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Amphibian Diversity and Habitat Associations in Nongkhylllem Wildlife Sanctuary and Adjacent Reserved Forests of Meghalaya, India

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Abstract: Amphibians act as sensitive indicators of ecosystem health owing to their intricate life histories and dependence on both terrestrial and aquatic environments. This research evaluated the diversity, abundance, and habitat utilization patterns of amphibians within the Nongkhylllem Wildlife Sanctuary (NWLs) and the adjacent Reserved Forests (NRF) in Meghalaya, India. Field surveys were performed utilizing Visual Encounter Surveys and active searches across various microhabitats, leading to the documentation of 30 species comprising 322 individuals. The diversity indices indicated a high level of species richness ($S = 30$), Shannon–Wiener diversity ($H' = 3.08$), Simpson's index ($1-D = 0.946$), and Pielou's evenness ($J' = 0.906$), suggesting a well-balanced and ecologically resilient community. Seasonal analyses indicated peak richness during the post-monsoon period, while habitat comparisons revealed greater evenness in NRF despite a slightly lower species richness. Evergreen forests were predominant in species occurrence, whereas agricultural and waterbody habitats contributed minimally. In summary, the study emphasizes the necessity of preserving heterogeneous forested habitats and ensuring microhabitat connectivity to sustain amphibian diversity in northeastern India, thereby providing foundational data for conservation planning.

Keywords: Amphibian diversity, Nongkhylllem Wildlife Sanctuary, Nongkhylllem Reserved Forest, Diversity index, Community structure, Shannon–Wiener diversity, Simpson's index, Pielou's evenness

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Introduction

Amphibians are considered as one of the most ecologically sensitive groups of vertebrates, serving as crucial indicators of ecosystem health

because of their biphasic life cycle, porous skin, and dependence on both aquatic and terrestrial habitats (Duellman and Trueb, 1994; Wells, 2007).

Their physiological and ecological characteristics render them particularly vulnerable to environmental disturbances such as habitat degradation, changes in hydrology, and fluctuations in microclimates. Worldwide, amphibian populations are declining at rates surpassing those of any other vertebrate group, with nearly one-third of all species facing the threat of extermination (Collins and Storfer, 2003; Stuart *et al.*, 2004). This concerning trend highlights the necessity of documenting species richness, distributions, and habitat associations to guide conservation efforts.

Northeast India is recognized as one of the most diverse amphibian regions in South Asia, attributed to its biogeographical location at the intersection of the Eastern Himalayas and Indo-Burma biodiversity hotspots, two of the globe's most significant centers of endemism (Myers *et al.*, 2000; Ahmed *et al.*, 2009). The region is home to nearly 200 amphibian species and continues to discover new taxa through ongoing field surveys and molecular research (Biju and Bossuyt, 2009; Dinesh *et al.*, 2020). The area's exceptional species richness is further enhanced by high rainfall, intricate topography, and varied forest ecosystems.

Meghalaya, specifically, serves as a bastion of amphibian diversity in Northeast India. The region's rugged landscape, varying altitudes, extensive cave networks, and diverse forest types from tropical evergreen to subtropical pine forests contribute to a varied environment that sustains over 60 species of frogs, toads, and caecilians (Biju *et al.*, 2007; Hussain, 2011; Dinesh *et al.*, 2020; Warjri *et al.*, 2025; Saikia *et al.*, 2025). Numerous species are endemic or ecological specialists with restricted ranges, residing in microhabitats such as leaf litter, rocky streams, underground waterways, tree cavities, and temporary monsoon pools (Inger, 1966; Wells, 2007). Noteworthy taxa, including *Rhacophorus bipunctatus*, *Zhangixalus suffry*, *Amolops assamensis*, and *Ichthyophis garioensis*, are significant elements of the region's endemic

herpetofauna (Pillai and Ravichandran, 1999; Bordoloi *et al.*, 2007; Sengupta *et al.*, 2008).

Amphibian communities in Meghalaya demonstrate significant seasonal and spatial variability. Species such as *Rhacophorus maximus*, *R. bipunctatus*, *Polypedates teraiensis*, and various *Minervarya spp.* exhibit strong breeding patterns linked to the monsoon, with reproductive activities peaking during heavy rainfall that affects microhabitat moisture and larval development (Dinesh *et al.*, 2020). Elevational gradients further influence species distributions: lowland wetlands and moist deciduous forests support species like *Euphlyctis cyanophlyctis* and *Hoplobatrachus tigerinus*, while mid-elevation and montane forests are home to torrent-adapted species such as *Amolops formosus*, *Odorrana chloronota*, *Clinotarsus alticola*, and *Humerana humeralis* (Savage, 2002; Bora *et al.*, 2018).

The diversity of herpetofauna in Meghalaya has been documented since the colonial era, with notable contributions from various researchers such as Gunther (1858, 1875), Anderson (1871), Alcock (1904), Annandale (1912), Boulenger (1919), and Smith (1931). These foundational studies laid the groundwork for contemporary amphibian systematics in Northeast India. In recent years, integrative methodologies that combine field surveys, bioacoustics, and molecular taxonomy have facilitated the identification of cryptic species and clarified species identities within genera such as *Polypedates*, *Raorchestes*, *Zhangixalus*, and *Xenophrys* (Bossuyt and Dubois, 2001; Biju and Bossuyt, 2009; Fei *et al.*, 2009).

Despite this considerable diversity, amphibians in Meghalaya are confronted with increasing threats. Human-induced pressures including deforestation, forest fragmentation, agricultural expansion, road construction, pollution, and stream modification disrupt essential breeding and foraging habitats (Arntzen *et al.*, 2017; Hebbar *et al.*, 2019). Climate change further intensifies vulnerability by altering rainfall patterns, diminishing the hydroperiods of breeding pools, and heightening the occurrence of

extreme weather events. These combined pressures pose a significant risk to the survival of both common and range-restricted species.

Nongkhylllem Wildlife Sanctuary (NWLS) and the adjacent Nongkhylllem Reserved Forest (NRF) in the Ri-Bhoi district constitute an ecologically important landscape that sustains a variety of habitat types, including semi-evergreen forests, moist deciduous areas, riparian corridors, and grasslands. While NWLS enjoys a degree of protection, the surrounding NRF is subject to considerable human disturbance, resulting in data deficiencies concerning amphibian community structure in these transitional habitats. Given the ecological significance of amphibians and their sensitivity to habitat alterations, it is essential to comprehend their diversity, abundance, micro-habitat preferences, and spatial distribution in this area.

The current research intends to deliver a thorough evaluation of amphibian diversity and distribution throughout NWLS and the neighboring NRF by combining systematic field surveys with analyses of habitat and microenvironments. In particular, the study aims to: (i) record species richness and community structure; and (ii) investigate the connections between habitat characteristics and species presence. This research plays a vital role in enhancing amphibian conservation efforts in Meghalaya by producing baseline data that is crucial for monitoring, management strategies, and protecting the rapidly evolving forest ecosystems in the region.

Materials and Methods

Study area

Nongkhylllem Wildlife Sanctuary and Reserved Forest (Fig. 1) are located in Ri-Bhoi district of Meghalaya, India and lies between 25°45' - 26°00' N latitude and 91°45' - 92°00' E longitude. The wildlife sanctuary was declared in 1981 prior to which it was part of the reserve forest. It covers 29 km² areas on a steep hill slope (20° to > 65°) with

an elevation ranging from 208 to 295 m. The altitude ranges from 200 m to 950 m above mean sea level.

The predominant area of the sanctuary consists of tropical moist deciduous forest, interspersed with sections of tropical semi-evergreen forest, particularly located in the river valleys and along the streams. The Umtrew is the main river of the area, and Umran, Umling and Umtasor are its tributaries. The Umtrew also marks the western boundary of the reserve forest and sanctuary.

The climate of the study area is monosomic with distinct warm-wet (May-October) and cold-dry (December-February) periods. The monsoon starts from May and extends up to October. Approximately 90% of the yearly rainfall takes place during the monsoon season. The average maximum temperature of 35°C was noted in August, while the average minimum temperature of 14°C was observed in January.

Methodology

As amphibians inhabit both terrestrial/arboreal and aquatic environments, the survey encompassed both types of habitats. Prior to this, a GIS-based vegetation map was created, and an equal number and size of trails were established to cover all vegetation types within the sanctuary. The survey was conducted along these forest trails, which included all habitat types (evergreen, semi-evergreen, shrub, deciduous, riverine, grassland), and the number of individuals of each species was documented in relation to their respective habitats. Existing forest trails were explored during the evening hours. For this study, two methods were employed during the survey: (i) Visual Encounter Survey: This method involved searching for amphibians while walking along the forest trail during the evening hours (from 15:00 to 22:00) (Crump and Scott, 1994; Heyer *et al.*, 1994; Naniwadekar and Vasudevan, 2014), and (ii) Active Search: In this method, amphibians were actively sought in various microhabitats,

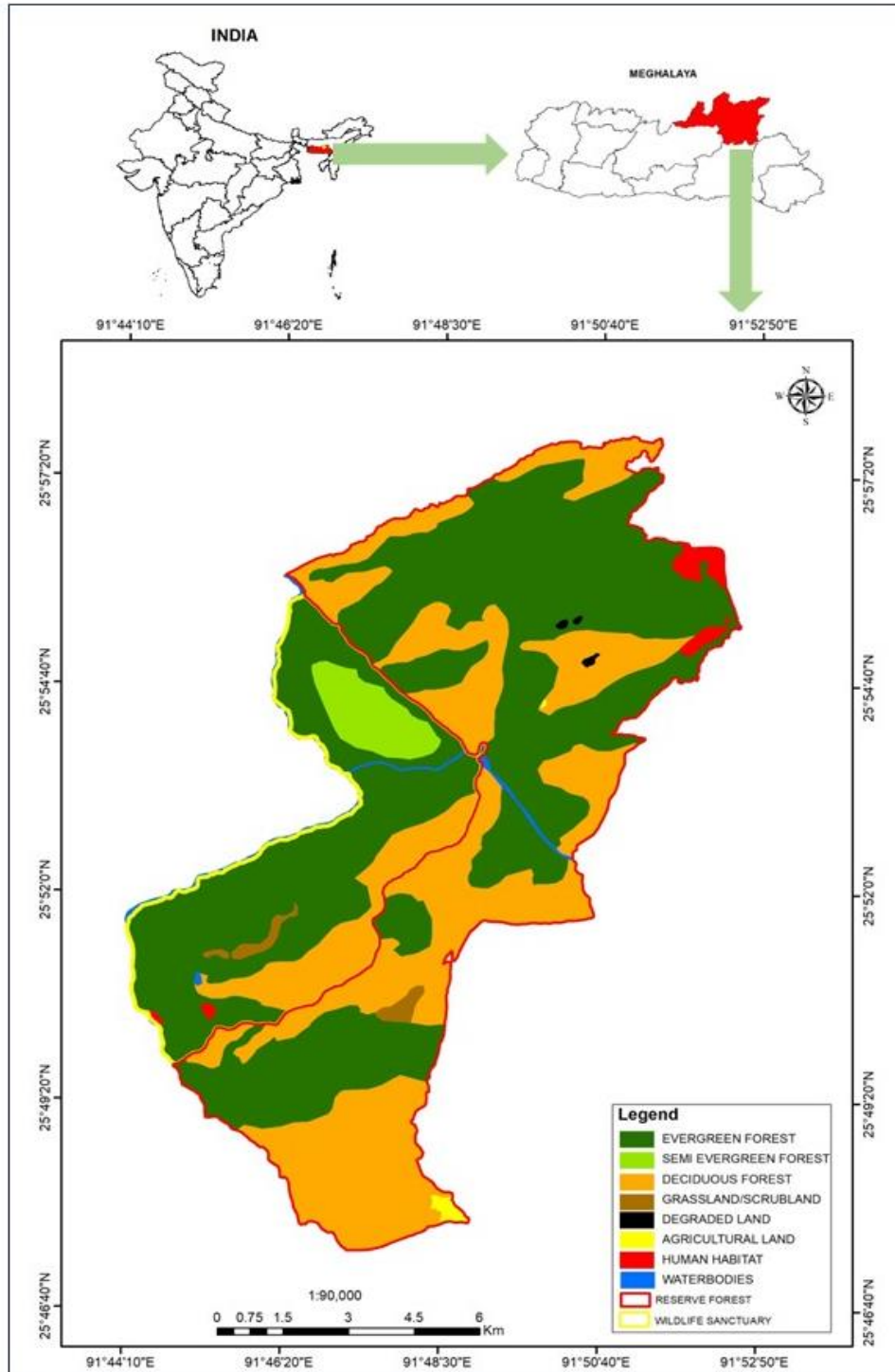


Fig. 1: Nongkhylllem Wildlife Sanctuary and Reserved Forest in Meghalaya, India (Source: Meghalaya Forests and Environment Department).

such as epiphytes, beneath loose bark and logs, under leaf litter, root buttresses, the overhead canopy, under rocks, in tree holes, puddles, springs, and along streambeds, considering the

vegetation outside the designated forest trails. This search operation was conducted both in the evening and during daytime hours (Sarkar *et al.*, 1992).

Representative samples of every species were methodically recorded using colour photographs. Images were captured from dorsal, dorsolateral, ventral, and lateral perspectives. Key features, including the color of the web and markings, were also photographed, as many of these details typically fade upon preservation. All images were digitized and labeled according to the specimen number for future reference. Subsequently, the observed species were returned to their natural habitat. Further, various diversity indices were used to calculate the diversity at various levels.

(i) **Shannon-Wiener Species Diversity Index, H'** (Shannon and Weaver, 1949): The diversity was calculated using Shannon-Weiner Index given below:

$$H' = -\sum (p_i \ln p_i)$$

Where, H' =Shannon-Wiener Species Diversity Index; p_i =Relative abundance of each group of organisms= n/N ; n =Abundance of each species; N =Total no. of individuals of all species

(ii) **Simpson's Diversity Index (Gini, 1912; Simpson, 1949; Herfindahl, 1950; Hirschman, 1964)**: The diversity in different habitats was also calculated using Simpson's Diversity Index, given below:

$$D = 1 - \sum (p_i)^2$$

Where, D =Simpson's Diversity Index; $p_i = n/N$; n =Abundance of each species; N =Total no. of individuals of all species

(iii) **Margalef's Diversity Index (Margalef, 1958)**: The diversity in different habitats was also calculated using Margalef's Diversity Index, given below:

$$d = (S-1)/\ln N$$

Where, d =Margalef's Diversity Index; S =Species Richness; N =Total number of individuals of all species

(iv) **Pielou's Evenness Index (Pielou, 1966)**: The distribution of species was calculated using Pielou's Evenness Index, given below:

$$J' = H/H_{\max}$$

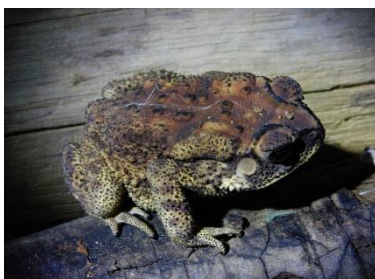
Where, J' = Pielou's Evenness Index; H = Shannon-Weiner Index; $H_{\max} = \ln N$; N =Total number of individuals of all species

Results

The amphibian community (Figs. 2A, B) exhibits a significant degree of species diversity as indicated by various widely recognized ecological indices. The species richness ($S = 30$) reflects a well-represented community. The Shannon-Wiener index ($H' = 3.08$) indicates a high level of diversity, which is a result of both the considerable number of species and their relatively equitable distribution across abundance categories. Simpson's Diversity ($1-D = 0.946$) reinforces this observation, suggesting that the likelihood of two randomly chosen individuals belonging to different species is quite high. This indicates low dominance and a high degree of community heterogeneity. Additionally, Margalef's Index ($d = 5.02$) signifies high richness in relation to sample size, demonstrating that the habitat sustains a structurally intricate amphibian community with a wide array of species niches. Further, the Pielou's Evenness value ($J' = 0.906$) indicates that species are distributed fairly evenly, with no single taxon disproportionately dominating the community (Table 1). Such a high level of evenness generally signifies a stable and minimally disturbed habitat, which supports a variety of ecological guilds. This indicates that the combination of high richness, high diversity, and high evenness suggests that the amphibian community is ecologically resilient, diverse, and well-balanced, indicating favourable habitat conditions and relatively low levels of anthropogenic impact.

Diversity Indices Across Seasons

The post-monsoon (PM) season demonstrated the highest species richness (30 species), signifying peak amphibian activity and detectability following the breeding period. The monsoon (M) season recorded a moderately high richness (18 species), aligning with the peaks of breeding activity. In contrast, the winter (W) and



Duttaphrynus melanostictus



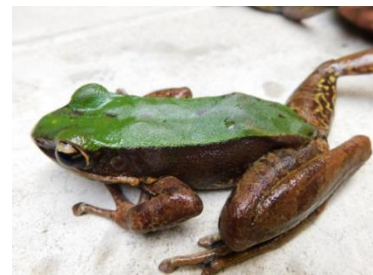
Hoplobatrachus tigerinus



Euphylyctis cyanophlyctis



Minervarya pierrei



Odorrana chloronata



Minervarya syhdrensis



Minevarya teraiensis



Euphylyctis adolfi



Limnonectes khasianus



Xenophrys major



Minervarya asmati



Minervarya nepalensis



Hylarana leptoglossa



Amolophs assamensis



Clinotarsus alticola

Fig. 2A: Amphibian fauna of Nongkhylllem Wildlife Sanctuary and Reserved Forest, Meghalaya, India.



Microhyla ornate



Microhyla berdmorei



Zhangixalus suffry



Polypedetes himalayensis



Polypedetes leucomystax



Polypedetes sp.



Kurixalus naso



Rhacophorus bipunctatus



Zhangixalus smaragdinus

Fig. 2B: Amphibian fauna of Nongkhylllem Wildlife Sanctuary and Reserved Forest, Meghalaya, India.

Table 1: Overall amphibian diversity together in Nongkhylllem Wildlife Sanctuary and Reserved Forest

Index	Value
Shannon–Wiener Diversity (H')	3.082
Simpson's Diversity ($1-D$)	0.946
Margalef's Index (d)	5.022
Pielou's Evenness (J')	0.906

reproductive-monsoon (RM) seasons displayed lower richness levels (13 and 12 species, respectively), indicative of reduced activity and lower detectability during colder and transitional phases. Shannon diversity reached its peak in PM ($H' = 3.13$), reflecting a highly heterogeneous community characterized by both high richness and balanced abundance. The monsoon (M) season also exhibited considerable diversity ($H' =$

2.76). The lower values observed in W (2.47) and RM (2.22) suggest more uneven distributions and diminished activity during these periods. Simpson's values mirrored this trend, with the highest values recorded in PM (0.949) and M (0.931), confirming that species dominance was low and that most species made significant contributions to the community structure. The lowest values were noted during RM (0.862),

Table 2: Seasonal variation of amphibian diversity

Index	Value			
	Retreating monsoon	Winter	Pre-monsoon	Monsoon
Shannon–Wiener Diversity (H')	2.223	2.47	3.133	2.762
Simpson's Diversity ($1-D$)	0.862	0.909	0.949	0.931
Margalef's Index (d)	3.508	3.728	5.859	3.371
Pielou's Evenness (J')	0.895	0.963	0.93	0.956

Table 3: Comparative variation of amphibian diversity between Nongkhyllem Wildlife Sanctuary and Reserved Forest

Index	Value	
	NWLS	NRF
Shannon–Wiener Diversity (H')	2.934	2.991
Simpson's Diversity ($1-D$)	0.9403	0.9418
Margalef's Index (d)	4.113	4.747
Pielou's Evenness (J')	0.949	0.929

indicating a higher dominance by a limited number of species following breeding dispersal. Margalef's index peaked in PM (5.86), indicating substantial richness relative to the sample size. The lower richness observed in RM (3.51) and W (3.73) reflects a decrease in species presence or detectability during these times. Evenness was highest in W (0.963) and M (0.956), suggesting a very balanced distribution of species. Although PM exhibited high richness, its evenness was slightly lower ($J' = 0.93$) due to certain species (e.g., *Minervarya* species, *Microhyla*, *Duttaphrynus melanostictus*) being more abundant than others. RM displayed the lowest evenness (0.895), indicating that the assemblage was dominated by a few species (Table 2).

Diversity Indices between WLS and RF

The analysis of amphibian community structure between NWLS and NRF uncovers significant patterns of diversity and distribution. The species richness (S) is marginally greater in NRF (25 species) compared to WLS (22 species), indicating that NRF accommodates a wider array of amphibian taxa. This observation is further corroborated by Margalef's Index, which is elevated in NRF (4.75) relative to NWLS (4.11), suggesting that RF contains a more diverse

assemblage in relation to sample size. The Shannon–Wiener diversity index (H') is also slightly higher in NRF (2.99) than in NWLS (2.93), indicating a marginally greater heterogeneity and equitable representation of species in NRF. Likewise, the Simpson's diversity ($1-D$) is notably high in both locations, although it is slightly greater in NRF (0.9418) than in NWLS (0.9403), confirming that both environments support highly diverse communities without significant dominance by any single species. Conversely, Pielou's Evenness (J') is superior in NWLS (0.949) compared to NRF (0.929) (Table 3). This suggests that while RF accommodates a greater number of species, the abundance distribution in NWLS is more balanced, with species being more evenly represented. NRF exhibits slightly greater variability in abundances, influenced by higher counts of certain *Minervarya* species and *Duttaphrynus melanostictus*. This indicates that the NRF seems to support greater richness and slightly enhanced diversity, while NWLS exhibits superior evenness in species distribution. These trends may reflect variations in microhabitat complexity, hydrological variability, canopy structure, or anthropogenic influences across the two sites. Collectively, the diversity indices

Table 4: Amphibian diversity across habitats

Index	Value
Shannon–Wiener Diversity (H')	1.011
Simpson's Diversity ($1-D$)	0.518
Margalef's Index (d)	0.693
Pielou's Evenness (J')	0.628

Table 5: Habitat level variation of amphibian diversity between Nongkhyllem Wildlife Sanctuary and Reserved Forest

Index	Value	
	NWLS	NRF
Shannon–Wiener Diversity (H')	0.935	1.02
Simpson's Diversity ($1-D$)	0.478	0.552
Margalef's Index (d)	0.783	0.593
Pielou's Evenness (J')	0.581	0.736

emphasize that both landscapes are crucial for sustaining amphibian diversity, with NRF offering a broader species pool and NWLS ensuring a more stable population balance among species.

Diversity Indices for Habitat Types

The five habitat categories (Evergreen, Semi-Evergreen, Deciduous, Water Bodies, and Agricultural Land) exhibit significant variation in amphibian abundance, which directly affects the calculated diversity indices. Species richness is related to the number of habitat categories sampled. With five distinct habitat types, the dataset offers a comprehensive representation of landscape-level environmental heterogeneity. The Shannon diversity value reflects moderate habitat diversity. This is primarily influenced by the strong dominance of evergreen forest (EVG), which comprises 212 records, significantly surpassing the other habitats. The lower contributions from waterbody (11) and agricultural area (8) diminish overall diversity by disrupting the balance among categories. A Simpson value of 0.52 indicates a moderate likelihood that two randomly selected amphibian occurrences will be found in different habitat

types. This again highlights the substantial contribution of evergreen forest, which distorts the proportional distribution. Margalef's richness index is relatively low because of disparity between total abundance (322 individuals) and habitat categories (5) (Table 4). A high sample size coupled with low richness leads to reduced Margalef values. Evenness is moderately low, suggesting an unequal representation of amphibian occurrences across habitats. The dominance of evergreen forest significantly lowers evenness, while semi-evergreen forest and deciduous forest provide moderate contributions, and waterbody/ agricultural area offer minimal contributions.

The comparative evaluation of habitat-use diversity between NWLS and NRF reveals distinct patterns in both richness and evenness. Species/habitat-use richness (S) is greater in NWLS ($S = 5$). Nevertheless, Shannon's diversity (H') is higher in RF (1.02) compared to NWLS (0.93), indicating a more equitable distribution of individuals across habitats. This is further corroborated by Pielou's Evenness (J'), which is significantly higher in NRF (0.736) than in NWLS

(0.581), indicating that habitat utilization in NRF is more uniformly spread instead of being predominantly influenced by one specific habitat. In a similar vein, Simpson's diversity index (1-D) is also greater in NRF (0.552), indicating a lower dominance effect and a higher likelihood that two randomly selected individuals were found in different habitats. In contrast, Margalef's index is elevated in NWLS (0.783) due to its increased richness, despite the pronounced dominance of evergreen forest (Table 5). The findings suggest that while NWLS possesses greater richness, NRF demonstrates superior evenness and overall diversity, indicating a more balanced utilization of the available habitats within the NRF landscape.

Discussion

The comparative evaluation of habitat-use diversity between NWLS and NRF highlights significant ecological distinctions that mirror the fundamental habitat structure, human-induced pressures, and landscape variability impacting amphibian communities. While NWLS demonstrated greater habitat richness ($S = 5$) due to the inclusion of all five habitat categories, its overall diversity was notably lower compared to NRF. The predominance of a single habitat type, specifically evergreen forest, in NWLS resulted in diminished evenness in habitat utilization, as evidenced by the lower Shannon diversity values ($H' = 0.935$) and Pielou's evenness ($J' = 0.581$). These trends imply that amphibian populations in WLS may be confined to a limited number of optimal habitat patches, potentially due to habitat specialization, structural variations, or decreased environmental heterogeneity. Similar findings were documented by Ernst and Rodel (2005), who observed that forest-dwelling amphibians in tropical ecosystems frequently display reduced habitat-use diversity when one habitat type predominates the landscape. Conversely, NRF displayed lower habitat richness ($S = 4$), primarily due to the absence of agricultural land (AG), yet it exhibited significantly higher Shannon diversity ($H' = 1.020$) and evenness ($J' = 0.736$). This indicates that amphibians in RF utilize the

available habitat types in a more uniform manner, reflecting a more equitable distribution across evergreen forest, semi-evergreen forest, deciduous forest, and wetland habitats. Elevated evenness values typically signify enhanced ecological stability and diminished competitive exclusion (Magurran, 2004). The increased Simpson diversity index (1-D = 0.552) in RF further corroborates this, indicating lower dominance effects and suggesting that the available habitats facilitate a wider distribution of species and individuals. These patterns align with earlier research by Wells (2007), who noted that amphibian assemblages are generally more diverse and evenly distributed in forest patches where multiple microhabitats are well interconnected.

The elevated Margalef's richness index (0.783) in NWLS indicates a higher number of habitat categories. Various ecological factors could interpret for the observed differences between NWLS and NRF. Firstly, NRF seems to sustain a more varied habitat structure and potentially a greater level of microhabitat connectivity, enabling amphibians to more efficiently utilize available resources. Diverse forest patches are recognized for supporting more varied amphibian populations due to the increased availability of breeding sites, moisture gradients, and refugia (Das *et al.*, 2008; Hillers *et al.*, 2008). Secondly, the lack of agricultural land in RF may mitigate disturbances such as pesticide runoff, soil compaction, and grazing pressure, all of which adversely impact amphibian survival (Stuart *et al.*, 2004). In contrast, the existence of agricultural patches within or adjacent to NWLS may create ecological traps or diminish habitat suitability, thereby limiting amphibian distribution.

Moreover, the more balanced diversity pattern observed in NRF indicates improved ecological functioning and enhanced resilience of amphibian communities. Ecosystems characterized by greater evenness tend to be more stable and better equipped to endure environmental fluctuations, especially in areas susceptible to periodic

variations in temperature and rainfall (Tilman *et al.*, 2006). This stability is crucial for amphibians, whose population dynamics are intricately linked to moisture availability, hydrological cycles, and microclimatic conditions (Duellman and Trueb, 1994).

The diversity patterns observed suggest that reserved forest fosters a more resilient and evenly distributed amphibian community, likely attributable to increased habitat heterogeneity and diminished dominance of any single habitat type. Conversely, WLS, despite exhibiting greater habitat richness, is ecologically more imbalanced, with amphibians heavily dependent on evergreen forest patches. These results underscore the significance of preserving habitat complexity and microhabitat diversity for amphibian conservation in Northeastern India. Consequently, conservation strategies should focus on habitat restoration, minimizing anthropogenic disturbances, and improving habitat connectivity to ensure the long-term survival of amphibians in both landscapes.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the Department of Zoology, University of Science and Technology Meghalaya, India.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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