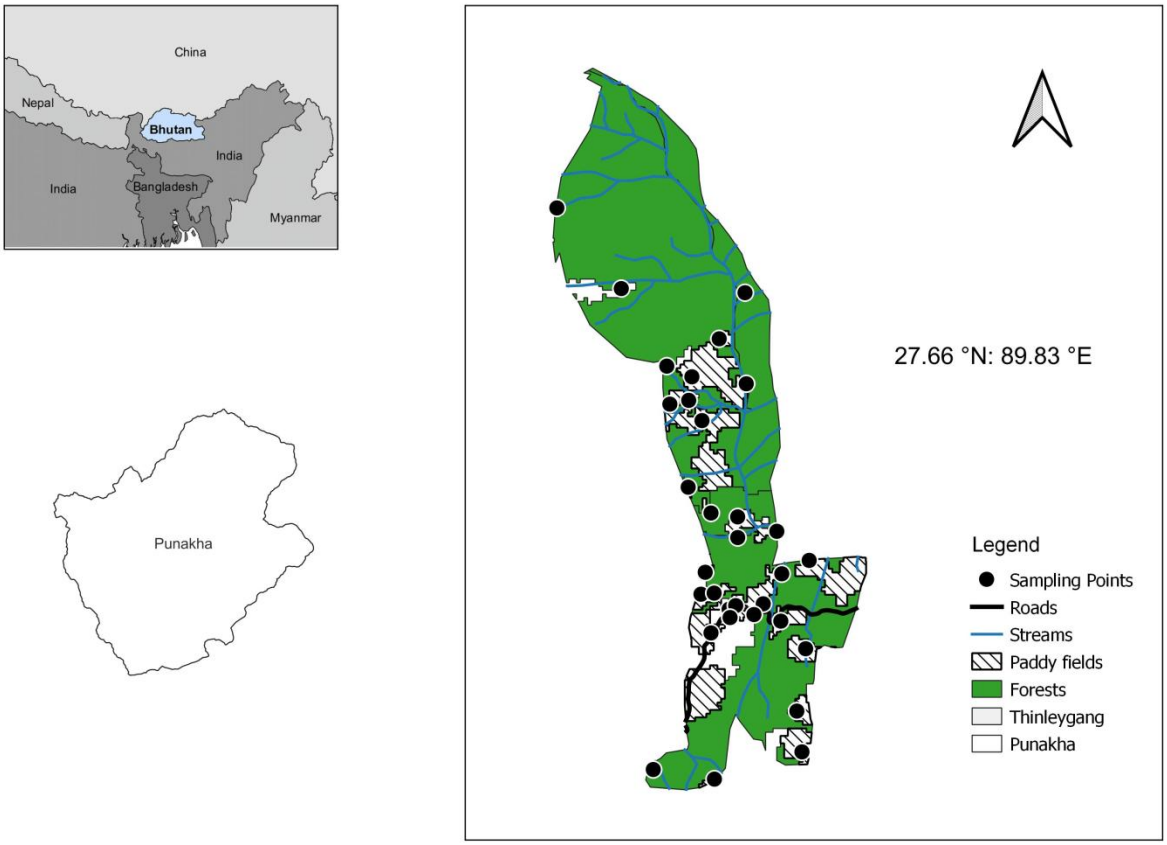

Figure 1. Map of the study area

2.2. Study Design

2.2.1. Survey design

The primary method employed was opportunistic collection based on the measurement of replicated sample units referred to as quadrats or plots of the study area's diverse habitat including paddy fields, roads, streams, and forest. Random plots within the study area were generated using arc GIS (geographical information system) software with equal plots in each habitat type laid systematically. For the distributional study, a time-constraint visual encounter technique described by (10)was used due to the study area's diverse habitat types such as paddy fields, roads, streams, and forests. A survey team of three people used a walk-and-turn method to survey all the habitats within the study area.

The data was collected during the post-monsoon seasons (September – October) as the newts leave the ponds after spawning (2). Personal communication with local residents and the preliminary survey were also carried out to understand the people's perception ofthe prevalence of the newts in the area.

2.2.2. Data collection

Opportunistic data collection was used in the study including turning objects that can be easily lifted and replaced without significant disturbance to the forest floor. The geo-coordinates of sites of encounter and elevationwere documented. A data logger (HOBO Logger) was used in the field during the survey to record the humidity and temperature of the habitats. For further analysis, each individual was weighed, measured, and released back to their capture sites. The identification of individual sexes and maturitywas done in reference to (11).

Information on the perception of local people on the occurrence of HN in the locality was collected through a semi-structured questionnaire survey. Respondents were randomly interviewed by drawing samples using the Yamane Formula (12) about their perception of the occurrence of HN in their locality. 30 respondents were randomly selected from the population during the preliminary survey to serve as respondents based on their willingness and availability during the data collection period. Data collected from the respondents were documented to see their willingness to conserve the HN in their locality.

2.3. Data Analysis

The number of individuals of males, females, and immature newts (efts) was recorded during the data collection period. Sex ratios of newts collected during the sampling periods were compared using the contingency table and the Chi-square test of independence to enumerate the sex of newts and the frequency of distribution. Absolute density (newts/100 m2) of newts including all newt groups (females, males, immature newts) in four habitat types (forest, paddy fields, stream, and roads) was calculated using the actual number of newts encountered during each sampling period divided by the area of each habitat type. The sex of newts was determined based on the presence or absence of cloacal character found only in males (13).

The chi-square test of independence was performed to examine the relation between the sex of adult newts and the habitats preferred by the newts. Sexual dimorphism in newts was enumerated through measurements from adult newts to the nearest 1mm scale: SVL = snout–vent length from the tip of snout to the edge of vent, TL= tail length, TTL = total length. The ratio of male: to female was calculated by dividing the number of male or female newts by the total newts encountered during the study field.

Sexual dimorphism in the newts encountered was computed using an independent t-test using the morphological measurements of individual newts including snout-vent length, tail length, total length, and weight of the newts. The relationship between body weight and habitat of occurrence was compared using One-way between groups Anova. Similarly, the relationship between the total length and habitat of occurrence was computed using the One-way between groups Anova.

No tests were performed as the objective of the study was to document the perception of people and threats faced by HN in the locality. However, the documentation of people's understanding and visible threats to the HN populations listed were based on questionnaire surveys, personal observation, and frequent visits to the encounter sites in the study area. Data analysis was conducted in Microsoft Office Excel and R Studio.

3. Results and Discussions

3.1. Population structure

A total of 70 individuals (24 males, 35 females, 11 efts) were sampled in the 3200 m2 study site (32 plots of 10 m X 10 m) at Thinleygang during the September and October months. Each Newt was sexed according to the presence or absence of the enlarged and rounded cloaca, which is found only in males. Newts lacking cloacal characters were identified as efts.

Newts were encountered more often during the rainy days, early morning, and late evenings in the study area. Similar microhabitats were also reported to be used by *Tylototritonvietnamensis* in Vietnam (14) and *T. verrucosus* in Bhutan (4). Early mornings and late evenings provided suitable timings for foraging in these habitats in the study area. It was also reported by (15) that the *T. yangii* newts were more active in

the early hours of the day (9.30- 11.00 a.m.) and during the beginning of the nighttime (6.30- 11.30 p.m.) in China.

Table 1. Cross-tabulation of sex and number of HN in the study area

		Forest	Paddy field	Streams	Roads	Total
Gender	**Male**	5	13	5	1	24
	Female	9	18	8	0	35
	Total	14	31	13	1	59

X^2 (3, N= 59) = 1.64, p > .05

A chi-square test of independence(16) was performed to examine the relation between the sex of Newts and the habitat preferred by the newts. The relation between the sex of the Newts and its occurrence in different habitats was not significant, X^2 (3, N= 59) = 1.64, p > .05 (Table 1.). It was evident that the habitats preferred by adult newts are irrespective of sex. This phenomenon arises as newts use various microhabitats for mating around permanent ponds, temporal rainfall ponds, and along shallow streams during the breeding season (June to August). During the non-breeding season, newts were found sheltered under leaf litter, debris, and edges of paddy fields. Similar observations were also reported by (4) in which a greater number of newts individuals were caught from the paddy fields.

The sex ratio of adult newts (male: female) in the study area revealed higher female newts than male newts by a small margin (1: 1.45). These findings are consistent with the greater number of male than female newts of *Tylototritonuyenoi* in northern Thailand (17). This observation of the sex ratio within the study area can be assumed fairly skewed as mating and courtship in newts ends by July and individual newts were mostly found on their own or in groups of similar sexes.

3.2. Population size and density

A total of 70 individual newts were encountered in four different habitats during the post-monsoon months of September and October. The newts were found most abundant during the preliminary survey in the breeding season (June - July). The absolute density of newts in each habitat was calculated from the actual number of newts encountered during the sampling period divided by the area of each habitat type. The maximum density of 31.05 newts/100 m^2 was found in the paddy field during the sampling period. The HN density decreased outside the paddy field with the lowest density of 1 newt/100m2 along the roads. (4) also reported a higher number of newts in the paddy field throughout their survey in July and August. The maximum number of newts were encountered in the paddy field (52%), forest (24%), streams (22%), and roads (2%) during the sampling period. The maximum density of newts in the paddy field can be related to the abundance of stagnant water and prey availability including tadpoles of anurans (Order: Anura), earthworms (Family: Megacoledia), damselflies larvae (Order: Odonata) and dragonfly larvae (Order: Odonata) observed in the study site during the sampling period and preliminary survey.

3.3. Sexual Dimorphism

A Shapiro-Wilk test (p> .05)(18) showed that the morphological measurements for both male and femaleHN were approximately normally distributed. An independent sample t-test was conducted to compare the snout-vent length difference in adult male and female newts. There was not a significant difference in snout-vent measurements for adult male newts (M= 62.79, SD= 8.26) and adult female newts (M= 62.74, SD= 7.71); t (57) = .023, p= .98. These results suggest that the snout-vent length and sex of adult newts are not related (Table 2.). Specifically, the results suggest that the snout-vent length of newts is irrespective of sex.

Table 2. Morphological measurement of HN in the locality

	Male (n=24)		Female (n=35)		
Measurements	**Mean**	**SD**	**Mean**	**SD**	***P value***
SVL (mm)	62.79	8.26	62.71	7.713	>0.05
TL (mm)	54.88	8.34	56.06	7.635	>0.05
TLL (mm)	117.71	16.02	118.83	14.883	>0.05
Wt. (g)	66.75	10.20	73.51	10.517	>0.05

SVL= snout-vent length, TL= tail length, TLL= Total Body length, Wt= weight of newt

An independent sample t-test was conducted to compare the tail length in adult male and female newts. There was no significant difference in the means of the tail length of adult male (M=54.88, SD= 8.34) and female newts (M= 56.06, SD= 7.63); t (57) = .56, p=.57 (Table 2.). These results suggest that the length of the tail in adult male and female newts is not related to the sex of the newts. (19)suggest that the difference in the growth rate or age of maturity is the proximate cause of sexual dimorphism difference in the adult male and female newts. In agreement with the general trend, the body size difference which was not observed in thenewts within the study area may be due to the differential age at maturity. However, according to (20)male salamanders and newts show considerably longer tails than females, but this was not observed in the measurements of the individual newts during the sampling period due to a lack of skeletochronological apparatus to determine the age of newts.

3.3.1. Relationship between body weight and different habitats of HN

A one-way between-groups ANOVA was performed to compare the effect of habitat type on the weight of the newts. The outcome variable was found to be normally distributed and equal variances were assumed based on the results of Levene's test (*F* (55) = .793, *p*= .45). There was not a statistically significant difference in the body weights of the newts found in different habitats (*F* (3, 55) = .348, *p*= .79) (Table 3.). These observations within the study area found that female newts were slightly heavier than male newts.

Table 3. One way between group Anova results of weight to the habitat of HN

Weight (g)	*SS*	*df*	MS	*f*	*P*
Between Groups	126.9	3	42.32	0.34	0.79

Female newts being heavier than male newts in the study area may be related to the setting of the individual newts in different micro-habitats. Within the study area, female newts were found on the edges of the paddy fields or seasonal streams which provided readily available food. Similarly, male adult newts were found mostly in terrestrial micro-habitat such as under boulders, burrows, and leaf litter which may have limited the availability of food in these habitats.

Morphological traits including colors of individual sexes did not significantly differ from the observation in the study area. The newts irrespective of sex depicted the same color variation from dark brown to light brown. These observations are consistent with the findings from a comparative study on Himalayan topotypic*T. verrucosus*specimens in which the dorsal region of the individuals both in life and preservation are blackish brown with dark to light brown warts (21).

3.3.2. Relationship between total count and slope

A bivariate Pearson correlation coefficient was computed to assess the relationship between the total count of newts and the slope of habitats within the study area. Preliminary analysis showed that there

were no violations in the assumptions of normality, linearity, or homoscedasticity. There was no significant correlation between the count of newts (2.22 ± 2.37) and the slope of the micro-habitats (22.81 ± 10.60), r = -.172, n = 32, *p* = .34. No other studies on the genus *Tylototriton*have recorded or observed the effect of slope on the distribution of the species.

3.4. People's perception of the conservation of Himalayan Newts in Thinleygang

A total of 30 respondents were interviewed for a social survey to study their perception of the conservation of Himalayan Newt in the locality. Out of these, 15 respondents were male, and 15 other females. The eldest respondent was 65 years old and the youngest was 12 years old. The maximum respondents were from the age class of 21- 30 and 31- 40 years (30%). The second highest respondents were in the age class of 11-20 years (17%) and (10%) from the age class of 41- 50 years. The least respondents were from the age class of 51- 60 and above 60 years old (7%). The respondents for the survey were selected in such a way that the respondents are either permanent inhabitants or have settled in the area for more than five years. On the occupation level of the respondents, 73% of the total respondents were farmers and 17% of the respondents were students.

3.4.1. Local people's awareness of Himalayan Newt

In general, all respondents were aware of the presence of HN in their locality. The observation of HN in the locality by the respondents was 100% which confirmed the presence of HN in the locality. About 77% responded that the HN was mostly seen in paddy fields, 16% in the forest adjacent to agricultural fields, and 7% in the streams and temporary rain ponds. None of the respondents recorded the first occurrence of HN in the locality. All respondents irrespective of age class responded the presence of HN in their locality was invasive with no probable negative impact on their crop production.

Abundant encounter with the HN in the locality was recorded during the spring and summer seasons. This observation of HN occurrence in the locality during the particular seasons relates best with the ecology of the HN in which the newts were seen to emerge from hibernation at the beginning of the monsoon seasons and breed in water from May to June (1,3,4) All respondents irrespective of sex and occupation responded that the conservation of HN in the locality was important. The reason behind such statements was mainly supported by religious views and reduction in the rate of encounter in the locality with changing land use practices.

3.4.1. Local people's perception on the population trend of HN in the locality

Household interview results showed that out of 30 respondents, 60% have perceived that populations of HN in their area have decreased compared to the past years. About 33% responded that the population of HN remained the same and 7% responded that the population of HN increased in the locality (Figure. 2.). The respondents also reported the presence of HN in other localities including Kabjisa Geog and Kazhi gewog in Wangdue Phodrang Dzongkhag. These statements were well validated as reports of the presence of species in these localities were reported by (4)However, majority (70%) of the respondents could not confirm the population of HN in their locality as they have considered HN to be same as other frogs and toads in the locality.

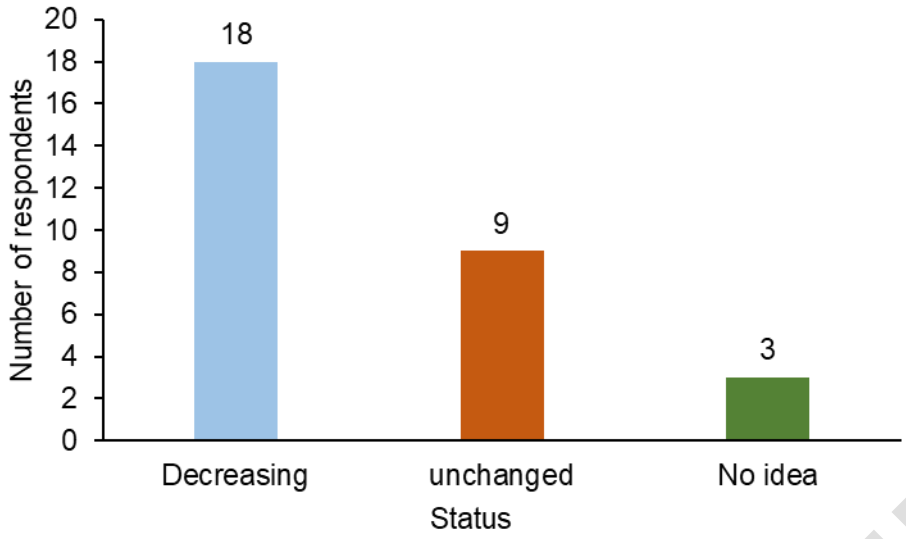

Figure 2. Populationstatus of HN as perceived in the study area

3.5. Perceived survival threats of HN in the locality

3.5.1. Anthropogenic threats

The household survey results revealed that (60%) of the respondents observed the population of HN in the locality was decreasing. Most respondents responded to the changing land use practices as the major threat for the decrease in the number of newts in the locality. All respondents reported being aware of the ecological importance of HN present in their locality. The most common cause of survival threat to HN is the draining by local people for utilizing the land for cultivation. Furthermore, suitable micro-habitats including swamps, seasonal streams, and rainfall ponds were observed to be polluted with waste, detergents, and drainage from households. Slash and burn practices in the agricultural fields adjacent to the forest posed greater threats to the HN as the most suitable micro-habitats were observed in these areas.

Other threats to HN survival in the locality included killing for amusement by local people, clearing of submerged vegetation along the paddy field edges, and disposition of newts to new sites outside its range of occurrence. Despite the above threats faced by newts in the locality, no respondents reported consumption, trade, or medicinal use of the species to date.

3.5.2. Natural threats

Emerging infectious diseases especially caused by fungi (chytrids) and viruses (rana viruses) are among the suspected causes of amphibian declines (3). Other threats to newt survival include the introduction of common carp into the natural streams and ponds (2). Within the study area, the most common natural threat to HN abundance was observed from the drying up of ponds and streams created by rain. Efts of newts were observed to be predated by birds and loss of suitable habitat were the most significant threats to the survival of newts in the locality. Tapping of streams for drinking water, destruction of moist areas within the forest by cattle, clearance of leaf litter, and forest clearance for roads significantly reduce the habitat area for newt's survival.

4. Conclusion

Out of the 32 plots, newts were encountered in 21 plots. The maximum number of newts including male, female, and efts were encountered in the paddy field (52%), followed by forest (24%), streams (22%), and the least along the roads (2%). Newts were encountered more often during the rainy days, early morning,

and late evenings in the study area. During the non-breeding season, newts were found sheltered under leaf litter, debris, and edges of paddy fields. The maximum density of 31.05 newts/100 m^2 was found in the paddy field during the sampling period. The newt's density decreased outside the paddy field with the lowest density of 1 newt/100m^2 along the roads.

Observations within the study area found that female newts were slightly heavier than male newts. Newts were encountered within the study area at the lowest elevation of 1895 masl and the highest encounter at the elevation of 2800 masl. Female newts in the study area recorded slightly longer tails than male newts. SVL and body weight of newts were recorded with less difference despite the sex. Female adult newts and efts were detected mostly near the streams or paddy ponds whereas male newts spread across various habitats sometimes 10-100 m away from the streams. The slope and total count of the newts did not significantly differ in all habitats.

A total of 30 respondents were interviewed for a social survey to study their perception of the conservation of Himalayan Newt in the locality. Out of these, 15 respondents were male and 15 other females. Household interview results showed that out of 30 respondents, 60% have perceived that populations of HN in their area have decreased compared to the past years. About 33% responded that the population of HN remained the same and 7% responded that the population of HN increased in the locality. The most common cause of survival threat to HN is the draining by local people for utilizing the land for cultivation. Furthermore, suitable micro-habitats including swamps, seasonal streams, and rainfall ponds were observed to be polluted with waste, detergents and drainage from households. Slash and burn practices in the agricultural fields adjacent to forest posed greater threats to the HN as the most suitable micro-habitats were observed in these areas.

Within the study area, the most common natural threat to HN abundance was observed from the drying up of ponds and streams created by rain. Efts of newts were observed to be predated by birds and loss of suitable habitat were the most significant threats to the survival of newts in the locality. Conservation of existing habitats, building of new ponds, and continuity of traditional agricultural practices can help conserve the species in the locality.

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