Zoology and Ecology, 2024, Volume 34, Number 2
Online ISSN: 2165-8013
https://doi.org/10.35513/21658005.2024.2.3

DIVERSITY, ECOLOGY AND CONSERVATION OF ANURANS FROM RAMNAGAR, WEST BENGAL, INDIA

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Chanda, A.K. 2024. Diversity, ecology and conservation of anurans from Ramnagar, West Bengal, India. *Zoology and Ecology* 34(2), 102–110. https://doi.org/10.35513/21658005.2024.2.3

Article history
Received: 16 June 2024;
accepted: 16 July 2024

Keywords:
Anuran heterogeneity; ecology; species richness; evenness; protection

Abstract. This study aimed to assess and understand the amphibian biodiversity and conservation status in the Ramnagar area, West Bengal, India. Amphibians play crucial roles in ecosystem functioning and serve as indicators of environmental health. However, their populations worldwide are facing significant threats, highlighting the need for comprehensive assessments and conservation strategies. In this study, extensive field surveys were conducted in the Ramnagar area to document amphibian species richness, evenness, diversity and asses the influence of environmental factors (vegetation cover, pH, dissolved oxygen in water, humidity, and temperature) on species diversity. A combination of visual encounter surveys and passive sampling techniques was employed to ensure a comprehensive coverage of the study area. The results of the study revealed a moderate diversity with a total of 10 species and 4 families identified. *Duttaphrynus melanostictus* was the most abundant, whereas *Fajervarya orissaensis* was the least observed. Shannon and Simpson indexes suggest a moderately diverse community of anurans, Margalef's Index indicates a more even distribution of individuals across species, suggesting a balanced community, while the Shannon Equitability Index suggests that the amphibian community is moderately diverse, with a reasonable variety of species present. Of the tested ecological factors, dissolved oxygen and humidity have the most significant effect on species diversity.

INTRODUCTION

The Ramnagar area, situated in the Purba Medinipur district of West Bengal, India, is a region known for its rich biodiversity and ecological significance. Among the various organisms inhabiting this area, amphibians play a vital role in maintaining the delicate balance of the ecosystem (Hopkins 2007). Amphibians are a diverse group of vertebrates that are highly sensitive to environmental changes and are considered excellent bioindicators of ecosystem health (Simon et al. 2011).

The Ramnagar area is ideal for amphibians due to its diverse array of ecosystems, including wetlands, paddy fields, rivers, and forests. These different habitats encompass a variety of microhabitats, which are essential for different stages of the amphibian life cycle. Amphibians are known to exhibit a wide range of adaptations to survive in both aquatic and terrestrial environments (Çömden et al. 2023) making them a fascinating group to study.

Understanding the diversity of amphibians in the Ramnagar area is crucial for several reasons. Firstly, amphibians serve as important prey for many other organisms, including birds, reptiles, and mammals, contributing to the overall food web dynamics. Secondly, they play a significant role in controlling insect populations, including pests that affect agriculture and human health. Additionally, certain amphibian species possess unique bioactive compounds in their skin secretions, which have shown potential in medical research for their antimicrobial and therapeutic properties (Gomes et al. 2007; Ibarra-Vega et al. 2023).

Despite their ecological importance, amphibian populations worldwide face numerous threats, including habitat loss, pollution, climate change, and emerging infectious diseases (Catenazzi 2015). West Bengal, including the Ramnagar area, is not exempt from these challenges. As a result, studying the diversity and distribution of amphibians in this region becomes even more critical for effective conservation.

By conducting field surveys, this study aims to explore and document the amphibian species diversity and their ecology in the Ramnagar area of Purba Medinipur district, West Bengal, India. These data can contribute to the development of conservation initiatives, habitat management plans, and raising awareness about the importance of preserving amphibian populations.

MATERIALS AND METHODS

Study area. Ramnagar, with a latitude of 21.6745°N and longitude of 87.5593° E, is located in the Purba Medinipur district of West Bengal. It comprises Ramnagar I (to the west) and Ramnagar II (to the east) (R-I and R-II) blocks (Figure 1). According to a 2011 census, a total of 323,384 people inhabit the area (Census 2011). Most people live in villages. The Ramnagar area offers a diverse range of habitats, including wetlands, forests, and agricultural lands, making it a suitable environment for various amphibian species to thrive.

Study period. The study was conducted from 01 September 2022 to 30 November 2023 in various microhabitats of Ramnagar.

Study design. An observational approach was taken for this particular study. The Ramnagar area consists of Ramnagar -I (R-I) and II (R-II) blocks. A total of 10 sampling sites were selected for each block (Figure 1). The sampling sites included various microhabitats like wetlands, forests, agricultural area, etc.

Data collection. Visual encounter surveys (VES) were conducted mostly during evening, night and early morning for detection of amphibian species (Pradhan et al. 2018). Species present at each sampling site were recorded by slowly and quietly walking through the sites and scanning the surroundings for amphibians. Data on habitat characteristics like vegetation cover, water quality, humidity, temperature were also collected at each sampling location to correlate with amphibian presence and abundance (Decena et al. 2020). Vegetation cover data were collected from the Block Land Records Office, whereas humidity, temperature and pH were measured using digital meters: a digital pH meter (ACU-CHECK) and a digital humidity and temperature meter (The Greenland Mushroom, HTC-01 clock). Dissolved oxygen was estimated by using Winkler's method. Photographs of each species were also taken. Geographical co-ordinates for each site were noted. Potential threats or disturbances to amphibian habitats were identified and recorded. Construction of new buildings, use of excessive pesticides and insecticides in agriculture, release of polluted water into ponds or other water channels, off-road use of bike and machine van were the potential threats observed during survey. The book 'Amphibians of Penninsular India' by Daniels, R.J.R. (2005) was used for identification of amphibian species.

Data analysis. All the data gathered from the survey were used for estimating species diversity, richness, and evenness of amphibian species. Statistical analysis to obtain frequencies, percentages, indexes like Shannon, Simpson, Margalef's and Shannon Equitability, preparation of charts, and Pearson correlation (to uncover relationships between environmental factors and species diversity) were done with MS-WORD, MS-EXCEL, and GraphPad Prism 8 statistical software.

Estimation of species diversity. For the estimation of species diversity, the following indices were used (Sharashy 2022):

Simpson Index, i.e., $D = 1\text{-}[\sum n(\underline{n}\text{-}1)/N(N\text{-}1)]$,

Shannon Index, i.e., $H = -\sum p_i \ln p_i$,

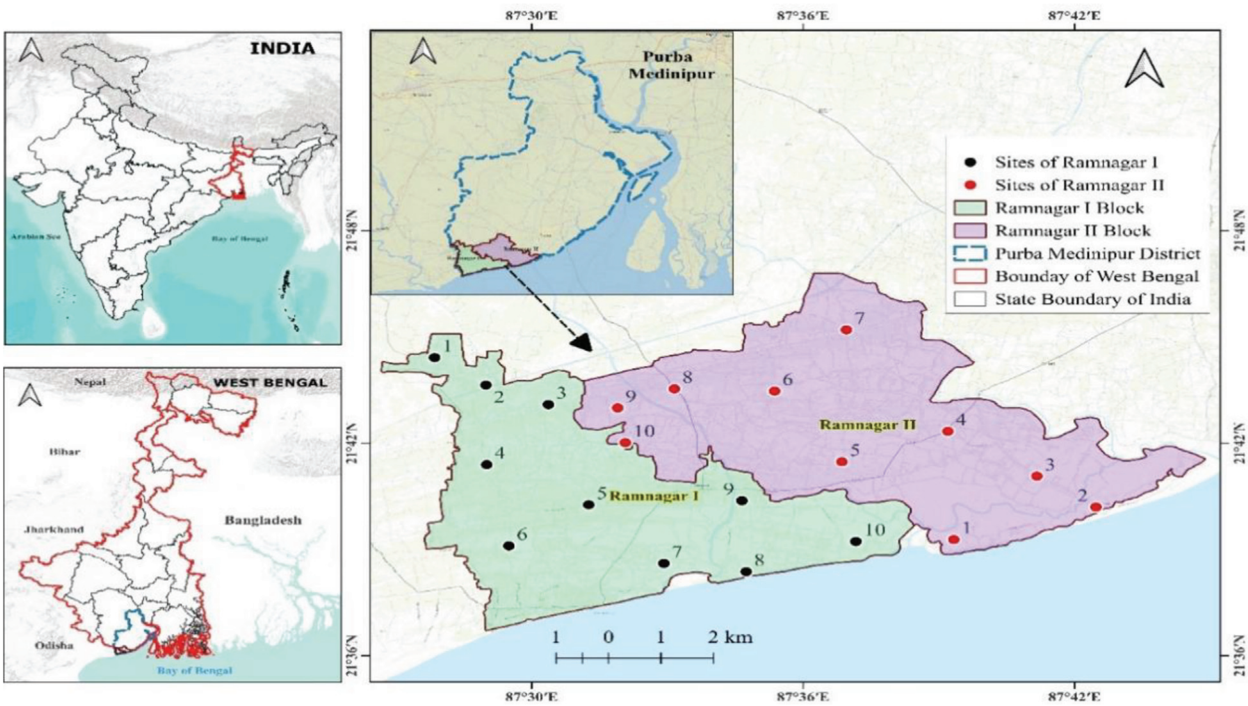

Figure 1. Map of the study area.

where n = the number of members of the species,

N = the number of all members of all the species, and $n/N = p_i$.

Estimation of species richness. In the current study, species richness was estimated by the use of Margalef's Index (Fauzi et al. 2021):

Margalef's Index = $(S-1)/\ln N$,

where S = the number of species.

Estimation of species evenness. In the current study, species evenness was estimated by the use of Shannon Equitability Index (Sharashy 2022):

$E_H = H/\ln K$,

where K = number of species.

RESULTS

Species richness and abundance

A total of 1757 individuals belonging to 10 species and 4 families of anurans were observed during the study (Table 1, Figure 2). *Duttaphrynus melanostictus* was the most abundant (17.76%) species and *Fajervarya orissaensis* the least (0.68%) observed one.

The second most frequently observed species was the Indian bull frog (*Haplobatrachus tigerinus*), with 295 observations, making up about 16.79% of the total. The common tree frog (*Polypedates maculatus*) and the Indian marbled toad (*Duttaphrynus stomaticus*) also had relatively high numbers of observations, with 278 (15.82%) and 217 (12.35%) instances, respectively

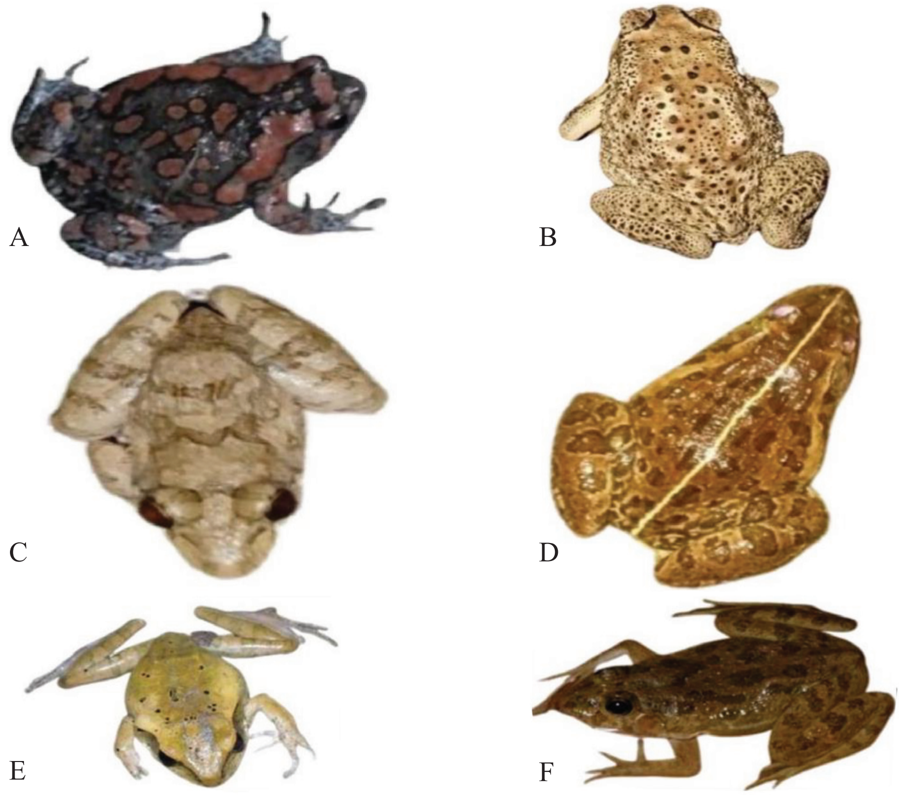

Figure 2. Some of the observed species during the survey. A) *Uperodon taprobanicus*, B) *Duttaphrynus melanostictus,* C) *Fejervarya limnocharis*, D) *Haplobatrachus tigerinus*, E) *Polypedates maculatus*, F) *Euphlyctis* sp.

Table 1. Family-wise distribution of various species and the number of individuals per species in Ramnagar I and II.

Species	Common Name	Family	Number of observed individuals		
			R-I	R-II	Total
Polypedates maculatus	Common tree frog	Rhacophoridae	198	80	278
Haplobatrachus tigerinus	Indian Bull frog	Dicroglossidae	195	100	295
Haplobatrachus crassus	Jerdon's Bull frog	Dicroglossidae	111	90	201
Duttaphrynus melanostictus	Asian Common toad	Bufonidae	195	117	312
Duttaphrynus stomaticus	Indian Marbled toad	Bufonidae	120	97	217
Fejervarya limnocharis	Asian grass frog or rice field frog	Dicroglossidae	107	65	172
Uperodon taprobanicus	Indian Painted frog	Microhylidae	105	74	179
Minervarya sp.	Cricket frog	Dicroglossidae	40	12	52
Euphlyctis sp.	Indian skipperfrog or skittering frog	Dicroglossidae	28	11	39
Fajervarya orissaensis	Orissa frog	Dicroglossidae	12	0	12

(Figure 3). Dicroglossidae was the most abundant family having 6 species, whereas Rachophoridae and Microhylidae were represented by single species (Figure 4).

Diversity indices

The estimated Shannon Index value and Simpson Index value (for species diversity), Shannon Equitability Index value (for species evenness) and Margalef's Index value (for species richness) of the study area were 2.09, 0.87, 0.91 and 1.20, respectively. The Shannon Index values for R-I and R-II exhibited minimal differences, with R-I slightly edging ahead at 2.14 in contrast to 2.05 for R-II (Figure 5). The Margalef's Index value (D_{Mg}) for Ramnagar I and Ramnagar II blocks were 1.28 and 1.24, respectively (Figure 6).

Species diversity at various locations (Figure 5) of R-I ranged from 1.98 (site-10) to 2.15 (site-7), and for R-II between 1.77 (site-10) to 2. 13 (site-8). Species richness values ranged from 1.66 (site-3) to 1.96 (site-9) in R-I and from 1.31 (site-10) to 1.95 (site-6) in R-II. The highest value of species evenness was 0.96 in R-I and 0.98 in R-II.

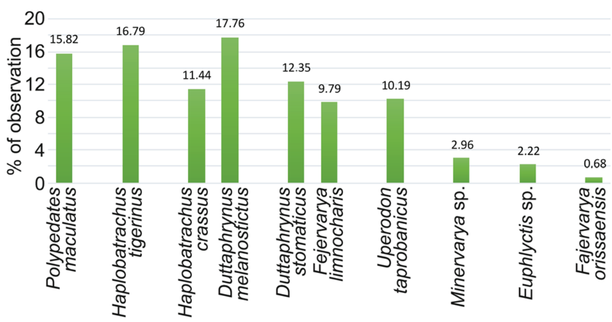

Figure 3. Frequency of observed species.

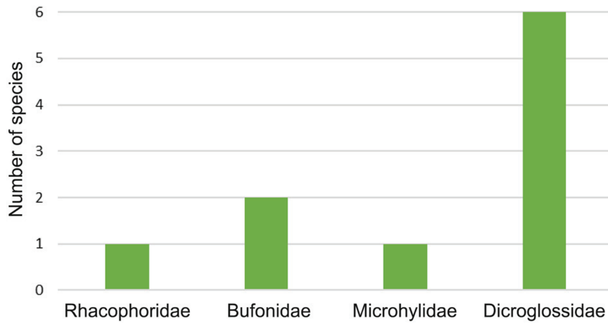

Figure 4. Number of species per family.

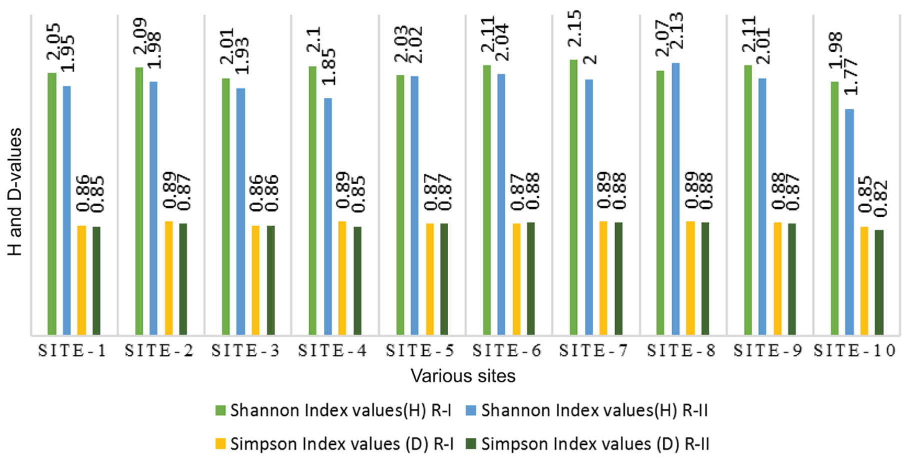

Figure 5. Shannon Index (H) and Simpson Index (D) values for each sampling site in Ramnagar I and II.

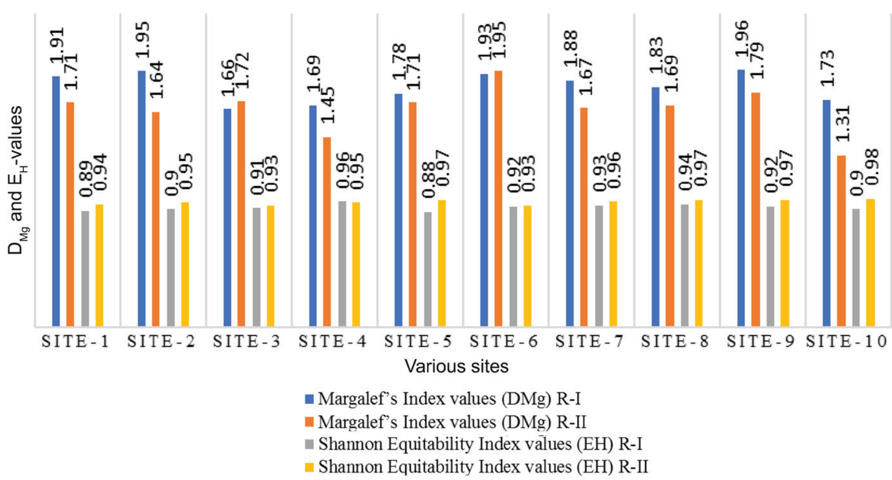

Figure 6. Margalef's Index (D_{Mg}) and Equitability Index (E_H) values at each sampling site in Ramnagar I and II.

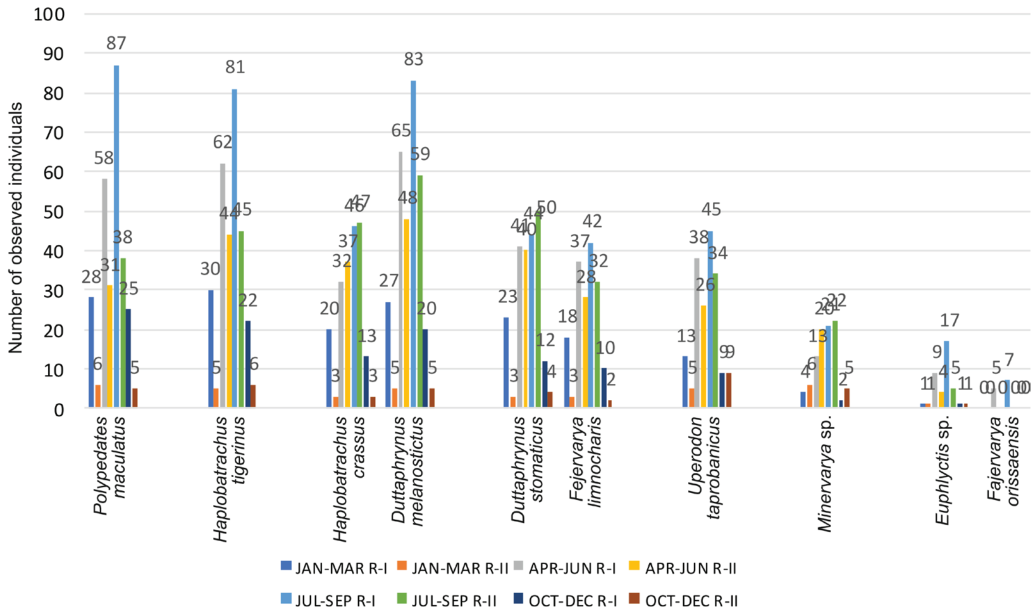

Figure 7. Seasonal variation in the number of observed individuals in Ramnagar I and II.

Table 2. Habitat variables at sampling sites in Ramnagar I and II.

Location	Vegetation cover (%)		pH		Dissolved oxygen		Temperature (°C)		Humidity (%)	
	R-I	R-II	R-I	R-II	R-I	R-II	R-I	R-II	R-I	R-II
Site-1	55	64	6.9	7.2	6.6	6.1	27	27	74	74
Site-2	60	65	7.2	7.2	7.3	6.1	25	28	75	74
Site-3	50	60	7.1	6.8	6.3	5.6	28	30	70	72
Site-4	55	60	7.0	6.8	6.7	5.9	27	30	74	72
Site-5	50	65	6.9	7.1	6.5	6.8	26	28	71	74
Site-6	65	68	7.1	7.1	7.4	7.4	25	25	76	77
Site-7	65	66	7.1	7.1	7.4	7.3	25	25	75	77
Site-8	55	73	7.0	7.0	6.8	7.4	27	24	76	80
Site-9	70	70	7.2	7.2	7.5	7.3	25	26	76	75
Site-10	45	43	6.8	6.5	5.9	5.1	30	32	69	64

Seasonal variation

Seasonal variation in amphibian numbers was also observed in both Ramnagar I and II blocks (Figure 7). During July–September the maximum numbers of amphibians were recorded, whereas during October–December the least numbers were recorded. Ramnagar I (R-I) generally shows higher counts for most species compared to Ramnagar II (R-II) across all seasons.

January–March: This period showed varied numbers across species, with some like *Polypedates maculatus, Haplobatrachus tigerinus*, and *Duttaphrynus melanostictus* showing relatively higher counts.

April–June: Counts generally increased across species, with many reaching their peak during this season.

July-September: There was a continuation of high counts, especially for species like *Polypedates maculatus* and *Duttaphrynus melanostictus*.

October–December: Counts decreased for most species during these months.

Table 3. Pearson correlation analysis results for R-I and R-II.

	Species diversity (H) of R-I	Species diversity (H) of R-II
Vegetation cover (%)	0.889	0.924
pH	0.667	0.728
Dissolved oxygen	0.918	0.871
Temperature (°C)	–0.837	–0.903
Humidity (%)	0.864	0.935

Habitat variables

The values of recorded habitat variables are presented in Table 2. In R-I, the vegetation cover ranged from 45% to 70%. In R-II, the vegetation cover ranged from 43% to 73%. Overall, the vegetation cover in R-II was slightly higher compared to R-I. In R-I, pH ranged from 6.8 to 7.2. In R-II, pH ranged from 6.5 to 7.2. The pH values in R-II were generally lower compared to R-I, indicating slightly more acidic conditions. In R-I, dissolved oxygen ranged from 5.9 to 7.5. In R-II, dissolved oxygen ranged from 5.1 to 7.4. The range of dissolved oxygen values was similar between R-I and R-II, with slightly lower minimum values in R-II. In R-I, temperature ranged from 25°C to 30°C. In R-II, temperature ranged from 24°C to 32°C. R-II showed a wider range of temperature values compared to R-I. In R-I, humidity ranged from 69% to 76%. In R-II, humidity ranged from 64% to 80%. The humidity values in R-II were more variable and had a wider range compared to R-I.

Pearson correlation analysis results (Table 3) suggested relationship of various habitat variables like vegetation cover, dissolved oxygen and pH of water, temperature and humidity with the species diversity (H). From the analysis it can be concluded that dissolved oxygen in Ramnagar I and humidity in Ramnagar II had the most significant effect on species diversity. Overall, the data confirmed that vegetation cover, water quality (pH and dissolved oxygen), and humidity were positively correlated with amphibian species diversity. On the other hand, temperature showed a negative correlation with species diversity.

DISCUSSION

Among the 10 observed species, the Orissa frog (*Fejervarya orissaensis*) was the least observed one, with only 12 observations, making up 0.68% of the total. This species is generally found in the adjacent Orissa state (Mahapatra et al. 2019) but may have entered the habitats within Ramnagar I block as some parts of it form an ecotone area with nearby villages of Orissa.

The Shannon Index value (H) (Kanieski et al. 2018) for species diversity was 2.09. This value suggests that the amphibian community in the studied area is relatively diverse. A higher Shannon Index indicates a greater variety of species and their abundances within the community (Supriatna 2018). The Simpson Index value (D) for species diversity (0.87) also indicates that the community is fairly diverse. Simpson Index ranges from 0 to 1, with high scores indicating high diversities (Shah and Pandit 2013). The Shannon Equitability Index value (E_H) for species evenness was 0.91. This index measures the evenness of the distribution of individuals among the different species. As the value is close to 1, it indicates a more even distribution of individuals across species, suggesting a balanced community (Sharashy 2022). The Margalef's Index (Pankaj 2021) value (D_{Mg}) for species richness was 1.20. It suggests that the amphibian community is moderately diverse, with a reasonable variety of species present. A higher value suggests a higher number of species in the community.

The Shannon Index (H) quantifies species diversity within a specific area, considering the abundance and variety of species present. The similarity of H values for R-I and R-II indicates a similar species diversity between the two locations. Margalef's Index, a measure of species richness based on total individuals and species count, presents a comparable scenario (Shah and Pandit 2013). R-I displays a marginal increase at 1.28, while R-II records a value of 1.24. This subtle distinction implies akin levels of species richness relative to the total sampled population for both sites.

Ramnagar I consistently had higher total counts across all seasons. Ramnagar II generally showed lower counts for most species, with some exceptions. Seasonal variations could be due to breeding patterns, migration, food availability, and environmental factors. The differences between R-I and R-II could be due to habitat differences, human activity, or other ecological factors. Across all species, there was a noticeable pattern of seasonal variation in amphibian numbers. The result from the study by Villa et al. (2019) in a Cloud Forest of the Tropical Andes also suggested that seasonal variations in climatic factors drive fluctuations in both the overall abundance and species composition of the anuran community. Carvalho-Rocha et al. (2023), while studying seasonal variation in patterns of anuran diversity found that abundance increased between spring and summer but declined between autumn and winter. This is in agreement with the present study which also indicated that the numbers tend to be higher during the warmer months (April–June and July–September) compared to the colder months (January–March and October–December). This suggests that amphibian activity and breeding are influenced by temperature and environmental conditions (Mehra et al. 2021). This pattern is typical in many ecosystems, where warmer temperatures and increased rainfall can lead to a higher biological activity and species diversity. Understanding these patterns is crucial for ecological conservation and management, providing insights into the health and dynamics of local ecosystems. Further studies and monitoring efforts can delve deeper into the specific factors influencing these observed patterns.

The significant increase in numbers during the April–June and July–September periods suggests that these months correspond to the breeding season for many of the observed species. This is reflected in the increased counts of individuals during these periods, which is likely due to higher reproductive activities (Prado et al. 2005). Koskei (2023), while studying breeding ecology and population status of Kenyan amphibians, also opined that amphibian abundance was maximum during wet seasons. This finding is in agreement with the result of the present study. The lower numbers observed during the colder months (January–March and October–December) could indicate a period of reduced activity or even hibernation for some amphibian species. This is especially evident in the decreased numbers of certain species like *Minervarya* sp., *Euphlyctis* sp. etc. during these months. There seems to be some variation in response to seasons between the two regions (R-I and R-II), which could be attributed to local environmental factors. For instance, *Haplobatrachus crassus* shows a different pattern in the two regions, with higher counts during different periods.

The differences in the number of individuals for each species (Table 1) imply that the suitability of habitats and the influence of environmental factors differ among these areas, contributing to the varying presence and abundance of different species. Decena et al. (2020) also reported a similar effect of environmental factors on amphibian diversity and species composition. *Polypedates maculatus*, *Duttaphrynus melanostictus* and *Haplobatrachus tigerinus* stand out as the more prominent species among those observed, showing consistently higher numbers across most surveyed locations. This suggests that these species have likely adapted well to the local environmental conditions and have established thriving populations in the surveyed regions. Conversely, certain species like *Minervarya* sp., *Euphlyctis* sp., and *Fajervarya orissaensis* exhibit lower numbers across all surveyed locations. This lower abundance could indicate that these species are potentially more sensitive to changes in their surroundings or have more specific and selective habitat requirements. While the absolute numbers may differ between Ramnagar I and Ramnagar II, similar patterns of species abundance and spatial variation are observed in both areas.

Variations in habitat variables can influence ecosystem dynamics and the distribution of species within these habitats. R-II showed more variability in habitat variables, with some sites having higher or lower values compared to the corresponding sites in R-I. R-I generally exhibited more consistent values across variables, with less variability compared to R-II. Roach et al. (2020) reported that land or vegetation cover positively effects amphibian diversity. The current study also suggests a strong positive correlation (Table 3) exists between vegetation cover and species diversity (0.889), which implies that areas with higher vegetation cover tend to support a more diverse amphibian population (Smallbone et al. 2011; Boissinot et al. 2019). According to Sunar et al. (2023) amphibian abundance is positively correlated with water pH. In the current study, water quality in terms of pH showed a moderate positive correlation with species diversity (0.667). This indicates that more suitable pH levels in water are associated with a higher amphibian species diversity (Dolmen et al. 2017). It was claimed that decrease in dissolved oxygen level negatively effects species richness (Baskale, Kaya 2009). The present study also states that dissolved oxygen strongly and positively correlates with species diversity (0.918) (Calderon et al. 2019). This highlights the critical role of dissolved oxygen in supporting a diverse population of amphibians. Temperature displayed a strong negative correlation with species diversity (-0.837) (Ortiz-Yusty et al. 2013). This suggests that extreme temperatures negatively impact amphibian diversity, and moderate temperatures are more conducive to supporting diverse amphibian populations. Decena et al. (2020) also reported similar findings. Rainfall and other factors related to humidity have been demonstrated as significant environmental factors influencing the diversity and composition of amphibian communities (Duellman 1988). The level of humidity plays a crucial role in shaping the phylogenetic diversity observed among amphibian populations. Humidity exhibited a strong positive correlation with species diversity (0.864). This was also reported by Da Silva et al. (2012). This reinforces the importance of maintaining higher humidity levels to support a diverse amphibian community. A strong positive correlation (0.924) between vegetation cover and species diversity (in R-II) means that as vegetation cover increases, species diversity also tends to increase (Smallbone et al. 2011). There was a very strong positive correlation (0.871) between water quality (dissolved oxygen) and species diversity. It means that as dissolved oxygen content in water increases, species diversity also tends to increase (Steve et al. 2014). A very strong negative correlation (-0.932) between temperature and humidity indicates that if temperature increases, humidity tends to decrease (Thani et al. 2017). Data also suggested a very strong negative correlation (-0.903) between temperature and species diversity (Muhammad et al. 2022). It means that as temperature increases, species diversity tends to decrease.

On average, R-I has a slightly higher diversity than R-II. A large amount of man-made disturbances (like infrastructure development, agriculture, logging, water pollution, off-road use of bike and machine van, etc.) were observed at site-10 of R-II, which may be the cause of its low diversity (Kusrini et al. 2020). A comparison of habitat variables between R-I and R-II provides insights into the environmental conditions of the study sites. Understanding these variations is crucial for effective habitat management and conservation efforts. Further research and monitoring are necessary to assess the long-term trends and impacts on ecosystems.

CONCLUSION

The study conducted in the Ramnagar area of Purba Medinipur district revealed a moderate diversity of anurans, identifying 10 species across 4 families. *Duttaphrynus melanostictus* emerged as the most abundant species, whereas *Fajervarya orissaensis* was the least observed. The analysis using Shannon and Simpson diversity indices indicated a moderately diverse anuran community. Additionally, Margalef's Index suggested a more even distribution of individuals across species, pointing towards a balanced community. The Shannon Equitability Index corroborates this, highlighting moderate species diversity within the amphibian community. Among the ecological factors examined, water quality, specifically dissolved oxygen, and humidity were found to have the most significant impact on species diversity. A strong positive correlation between dissolved oxygen levels and humidity with species diversity underscores the critical role of water quality in sustaining a diverse anuran community. Similarly, temperature was also identified as a key factor, negatively influencing species diversity.

These findings emphasize the importance of maintaining high water quality, suitable humidity, and temperature levels to support a diverse and balanced anuran community. Conservation efforts should prioritize these factors to preserve and enhance amphibian diversity in the studied habitat. Various anthropogenic activities remain a cause of concern for anurans in this area. Given the complete lack of previous research in this area, the study findings may contribute valuable information to the existing knowledge on amphibian biodiversity in West Bengal, particularly in the Purba Medinipur district. The updated checklist of amphibians and the information on habitat variables presented in the present study would serve as a source of further research and help the conservation and management authorities set priorities and subsequent plans. Future research should continue to monitor these ecological parameters and explore their interactions with other environmental factors to develop comprehensive conservation strategies. The study's outcomes are expected to assist local authorities, conservation organizations, and policymakers in making informed decisions regarding the protection and sustainable management of amphibians and their habitats in the region. This study suggests strengthening state-wise conservation of amphibians and their habitats and running public awareness programmes to involve them in habitat management.

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