

Research article

Amphibian diversity and their association with water quality: A case study of the ponds of Bhaktapur, Nepal

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Suggested citation: Hyongaju S,, Karmacharya D.K. and Shrestha M.K. 2023. Amphibian diversity and their association with water quality: A case study of the ponds of Bhaktapur, Nepal. Nepalese Journal of Zoology, 7(2):29– 37.

https://doi.org/10.3126/njz.v7i2.60808

Article History: Received: 29 September 2023 Revised: 18 December 2023 Accepted: 18 December 2023

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Abstract

Amphibians are noteworthy bio-indicators and bio-control agents. They have an intimate relationship with their aquatic environment. Therefore, the present study was conducted aiming to investigate the association of amphibian diversity with the Physicochemical factors of pond water. The field surveys were carried out in Rani Pokhari, Bhajya Pokhari and Gonga Pokhari of Bhaktapur Municipality during the rainy season using repeated visual encounter surveys and opportunistic observations. Surveys were made around the ponds from 06:00 to 09:00 and from 18:00 to 21:00 once a week spending three hours per visit walking along the perimeter of ponds. Water samples were collected from the four corners of each pond. The physicochemical analyses of sample water were carried out as per the scientific instrumentations and standard procedures. A total of 77 individuals of five different species of amphibians belonging to three families were documented from the study areas. The higher values of the Shannon-Wiener diversity index (H=1.34), and Simpson's reciprocal index (1/D=3.656) were recorded for Rani Pokhari. All the ponds exhibited a high degree of Pielou's evenness index (e>0.9). The physicochemical analysis indicated a favourable aquatic environment for amphibians in Bhajya Pokhari and Rani Pokhari and the least favourable aquatic conditions in Gonga Pokhari which supported only a few tolerant species. Karl Pearson's correlation coefficient between Shannon-Wiener diversity index and physicochemical factors revealed a positive association with temperature (r=0.997), transparency (r=0.695) and dissolved oxygen (r=0.353) while a negative association with pH, electrical conductivity, free CO₂, total dissolved solids and alkalinity. The water quality of the ponds of Bhaktapur is in healthy condition for the existence of amphibians and other aquatic organisms. The information obtained is useful for implementing amphibian and pond water conservation management. In addition, this study has established baseline database of amphibians for Bhaktapur District which is valuable for the local government to maintain the biodiversity profile of the district.

Keywords: Diversity index; Frogs; Physicochemical parameters; Water analysis

1 | Introduction

Globally, there are about 8,676 species of amphibians around the world with the largest order Anura currently containing 7,632 (88%) recognized species followed by 9% Caudata and 3% Gymnophiona (AMNH 2023). Nepal comprises a remarkable amphibian diversity which houses 57 species of amphibians including one newt, one caecilian and 55 frogs and toads (Rai et al. 2022; Amphibiaweb 2023). Amphibians in Nepal are distributed across a wide elevational range from 100 meters to above 5,000 meters (Shah & Tiwari 2004). Amphibians form a crucial component in the food chain both as a prey and predator (Hocking & Babbitt 2014). They feed on invertebrates as well as small vertebrates and aquatic vegetation. Their larvae are mostly herbivores which help in regulating biomass of aquatic phytoplankton forming the important link in the food web of ecosystem (Duellman & Trueb 1986; Hocking & Babbitt 2014). Amphibians are the bioindicator of health of environment that indicate pollution and degradation of aquatic habitat (Quaranta et al. 2009). They are effective bio-control agents consuming largely on agricultural pest invertebrates (Khatiwada et al. 2016; Sapkota et al. 2022) which cause extreme damage to crops (Attademo et al. 2005). Amphibians also feed on mosquitoes and diseasecausing flies that are responsible for the transmission of many human diseases (Durant & Hopkins 2008).

Amphibians extremely depend upon their aquatic habitats for their survival. The Physicochemical factor of the aquatic environment exerts a substantial control over the abundance and diversity of amphibians. Water quality affects the population dynamics and community structure of aquatic ecosystems (Castaneda 2014). Amphibians have intimate contact with aquatic ecosystems; different factors associated with water have an impact on the density, distribution and abundance of amphibians (Griffiths et al. 2010). Therefore, the present study was conducted aiming to fill the research gap on the association of amphibian diversity with the physicochemical factors of pond water. In addition, this study has established a baseline database of amphibians for Bhaktapur district which is valuable for the local government to maintain the biodiversity profile of the district which is also valuable for the upcoming researchers to extend their studies.

2 | Materials and methods

2.1 | Study area

The study was conducted in the ponds of Bhaktapur, the most ancient and smallest district of Nepal, occupying 119 km² area in the eastern part of Kathmandu Valley having an altitude from 1,331 meters to 2,191 meters above the sea level (Bhaktapur district profile 2020). The climate is subtropical, cool- temperate and temperate, with March to May spring, June to August summer, September to November autumn and December to February winter season (ICIMOD et al. 2007). Among the 35 ponds (Pokhari) of Bhaktapur Municipality (Khaniya 2005), three ponds; Rani Pokhari (27°40'19.25"N,

85°24'54.01"E), Bhajya Pokhari (27°40'14.61"N, 85°25'15.61"E) and Gonga Pokhari (27°40'36.64"N, 85°26'6.58"E) were selected for the amphibian survey and water sampling representing the ponds of Bhaktapur Municipality (Fig. 1).

2.2 | Field surveys and laboratory analysis

An amphibian survey was carried out during the rainy season from July to October using repeated visual encounter surveys (Crump & Scott 1994; Gillespie 1997; Sunar et al. 2022) and opportunistic observation (Heyer et al. 1994; Bhattarai et al. 2018; Paudel et al. 2023). Surveys were made from 6:00 to 9:00 in the morning and 18:00-21:00 once a week, spending three hours per visit walking along the perimeter of ponds (Sunar et al. 2022). All the species encountered were photographed on the spot using a Nikon camera (model DX 18-55mm) and identified through consultation with experts and using the field guidebook "Herpetofauna of Nepal: A Conservation Companion" (Shah & Tiwari 2004).

Water samples were collected from the four corners of each pond. pH, temperature and transparency of the pond water were measured on the spot at study sites using universal litmus paper, standard mercury thermometer and Secchi-disk respectively. Electrical conductivity (EC) was measured using an Electrical conductivity meter.



Figure 1. Map of study area showing the study ponds

Total dissolved solids (TDSs), Dissolved Oxygen (DO) and Free carbon dioxide (CO_2) were measured on the same day of collection in the Laboratory of the Department of Zoology, Bhaktapur Multiple Campus using volumetric titration and instrumentation methods.

2.3 | Data analysis

The species diversity of amphibians was measured using the equation:

Shannon-Wiener diversity index (H) = $-\sum_{i=1}^{n} Pi \ln Pi$ (Shannon & Wiener 1963), and

Simpson's diversity index (D) = $1 - \sum Pi^2$ (Simpson 1949).

Species richness is the total number of various species present in a certain community which was measured using the equation:

Margalef's index (R) = $(S - 1) / \ln N$ (Margalef 1958).

Species evenness is the number of each species in a particular area. It was measured using the equation:

Pielou's evenness index (E) = H / ln S (Pielou 1966).

Where, Pi = Proportion of individuals belonging to the*i*th species, S= number of species and N= total number of individuals of all species

The association between the amphibian diversity and the Physicochemical factors was analyzed using Karl Pearson's Correlation coefficient on MS Excel 2010.

3 | Results

Altogether 77 individuals of five species of amphibians (Fig. 2) belonging to three families were recorded from the selected ponds of Bhaktapur. Among them, 53 individuals of four species of amphibians belonging to three families were recorded from Bhajya Pokhari. Similarly, 11 individuals of four species of amphibians belonging to two families were recorded from Rani Pokhari and 13 individuals of two species of amphibians

Table 1. Amphibian diversity in the ponds of Bhaktapur

Ponds	Family	Species	n	RD	(H)	(1/D)	R	Е
Bhajya Pokhari	Microhylidae	Microhyla ornata	23	43.4%	1.17	3.356	0.524	0.937
	Dicroglossidae	Limnonectus syhandrensis	12	22.6%				
	Bufonidae	Duttaphyrnus melanostictus	10	15.1%				
		Duttaphyrnus stomaticus	8	18.9%				
	Total		53	100%				
Rani Pokhari								
	Dicroglossidae	Limnonectus syhandrensis	2	18.2%	- 1.34	3.656	0.867	0.968
		Hoplobatrachus tigerinus	2	18.2%				
	Bufonidae	Duttaphyrnus melanostictus	4	36.3%				
		Duttaphyrnus stomaticus	3	27.3%				
	Total		11	100%				
Gonga Pokhari								
	Bufonidae	Duttaphyrnus melanostictus	8	61.5%	0.50	1.901	0.270	0.992
		Duttaphyrnus stomaticus	5	38.5%	0.59			
	Total		13	100%				

belonging to one family were recorded from Gonga Pokhari (Table 1).

The Shannon-Wiener diversity index was 1.17, 1.34 and 0.59 in Bhajya Pokhari, Rani Pokhari and Gonga Pokhari respectively; similarly, Simpson's reciprocal index was 3.356, 3.656 and 1.901; Margalef's index (species richness) was 0.524, 0.867 and 0.270; Pielou's evenness index was 0.937, 0.968 and 0.992 in Bhajya Pokhari, Rani Pokhari and Gonga Pokhari respectively. Relative dominance was higher (43.4%) for *Microhyla ornata* in Bhajya Pokhari, *Duttaphyrnus melanostictus* (36.3%) in Rani Pokhari and *Duttaphyrnus melanostictus* (61.5%) in Gonga Pokhari. Lower relative dominance (15.1%) was found for *Duttaphyrnus melanostictus* in Bhajya Pokhari, *Hoplobatrachus tigerinus* and *Limnonectus syhandrensis* (18.2% each) in Rani Pokhari and *Duttaphyrnus stomaticus* (38.5%) in Gonga Pokhari (Table 1).

The average pH of water was 8.8, 8.3 and 9.3 in Bhajya Pokhari, Rani Pokhari and Gonga Pokhari respectively. All the pH values were above 7.0, indicating the alkaline nature of all three ponds' water. The average surface water temperature was 26.1°C in Bhajya Pokhari 27.1°C for Rani Pokhari and 23.8°C for Gonga Pokhari showing the warmness of pond water. The transparency of Bhajya Pokhari, Rani Pokhari and Gonga Pokhari was 64 cm, 38 cm and 17 cm respectively. Gonga Pokhari had least value of transparency indicating higher turbidity due to the higher amount of pollutants in the water. Total dissolved solids (TDS) are the varieties of minerals present in water. The TDSs of Bhajya Pokhari, Rani Pokhari and Gonga Pokhari were 120 ppm, 260 ppm and 220 ppm, respectively. Electrical conductivity (EC) indicates the capacity of water to conduct an electric current. The EC of Bhaiva Pokhari, Rani Pokhari and Gonga Pokhari was 195µS/cm, 231µS/cm and 318µS/cm respectively indicating the higher concentration of ionized substances in Gonga Pokhari having comparatively more human induced pollutants released from human residents. Alkalinity is the ability of water that neutralizes acid. Alkalinity of Bhajya Pokhari, Rani Pokhari and Gonga

	Acceptable limits (APHA	Average value			
Physicochemical factors	2005; EPA 1986; Boyer and	Bhajya	Rani	Gonga	
	Christian 1995)	Pokhari	Pokhari	Pokhari	
рН	7.0-9.0	8.8	8.3	9.3	
Temperature (°C)	5-25	26.1	27.1	23.8	
Transparency (cm)	12 - 80	64	38	17	
Total dissolved solids (ppm)	50 - 250	120	260	220	
Electrical conductivity(µS/cm)	150 - 500	195	231	318	
Alkalinity(mg/lt)	50 - 150	30.3	14.2	26.3	
Dissolved oxygen (mg/lt)	6.5-8	7.2	21.2	10.4	
Free carbon dioxide (mg/lt)	12-25	3.3	4.5	6.9	

Table 2. Physicochemical factors of ponds of Bhaktapur



Figure 2. Amphibians recorded and study sites. a: *Microhyla ornata*, b: *Limnonectes syhandrensis*, c: *Duttaphrynus melanostictus*, d: *Duttaphrynus stomaticus*, e: *Hoplobatrachus tigerinus*, f: Bhajya Pokhari, g: Rani Pokhari, h: Gonga Pokhari

Pokhari was 30.3 mg/lt, 14.2 mg/lt and 26.3 mg/lt respectively. The concentration of dissolved Oxygen in Bhajya Pokhari, Rani Pokhari and Gonga Pokhari was 7.2 mg/lt, 21.2 mg/lt, 10.4 mg/lt respectively. The

concentration of free CO_2 was 3.3 mg/lt in Bhajya Pokhari, 4.5 mg/lt in Rani Pokhari and 6.9 mg/lt in Gonga Pokhari (Table 2).



Figure 3. Association of amphibian diversity with water quality

Shannon-Wiener diversity index showed the strong positive association with temperature (r=0.997) and transparency (r=0.695) but weak positive correlation with the concentration of DO (r=0.353), while strong negative relation with pH (r=-0.954), electrical conductivity (r=-0.874) and free CO₂ (r=-0.852) and weak negative association with total dissolved solids (r=0.025) and alkalinity (r=-0.480) (Fig. 3). The positive association between Physicochemical factors and diversity signifies the increase in the value of Physicochemical factors up to optimum limits also increases the diversity.

4 | Discussion

The present study recorded five species of amphibians belonging to three families similar records were obtained by Paudel et al. (2023) who also documented five species of amphibians in Ramaroshan wetland complex of Achham, Nepal. A total of 11 frog species belonging to five families were recorded by Shah and Tiwari (2004) while Aryal et al. (2020) recorded 10 frog species belonging to six families from Kathmandu Valley. Higher numbers of individuals of the amphibian species were found in Bhajya Pokhari compared to Rani Pokhari which is similar with the study carried out by Pawar et al. (2004) and Gillispie et al. (2015). The less abundance of species in Rani Pokhari in comparison to Bhajya Pokhari can be related with the presence of many predatory organisms in the areas. However, both the Bhajya Pokhari and Rani Pokhari comprised of four species of amphibians and the presence of a higher number of amphibian individuals in these ponds might be due to less human interferences in comparison with Gonga Pokhari.

The diversity of species in a region depends on both species richness and their total numbers (Munoz et al. 2018). The higher values of Shannon-Wiener diversity index (1.34), Simpson's reciprocal index (3.656) and Margalef's index (0.867) were reported from Rani Pokhari. The least values of diversity indices were reported from Gonga Pokhari which could be due to disturbance from anthropogenic activities including mixing of household waste. In Bhajya Pokhari, Rani Pokhari and Gonga Pokhari, Pielou's evenness index was found to be 0.937, 0.968 and 0.992 respectively. The ponds were characterized by high degree of species evenness indicating the occurrence of similar species in all the ponds.

Amphibians are sensitive to their aquatic habitat perturbation. It is important to have suitable Physicochemical factors in their aquatic environment for their survival (Blaustein et al. 1994). The pH range from 8.3 to 9.3 in this study indicated that the amphibian prefers slightly alkaline water rather than acidic one which is consistent with the study of Shaikh et al. (2016) and Sunar (2022). The permissible range of pH for amphibians is 7.0 to 9.0 (Boyer & Christian 1995). The pH values were within the tolerable range for amphibians in the ponds of Bhaktapur. The strong negative correlation (r= -0.954) of amphibian diversity with pH signified that amphibians do not prefer habitats with extreme high and low pH values because such conditions destroy the aquatic organisms (Serrano et al. 2016).

Temperature considerably impacts on both the biological and chemical nature of water (Dixit et al. 2015). The difference in water temperature may depend on the climatic condition as well as on amount of sunlight, time and rate of wind flow. The maximum temperature $(27.1^{\circ}C)$ was recorded in Rani Pokhari and minimum $(23.8^{\circ}C)$ in Gonga Pokhari. Water temperature from $13.5^{\circ}C$ to $32^{\circ}C$ is suitable for the development of life forms (Gaikwad et al. 2008). In the present study, amphibian diversity and temperature revealed strong positive correlation (r=0.997) between them indicating the survival of amphibians within the optimum limits of temperature.

Turbidity signifies the transparency of water due suspended solids in it. Lesser turbidity or higher transparency (64 cm) was found in Bhajya Pokhari whereas lesser transparency (17cm) was in Gonga Pokhari which implies fewer amounts of suspended solids present in Bhajya Pokhari than in Gonga Pokhari. The higher number of suspended solids in Gonga Pokhari might be due to the discharge of domestic wastes from human settlement areas. Turbidity limits the penetration of sunlight through the water which in turn reduces the photosynthetic activity of phytoplankton in a community (Nunes et al. 2022). The current study indicated strong positive correlation (r=0.695) between amphibian diversity and transparency that signified that amphibian prefers plankton-induced turbidity rather than suspended inorganic pollutants (Rahman et al. 1982).

The standard value of TDSs for pond water is 50.0 to 250.0 ppm (APHA 2005) which is also preferable for amphibians (Saikh et al. 2014). Higher TDSs in Rani Pokhari (260 ppm) and Gonga Pokhari (220 ppm) might be because of nutrients from decomposition and sedimentation. Bhajya Pokhari showed low TDSs among three ponds which indicated that the pond has less minerals compared to Rani Pokhari and Gonga Pokhari. However, all ponds have TDSs in tolerable limit for amphibians. Current study revealed the negative association (r= -0.025) between amphibian diversity and TDSs that resembled the Kupinde lake of Salyan district (Sunar et al. 2022).

Electrical conductivity (EC) is determined by the amount of salts present in water. As per Environmental Protection Act (EPA) - 1986, amphibians prefer the EC range from 150-500 μ S/cm. Present study recorded the least EC (195 μ S/cm) in Bhajya Pokhari and higher EC (318 μ S/cm) in Gonga Pokhari. The higher values of EC in Gonga Pokhari might be due to decomposition of organic waste and mixing of domestic sewages from settlement areas. The results from this study revealed strong negative association (r= -0.874) between amphibian diversity and electrical conductivity. Polluted water shows higher values of conductivity (Trivedy et al. 1985) which is reportedly destructive for the amphibians (Krenkal 1980).

Alkalinity implies hydroxide, carbonate and bicarbonate content in water. It is the buffering capacity (Durge et al. 2018) of water which measures total value of base present in water. Alkalinity in Bhajya Pokhari (30.3mg/lt), Rani Pokhari (14.2 mg/lt) and Gonga Pokhari (26.33 mg/lt) were found to be less than the permissible value of alkalinity that ranges from 50-150 mg/lt (Boyer & Christian 1995). However, comparatively higher value of alkalinity in Bhajya Pokhari indicated that the pond is moderately rich in nutrients (Spence 1964). Negative correlation (r= -0.480) was found in between amphibian diversity and alkalinity in the present study. Most aquatic organisms favor alkalinity between 50 mg/lt to 150 mg/lt but no less than 20 mg/lt (Wurts 2002).

DO is the critical factor regulating the survival of aquatic life. A normal pond would have a DO level of about 6.5-8 mg/lt (APHA 2005). Maximum DO (21.2 mg/lt) was found in Rani Pokhari whereas minimum (7.2 mg/lt) was recorded from Bhajya Pokhari. The variations of DO depend on the primary production and respiration of aquatic organisms. In the present study, amphibian diversity and DO show the positive association (r=0.353) identical with Calderon et al. (2019) that signified amphibians prefer the aquatic environment with sufficient amount of DO.

For the suitable environment of amphibians, the favorable range of CO₂ is 12.0 to 25.0 mg/lt (APHA 2005). The present study recorded maximum free CO₂ (6.9 mg/lt) from Gonga Pokhari and minimum (3.3 mg/lt) from Bhajya Pokhari. The least free CO₂ in the ponds might be the result of consumption of free carbon dioxide by the producers during photosynthesis. Amphibian diversity and free CO₂ exhibited a strong negative correlation (r= - 0.852) which indicated that amphibians are severely affected by extremely high or low levels of CO₂ in their aquatic environment.

5 | Conclusions

Physicochemical factors of pond water have a significant effect on amphibian diversity surviving in the pond ecosystem. Five species of amphibians were recorded from the ponds of Bhaktapur. Bufonidae were present in all the ponds. Rani Pokhari, Bhajya Pokhari and Gonga Pokhari all are slightly alkaline in nature which are within the tolerable range for amphibians. The temperature is favorable for the development and the survival of amphibians. Transparency, turbidity, TDSs, EC, alkalinity, DO and free CO₂ of the ponds are also within the optimum limits to survive the amphibians. Gonga Pokhari contains higher suspended solids which are mixed from the discharge of domestic wastes from human settlement. Bhajya Pokhari has rich nutrients and Rani Pokhari has sufficient amount of DO for the water living creatures. The water quality of ponds of Bhaktapur is in healthy condition for the existence of amphibians and other aquatic organisms. The information obtained from current study is very useful for the monitoring of amphibians and pond water quality for the local government and upcoming researchers. Physicochemical factors of the pond water should be regularly managed properly by maintaining its health to conserve diversity of amphibian and other aquatic organisms existing in the pond ecosystem. Regular monitoring is highly recommended to formulate policies in local level for implementing amphibian and pond water conservation management.

Acknowledgements

We are indebted to the local government, Bhaktapur Municipality for the research permission. We are grateful to our Bhaktapur Multiple Campus, Institute of Science and Technology, Tribhuvan University for providing the platform to carry out this research and laboratory facilities. Many thanks to Professor Karan Shah, Subarna Ghimire and Purna Man Shrestha for the species identification. Many appreciation to Rosary Karki, Anju Karmacharya, Minu Shilpakar, Rabi Bati, Jeewan Gwaccha, Sujan Khyaju and Krishna Kutuwaju for their support during fieldwork. We are also thankful to Professor Dr. Krishna Das Manandhar for the valuable comments, suggestions and evaluation of the work.

Authors' contributions

S.H., D.K.K., M.K.S. conceptualized the study. S.H. conducted the lab work. S.H. and D.K.K. performed data analysis and prepared the draft of the paper. All authors read, gave their inputs and approved the final manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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