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Effect of water physicochemistry on amphibian abundance in Sub-tropical Kupinde Lake of the Nepal Himalaya

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Abstract. *Sunar CB, Pandey N, Chand B, Upadhyaya LP, Thapa B, Pant RR, Khanal L. 2022. Effect of water physicochemistry on amphibian abundance in Sub-tropical Kupinde Lake of the Nepal Himalaya. Intl J Bonorowo Wetlands 12: 89-95.* Amphibians are the key bioindicators of ecosystem health. Their diversity and abundance are affected by the physicochemical factors of the environment. Wetland ecosystems of the Himalaya are under the threat of human activities and current climate change. However, hydrochemical status and faunal diversity are poorly documented in the wetlands of western Nepal. This study characterized the physicochemical parameters of water in Kupinde Lake in the Salyan District of Karnali Province, Nepal, and established the association between amphibian abundance and those parameters. A total of 24 samples of surface water were collected at the lake's edge during October 2021, and 18 physical and chemical parameters were analyzed. Visual encounter surveys were conducted at each water sampling site to count amphibians within a circle of two meters in diameter. The lake water was slightly alkaline (mean pH = 8.16±0.29), and the major dominating cation and anion were Ca^{2+} and HCO_3^-, respectively. Amphibian abundance was positively correlated with pH, HCO_3-, and NH_4^+ and negatively correlated with EC, TDS, CO_2, Cl^-, and Na^+. The polynomial regression analysis revealed amphibian abundance has a consistent positive association with water pH ($r^2 = 0.497$, $p < 0.05$) and unimodal relation with the temperature ($r^2 = 0.188$, $p < 0.05$). Low amphibian diversity and dominance of a single amphibian species, the Indian skipper frog (*Euphlyctis cyanophlyctis* Schneider, 1799), indicate poor ecosystem health of the lake. The findings of this study provide baseline information for monitoring and managing the Kupinde Lake.

Keywords: Bioindicators, *Euphlyctis cyanophlyctis*, herpetofauna, hydrochemistry, Karnali Province

INTRODUCTION

Amphibians are unique among vertebrates because they use aquatic and terrestrial habitats at different stages of their lives (Compton et al. 2007; Rittenhouse and Semlitsch 2007). They utilize a variety of terrestrial and aquatic habitats for breeding, larval development, and over-wintering (Shah and Tiwari 2004). They play important roles in the trophic levels of the ecosystems (Mifsud 2014; Thakuri and Pokhrel 2017; Ali et al. 2018). Amphibians are excellent indicators of wetland ecosystem health because they rely heavily on water (Hecnar 2004; Amankwaa et al. 2020; Riyanto and Rahmadi 2021; Paudel et al. 2023) and play a key role in forecasting environmental quality in their habitats (Mifsud 2014; Amarasinghe et al. 2021). They provide various ecosystem services, such as religious and cultural services, food, and medicine (Shah and Tiwari 2004; Paulding and Randhir 2021). In addition to regulatory functions, they also disperse seeds and control biological pests (Paulding and Randhir 2021).

Amphibian populations are declining due to reduced habitat and degraded microhabitat quality, climate change, pollution, anthropogenic activities, diseases, etc. (Rastegar-Pouyani et al. 2015; Blaustein et al. 2018; Muths et al. 2020; Paulding and Randhir 2021). In wetlands, factors such as permanent water, the spatial configuration of wetlands and upland habitats, the characteristics of local habitats, and water chemistry and quality have an extensive effect on the composition of amphibian communities and the abundance of individual species (Hecnar 2004). Dissolved oxygen, temperature, pH, salinity and water conductivity, organic carbons, and pollutants are important factors in their habitats that can affect survival, growth, maturation, and physical development (Sparling 2010). These wetland characteristics are influenced by hydroperiod, resulting in changes in the abundance, density, reproduction, and growth rates of amphibians (Brannelly et al. 2019; Boelter et al. 2020). In addition, fertilizer and pesticides are commonly applied to increase agriculture production, negatively affecting the amphibians and reptiles that inhabit agricultural fields and downstream water bodies (Ghosh and Basu 2020). Various agricultural chemicals, such as pesticides, herbicides, fungicides, and fertilizers, contribute to water pollution (Mann et al. 2009). Polluted water can affect amphibians through their permeable skin (Boyer and Grue 1995), and they become prone to infections, limb deformities and a decrease in their numbers (Linzey et al. 2003). In order to survive, reproduce and develop, amphibians, particularly anuran species, are highly dependent on suitable water quality (Pollet and Bendell-Young 2000; Calderon et al. 2019).

Wetlands with poor water quality may not sustain future generations of such sensitive amphibians.

Water is essential to the earth's uniqueness and provides all forms of life (Gorde and Jadhav 2013). Amphibians that inhabit the water have been affected by increasing salinization, nitrification, hydrocarbons, and pesticide contamination (Salman 2019). There has been evidence that amphibian development, growth, reproduction, and survivability are hampered by high levels of electrical conductance, nitrates, nitrites, total phosphates, chloride, and unionized ammonium, and low concentrations of dissolved oxygen (Serrano et al. 2016; Babini et al. 2018). In addition to these effects, pesticides are also destroying amphibians' fitness and survival as a result of their massive use (Mann et al. 2009). Aquatic communities in wetlands are potentially exposed to chemical stressors as well as road salt (Trombulak and Frissell 2000). There is a possibility that road salt can negatively impact ecosystem health, biological diversity, and the functioning of lake ecosystems due to its deposition (Lewis et al. 2021; Szklarek et al. 2022).

Wetlands of the Himalayan region are threatened due to anthropogenic pressure and climate change (Paudel et al. 2023). Lakes in the mid-hill region of the Nepal Himalayas are important ecosystems providing multifaceted services. For example, Kupinde Lake, a sub-tropical lake in western Nepal, is an important tourist destination of religious importance. Currently, the lake area is being developed with the construction of roads, making it easier accessibility to visitors. Consequently, road salt contamination and the spread of invasive species in lake ecosystems are possible results of this anthropogenic activity (Lewis et al. 2021; Ren et al. 2021; Szklarek et al. 2022). In order to understand the ecosystem health and provide baseline information for future management of the lake, it is crucial to characterize the physicochemical parameters and establish their association with the lifeform being supported by the lake. Therefore, this study aimed to i) characterize the physicochemical parameters of the Kupinde Lake water, ii) explore the amphibian community inhabiting the lake and, iii) establish an association between amphibian abundance and water quality parameters.

MATERIALS AND METHODS

Study area

The Kupinde Lake is in the Salyan District of Karnali Province, Nepal (Figure 1). It lies 28°24.701' N and 82°03.608' E, at an 1137 m asl and 15 km from the district headquarters, Khalanga. Salyan District is a hilly area covering an area of 1462 km^2 with a population of 241,716 (CBS 2011). The district is bounded by Rolpa to the east, Surkhet and Bardiya to the west, Rukum and Jajarkot to the north, and Dang and Banke districts to the south. The district has a sub-tropical to temperate climate with maximum temperatures of 31°C, minimums of 3°C and annual rainfall of 1100 mm. Kupinde Lake is one of the most popular tourist destinations in Bangad Kupinde Municipality of Salyan District. It occupies an area of 0.24 km^2 which is surrounded by sparse forest.

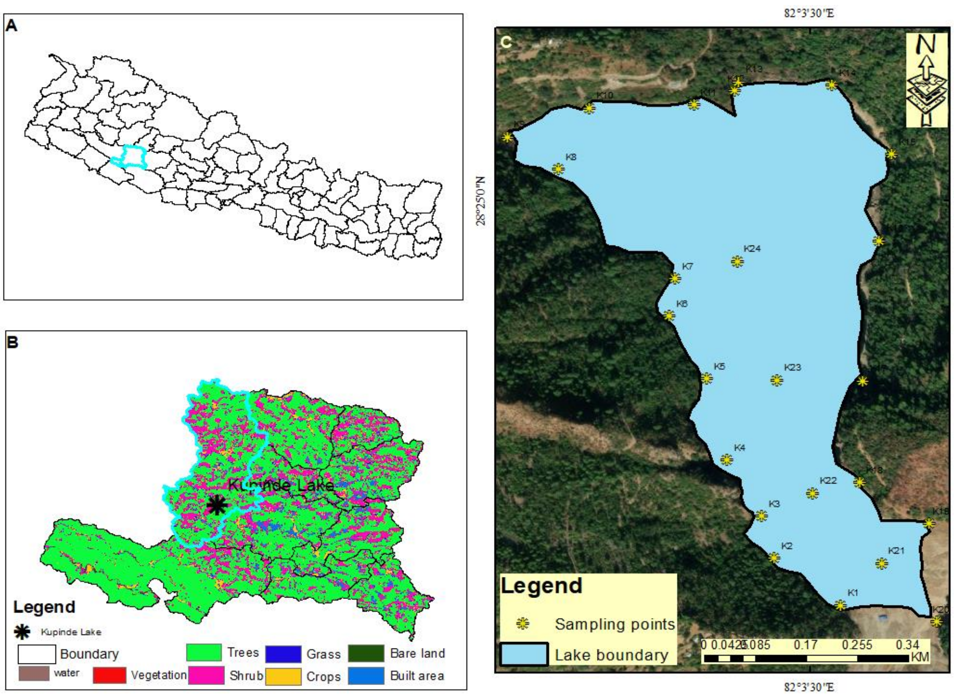

Figure 1. Map of the study area: A. Map of Nepal showing Salyan District, B. Map of Salyan District showing the Kupinde Lake, C. Map of Kupinde Lake showing sampling points

Water sampling and physicochemical parameters measurement

A total of 24 water samples were collected in October 2021 from the periphery and middle of the lake using a boat. The location of the water sample was recorded with Garmin e-trex10 GPS unit (Garmin, Chicago, IL). From each sampling site one liter water sample was collected in High Density Polyethylene (HDPE) bottle. Out of 24 samples, 20 were taken from the periphery and four were from the inner area of the lake such that it could represent the entire lake area (Figure 1C). Water samples were analyzed for eighteen important physicochemical parameters. The physical parameters like pH, temperature, (pH meter (Model No. pH55) Total Dissolved Solids (TDS) and Electric Conductivity (EC) (Multipurpose meter (SN: 11003290111) were measured on-site. On-site measurements were repeated until a consistent reading was obtained. Chemical parameters like Total Hardness (TH), Calcium Hardness (CaH), Magnesium Hardness (MgH), alkalinity (HCO_3^-), and chloride (Cl^-) were measured by titrimetric method. In addition, chemical parameters like ammonium (NH_4^+), nitrate (NO_3^-), and phosphate (PO_4^{3-}) were analyzed using an UV-visible spectrophotometer. Potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), and sodium (Na^+) were analyzed using a Flame Photometer in the laboratory of the Central Department of Environmental Science, under the Institute of Science and Technology, Tribhuvan University (CDES-TU), Kathmandu, Nepal.

Amphibian survey

Species richness of amphibian fauna was observed by visual encounter survey during October 2021. The survey was conducted from 5:00 to 10:00 hours in the morning, 2:00 to 5:00 afternoon and 7:00 to 9:00 evening. At each sampling point, 2-3 people visited twice each day for a total of 10 minutes each. A survey was conducted along the lake's perimeter, looking for individuals at its edge. The sites (1, 2, 9, 10, 11, 12, 13, 14, 15, 18, 19, 20 and 21) were visited 10 times during the study. The sampling sites (3, 4, 5, 6, 7, 8, 16, 17, 22 23 and 24) were visited four times. Because some sites were less accessible, survey efforts were not the same for all sites. Amphibian counts were performed within a circle of 2-meter diameter of each water sampling site prior to sampling and average value from repeated counts was used as amphibian abundance in downstream analysis. Photographs and samples of specimens were taken for both identification and evidence. The collected specimens were preserved in a plastic bottle with 70% ethanol for further identification. The collected specimens have been stored at the Central Department of Zoology, Tribhuvan University, Kathmandu.

Data analysis

Spearman's rank correlation analysis was performed to establish the relations between the physicochemical parameters of the lake water and amphibian abundance. The Shapiro-Wilk test was run to determine the collinearity of all the variables. Only one variable was selected for the Generalized Linear Modeling between the variables having pairwise correlation coefficient $r>|0.7|$. The GLM analysis (function 'glm' in R) with Poisson distribution and log-link function employed in the R software (R-Studio 2022) was used to analyze the relations between the selected physicochemical parameters of water and amphibian abundance. Polynomial regression (third order) was performed between the abundance of amphibians and major physicochemical parameters (pH and temperature). Third-order polynomial regression was selected based on the least AIC value.

RESULTS AND DISCUSSION

Physicochemical parameters of water in Kupinde Lake

The mean temperature of Kupinde Lake water was 22.58°C±0.69, and the pH was 8.16±0.29 (Figure 2). Temperature is one of the important regulators of the physicochemical and biological activities of the aquatic ecosystem. The average lake water temperature varies with seasons and geographical position of the lake. Several factors affect lake surface temperature, including the location of the lakes, morphometric parameters, wind speed, transparency, and human activities (Ptak et al. 2018; Yang et al. 2019). The Kupinde Lake water was slightly alkaline. A similar pH range was also reported from Lake Rara (Gurung et al. 2018), one of the high-altitude lakes in the Nepal Himalayas. Alkalinity is a common phenomenon in most freshwater ecosystems and that might be attributed to the presence of limestone rocks in the lake catchments (Ormerod et al. 1990).

The mean EC was 136.54 ±3.04 µS/cm, and TDS was 68.25 ±1.67 mg/L. Electrical conductivity effectively indicates human-induced changes in ion concentration in aquatic ecosystems (Wu et al. 2020). TDS measures the concentration of ionic constituents in water. It is believed that TDS and EC are related to the composition of water ions and the concentration of dissolved solids (Taylor et al. 2018). The lesser Himalayan lake in Pokhara valley had a conductivity of 120.48±2.52 µS/cm and TDS of 97.73±2.97 (Khadka and Ramanathan 2021), and the high-altitude lake in Rara had EC 193.85 µS/cm and TDS 96.85 mg/L (Kaphle et al. 2021) and the previous study by Pant et al. (2020) reported the EC of Ghodaghodi lake was 142 µS/cm and TDS 77 mg/L. Kupinde Lake had lower electrical conductivity and TDS levels than other lakes, except Phewa Lake, which indicates that it is less polluted. The most dominant cation and anion in Kupinde Lake were Ca^{2+} (27.97 ±4.33 mg/L) and HCO_3^- (157.71 ±69.09 mg/L), respectively. This finding is consistent with previous study Phewa lake of lesser Himalayan (Khadka and Ramanathan 2021), Rara Lake (Kaphle et al. 2021), and Ghodaghodi lake (Pant et al. 2020). The major ion chemistry of lake water provides valuable insight into the sources of dissolved ions, weathering, and hydrogeochemical processes (Singh et al. 2016). Mostly, major cations and anion levels are affected by carbonate weathering (Pant et al. 2020; Kaphle et al. 2021; Khadka and Ramanathan 2021), and anthropogenic activities (Zhao et al. 2021).

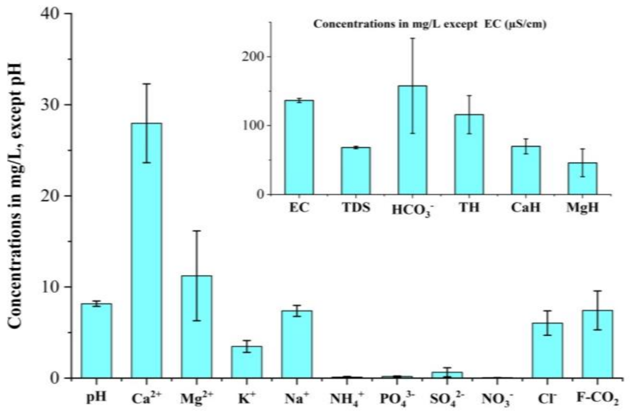

Figure 2. Concentration (mean values with standard deviation) of physicochemical parameters of water in Kupinde Lake, Nepal

Amphibian community structure in Kupinde Lake

Two species of Anuran amphibians- Indian skipper frog (*Euphlyctis cyanophlyctis* Schneider, 1799) and Syhadra frog (*Fejervarya syhadrensis* Annandale, 1919) were recorded from the Kupinde Lake during the study period. The *E. cyanophlyctis* was the most abundant amphibian species. Among the 24 study sites, *E. cyanophlyctis* was detected from the 20 sampling points. The highest average abundance of species was found at site 21 (n = 120), followed by site 20 (n = 115). Amphibians were not observed in the site six and center of the lake (i.e., Site 22, 23, and 24). A permanent water body would provide a suitable habitat for *E. cyanophlyctis*, since it prefers the littoral zone over deep water (Chowdhury et al. 2021). In this study, *E. cyanophlyctis* was the most abundant near the lake's periphery. There may be a reason for this, since plant leaves provide them with a shelter where they can lay eggs and food resources are nearby. In the study area, ecological conditions were problematic for amphibians because of the geographical position and the presence of rocky cliffs on the lake's periphery. This may contribute to the low amphibian diversity in the area.

Relationship between physicochemical parameters and amphibian abundance

A positive correlation was observed between amphibian abundance and pH, HCO_3^-, and NH_4^+ (Figure 3). In Kupinde Lake water, the chemical parameter HCO_3^-, and NH_4^+ have a strong positive correlation (Figure 3), which influences the pH. This positive association may be explained by the physicochemical parameter in the lake catchment interacting with limiting conditions. Alternatively, the amphibian abundance correlated negatively with EC, TDS, CO_2, Cl^-, and Na^{2+}. In these parameters, there was a positive correlation with (EC-TDS) and (CO_2 - Cl^-) (Figure 3). However, these parameters showed a negative correlation with amphibian abundance, possibly due to the relatively low concentration in lake water.

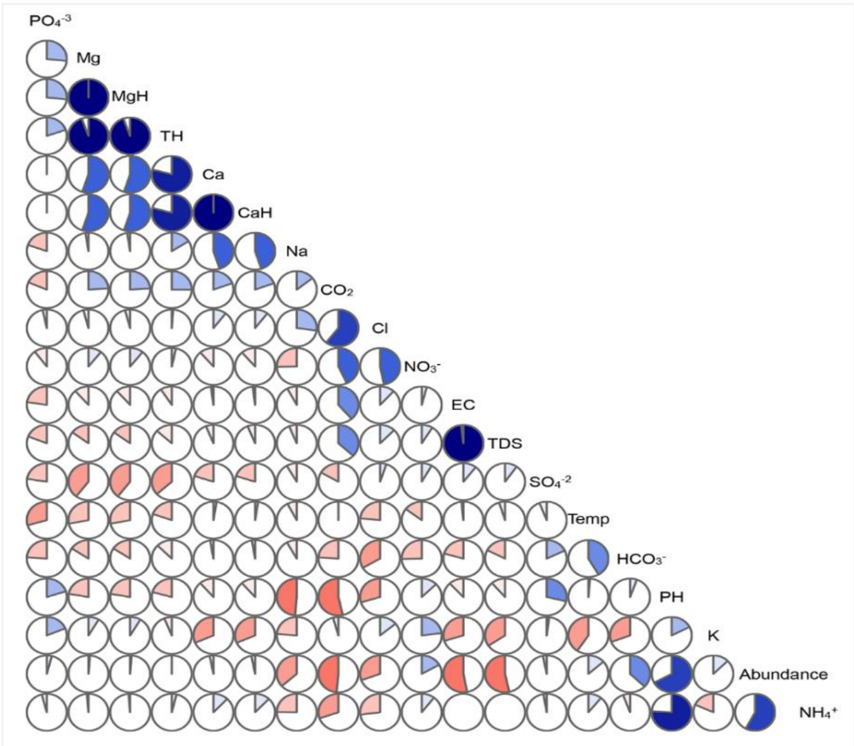

Figure 3. Correlogram of the pairwise correlation (r) between physicochemical parameters and amphibian abundance. Shaded blue and red colors within circles depict positive and negative correlations, respectively. Empty circle represents r = 0, full blue circle represents r = 1 and full red circle represents r = -1

Based on the physicochemical parameter testing, the Generalized Linear Model (GLM) (Table 1) indicated amphibian abundance was significantly affected by temperature, pH, TDS alkalinity, calcium, sulfate, and sodium. According to the test parameters, amphibian species abundance decreased with increasing temperature, TDS, sulfate, and sodium. Similarly, species abundance is positively related to pH, alkalinity, and calcium concentration.

The regression analysis between the abundance of amphibians and major physicochemical parameters i.e., temperature and pH based on the AIC values were better suited to the third order ($p < 0.05$) (Table 2). The polynomial regression analysis between several individuals and pH revealed a consistent positive association ($r^2 = 0.497$, $p < 0.05$). On the other hand, the association between amphibian abundance and temperature was unimodal ($r^2 = 0.188$, $p < 0.05$).

The results indicate that temperature, pH, TDS, alkalinity, calcium, sulfate, and sodium significantly associate with anuran abundance in Kupinde Lake. Amphibian abundance had a negative association with temperature, TDS, sulfate, and sodium; whereas a positive association existed with calcium, alkalinity, and pH (Figure 4). The lake micro-environment is influenced by these physicochemical factors that, in-turn, affect the species inhabiting the lake. A negative relationship was observed between amphibian abundance and temperatures. It might be due to the lake's water's relatively low temperature. Amphibian gonadal structures and sex ratio are generally affected by extremely low and high temperatures (Phuge 2017). The temperature of the water is influenced by the temperature of the air and the intensity of solar radiation (Oli et al. 2013). Temperature changes can affect breeding activities and early growth (Wheeler et al. 2015; Catenazzi and Kupferberg 2017). A rise in water temperature influences larval development and survival until metamorphosis (Skelly et al. 2002).

The hydrogen ion concentration (pH) levels were consistently normal at all sampling points (mean pH 8.16). The pH and calcium have a weak negative correlation, indicating that pH has consistently been within the normal range, while relatively high calcium concentrations enhance the pH values (Brown 1983). This is consistent with scientific studies on maintaining a balance of hydrogen ions in amphibian habitats within a pH range of 6.5 to 8.5. Water with low pH can affect amphibians' reproductive directly by killing embryos and larvae and disrupting trophic relationships between them and other aquatic animals (Serrano et al. 2016). Hence, the abundance of amphibians is negatively correlated with low and high pH values, which may affect the size of populations (Skei and Dolmen 2006). The species abundance was high, with a minimum TDS of 64 ppm. Amphibians need a TDS value between 50 and 250 ppm to survive and anything below this range will be detrimental (Shaikh et al. 2014). A negative association was found between TDS and amphibians. TDS solution contains several ions, including sodium, chloride, potassium, magnesium, sulfate, chloride, and bicarbonate (Chapman et al. 2000). different ions might achieve a similar effect at a similar concentration, however, it depends on their identity and concentration. The volume of TDS was extremely high compared to normal, which could lead to amphibian mortality due to excessive organic and inorganic components (Shaikh et al. 2014).

Table 1. Generalized Linear Model (GLM) with Poisson distribution and log link function test showing the relations between amphibian abundance and physical-chemical parameters of water quality in Kupinde Lake, Nepal

Parameter	Estimate	SE	z value	Pr (>|z|
Intercept	28.82	8.83	3.26	0.001*
Temp	-0.68	0.21	-3.23	0.001**
pH	1.80	0.91	1.97	0.047*
TDS	-0.35	0.07	-5.03	0.000***
Alkalinity	0.00	0.00	3.05	0.002**
Ca	0.06	0.01	3.54	0.000***
Sulphate	-0.62	0.21	-2.90	0.003**
Na	-0.60	0.17	-3.40	0.000***

Table 2. Polynomial regression of abundance of amphibians with temperature and pH

Regression	Temperature	pH
First order r^2	0.018	0.449
AIC	26440	14829
Second order r^2	0.116	0.452
AIC	23789	14743
Third order r^2	0.188	0.497
AIC	21867	13541

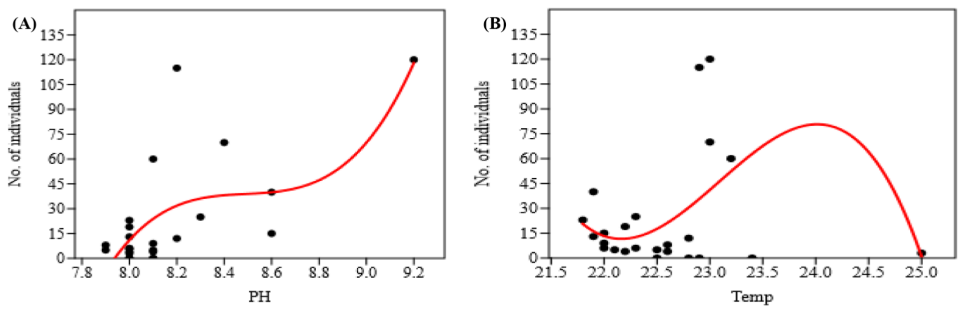

Figure 4. Relationship between amphibian abundance and major physicochemical parameters; A. Number of amphibian individuals and pH, and B. Number of amphibian individuals and temperature

An intriguing finding of this study was that Electrical Conductivity (EC) was negatively correlated with species abundance. The reason could be that the lake has low EC (mean 136) while aquatic organisms require ECs between 150 and 500 (Sparling 2010). In contrast, a positive association has been shown between the EC and amphibian abundance (Calderon et al. 2019). Evidence shows that a significant increase in conductivity negatively impacts amphibian behavior, growth, and development (Chambers 2011; Klaver et al. 2013). It appears that bicarbonate is the most dominant anion among the major anions. The results indicate that it has a significant effect on amphibian abundance. Generally, bicarbonate is derived from calcium carbonate rocks (CaCO3) and calcium magnesium carbonate rocks (dolomite) (Mallick 2017). A significant impact of amphibian abundance was found in the current study. In frog blood, the calcium concentration is similar to that of vertebrates. Calcium metabolism in amphibians seems to involve endocrine and humoral factors such as parathyroid hormone, calcitonin, vitamin D, and prolactin. Calcium amounts vary with the season, increasing in spring and summer and decreasing in winter (Stiffler 1993).

Amphibian abundance is negatively correlated with sulfate and sodium levels in the lake due to its extremely low levels. Water quality is considered the main factor influencing health and disease in all aspects of a biotic system. However, hydrochemistry is little studied concerning *E. cyanophlyctis*. In Kupinde Lake, the physio-chemical characteristics of water, such as temperature, pH, alkalinity, etc., showed a strong influence over the anuran survival, growth and reproduction, which influences how appropriate the lake environment is for the amphibians. For example, temperature or pH ranges are more conducive to anuran growth and reproduction whereas some levels of TDS, alkalinity, calcium, sulfate, and sodium may be necessary for proper physiological functioning. Therefore, a further detailed study is required to determine how these factors interact with each other. This should be acknowledged that we explored amphibian abundance by visual encounter method during the post-monsoon season. Although we tried to observe the amphibians carefully, we could have missed some species or individuals from the survey. Therefore, future studies during the monsoon season with a combination of different sampling methods could yield better results.

In conclusion, the Kupinde Lake in the sub-tropical climate of the Nepal Himalaya has slightly alkaline water dominated by Ca^{2+} and HCO_3^-. Amphibian abundance was negatively affected by the temperature, TDS, sulfate, and sodium, while significant changes in pH, alkalinity, and calcium. Low amphibian diversity and dominance of a single species (*E. cyanophlyctis*) indicate poor ecosystem health of the lake. The findings of this study could be important for monitoring and managing the Kupinde Lake.

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REFERENCES

Ali W, Javid A, Hussain A, Bukhari SM. 2018. Diversity and habitat preferences of amphibians and reptiles in Pakistan: A review. J Asia-Pac Biodivers 11: 173-187. DOI: 10.1016/j.japb.2018.01.009.

Amankwaa G, Yin X, Zhang L, Huang W, Cao Y, Ni X. 2020. Hydrochemistry and multivariate statistical analysis of the quality of water from Lake Bosomtwe for agricultural and human consumption. J Water Supply: Res Technol-Aqua 69: 704-719. DOI: 10.2166/aqua.2020.061.

Amarasinghe AAT, Putra CA, Henkanaththegedara SM, Dwiyahreni AA, Winarni NL, Sunaryo, Margules C, Supriatna J. 2021. Herpetofaunal diversity of West Bali National Park, Indonesia with identification of indicator species for long-term monitoring. Glob Ecol Conserv 28: e01638. DOI: 10.1016/j.gecco.2021.e01638.

Babini MS, Bionda CdL, Salinas ZA, Salas NE, Martino AL. 2018. Reproductive endpoints of *Rhinella arenarum* (Anura, Bufonidae): Populations that persist in agroecosystems and their use for the environmental health assessment. Ecotoxicol Environ Safety 154: 294-301. DOI: 10.1016/j.ecoenv.2018.02.050.

Blaustein A, Urbina J, Snyder P, Reynolds E, Dang T, Hoverman J, Han B, Olson D, Searle C, Hambalek N. 2018. Effects of emerging infectious diseases on Amphibians: A review of experimental studies. Diversity 10 (3): 81. DOI: 10.3390/d10030081.

Boelter T, dos Santos FM, Moreira LFB, Stenert C, Reichard M, Maltchik L. 2020. Effects of hydroperiod on morphology of tadpoles from highland ponds. Aquat Ecol 54: 1145-1153. DOI: 10.1007/s10452-020-09799-1.

Boyer R, Grue CE. 1995. The need for water quality criteria for frogs. Environ Health Perspect 103: 352-357. DOI: 10.1289/ehp.95103352.

Brannelly LA, Ohmer MEB, Saenz V, Richards-Zawacki CL, Vindenes Y. 2019. Effects of hydroperiod on growth, development, survival and immune defences in a temperate amphibian. Funct Ecol 33: 1952-1961. DOI: 10.1111/1365-2435.13419.

Brown DJA. 1983. Effect of calcium and aluminum concentrations on the survival of brown trout (*Salmo trutta*) at low pH. Bull Environ Contam Toxicol 30: 582-587. DOI: 10.1007/BF01610178.

Calderon MR, Almeida CA, González P, Jofré MB. 2019. Influence of water quality and habitat conditions on amphibian community metrics in rivers affected by urban activity. Urban Ecosyst 22: 743-755. DOI: 10.1007/s11252-019-00862-w.

Catenazzi A, Kupferberg SJ. 2017. Variation in thermal niche of a declining river-breeding frog: From counter-gradient responses to population distribution patterns. Freshwater Biol 62: 1255-1265. DOI: 10.1111/fwb.12942.

CBS. 2011. Government of Nepal Central Bureau of Statistics. Nepal.

Chambers DL. 2011. Increased conductivity affects corticosterone levels and prey consumption in larval amphibians. J Herpetol 45: 219-223. DOI: 10.1670/09-211.1.

Chapman PM, Bailey H, Canaria E. 2000. Toxicity of total dissolved solids associated with two mine effluents to chironomid larvae and early life stages of rainbow trout. Environ Toxicol Chem 19: 210-214. DOI: 10.1002/etc.5620190125.

Chowdhury MAW, Shil SR, Rahman MM. 2021. Temporary water holes may benefit the breeding of the common skipper frog *Euphlyctis cyanophlyctis* (Anura: Dicroglossidae). Ecologies 2: 138-149. DOI: 10.3390/ecologies2010007.

Compton BW, McGarigal K, Cushman SA, Gamble LR. 2007. A resistant-kernel model of connectivity for amphibians that breed in vernal pools. Conserv Biol 21: 788-799. DOI: 10.1111/j.1523-1739.2007.00674.x.

Ghosh D, Basu P. 2020. Factors influencing herpetofauna abundance and diversity in a tropical agricultural landscape mosaic. Biotropica 52: 927-937. DOI: 10.1111/btp.12799.

Gorde SP, Jadhav MV. 2013. Assessment of water quality parameters: A review. Intl J Eng Res Appl 3 (6): 2029-2035.

Gurung S, Gurung A, Sharma CM, Jüttner I, Tripathee L, Bajracharya RM, Raut N, Pradhananga P, Sitaula BK, Zhang Y, Kang S, Guo J. 2018. Hydrochemistry of Lake Rara: A high mountain lake in western

Nepal. Lakes Reserv: Sci Policy Manag Sustain Use 23: 87-97. DOI: 10.1111/lre.12218.

Hecnar SJ. 2004. Great Lakes wetlands as amphibian habitats: A review. Aquat Ecosyst Health Manag 7: 289-303. DOI: 10.1080/14634980490461542.

Kaphle B, Wang J-b, Kai J-l, Lyu X-m, Paudayal KN, Adhikari S. 2021. Hydrochemistry of Rara Lake: A Ramsar Lake from the southern slope of the central Himalayas, Nepal. J Mount Sci 18: 141-158. DOI: 10.1007/s11629-019-5910-0.

Khadka UR, Ramanathan AL. 2021. Hydrogeochemical analysis of Phewa Lake: A lesser Himalayan Lake in the Pokhara Valley, Nepal. Environ Nat Resour J 19: 68-83. DOI: 10.32526/ennrj/19/2020083.

Klaver RW, Peterson CR, Patla DA. 2013. Influence of water conductivity on amphibian occupancy in the Greater Yellowstone Ecosystem. West N Am Nat 73: 184-197. DOI: 10.3398/064.073.0208.

Lewis JL, Agostini G, Jones DK, Relyea RA. 2021. Cascading effects of insecticides and road salt on wetland communities. Environ Pollut 272: 116006. DOI: 10.1016/j.envpol.2020.116006.

Linzey D, Burroughs J, Hudson L, Marini M, Robertson J, Bacon J, Nagarkatti M, Nagarkatti P. 2003. Role of environmental pollutants on immune functions, parasitic infections and limb malformations in marine toads and whistling frogs from Bermuda. Intl J Environ Health Res 13: 125-148. DOI: 10.1080/0960312031000098053.

Mallick J. 2017. Hydrogeochemical characteristics and assessment of water quality in the Al-Saad Lake, Abha Saudi Arabia. Appl Water Sci 7: 2869-2882. DOI: 10.1007/s13201-017-0553-1.

Mann RM, Hyne RV, Choung CB, Wilson SP. 2009. Amphibians and agricultural chemicals: Review of the risks in a complex environment. Environ Pollut 157: 2903-2927. DOI: 10.1016/j.envpol.2009.05.015.

Mifsud DA. 2014. A status assessment and review of the herpetofauna within the Saginaw Bay of Lake Huron. J Great Lakes Res 40: 183-191. DOI: 10.1016/j.jglr.2013.09.017.

Muths E, Hossack BR, Campbell Grant EH, Pilliod DS, Mosher BA. 2020. Effects of snowpack, temperature, and disease on demography in a wild population of amphibians. Herpetologica 76: 132-143. DOI: 10.1655/0018-0831-76.2.132.

Oli BB, Jha DK, Aryal PC, Shrestha MK, Dangol DR, Gautam B. 2013. Seasonal variation in water quality and fish diversity of Rampur Ghol, a wetland in Chitwan, Central Nepal. Nepalese J Biosci 3: 9-17. DOI: 10.3126/njbs.v3i1.41420.

Ormerod SJ, Weatherley NS, Merrett WJ, Gee AS, Whitehead PG. 1990. Restoring acidified streams in upland Wales: a modelling comparison of the chemical and biological effects of liming and reduced sulphate deposition. Environ Pollut 64: 67-85. DOI: 10.1016/0269-7491(90)90096-U.

Pant RR, Pal KB, Bishwakarma K, Thapa LB, Dangol A, Dawadi B, Poudel P, Bhattarai B, Joshi TR, Bhatt YR. 2020. Application of multivariate approaches to the hydro-chemical assessment of the Ghodaghodi Lake, Sudurpaschim Province, Nepal. Nepal J Sci Technol 19: 46-54. DOI: 10.3126/njst.v20i1.39390.

Paudel J, Khanal L, Pandey N, Upadhyaya LP, Sunar CB, Thapa B, Bhatta CR, Pant RR, Kyes RC. 2023. Determinants of herpetofaunal diversity in a threatened wetland ecosystem: A case study of the Ramaroshan Wetland Complex, Western Nepal. Animals 13 (1): 135. DOI: 10.3390/ani13010135.

Paulding C, Randhir TO. 2021. An ecohydrological assessment of potential impacts of climate change on the herpetofauna. Sustain Clim Change 14: 22-34. DOI: 10.1089/scc.2020.0027.

Phuge SK. 2017. High temperatures influence sexual development differentially in male and female tadpoles of the Indian skipper frog, *Euphlyctis cyanophlyctis*. J Biosci 42: 449-457. DOI: 10.1007/s12038-017-9689-2.

Pollet I, Bendell-Young LI. 2000. Amphibians as indicators of wetland quality in wetlands formed from oil sands effluent. Environ Toxicol Chem 19: 2589-2597. DOI: 10.1002/etc.5620191027.

Ptak M, Sojka M, Choiński A, Nowak B. 2018. Effect of environmental conditions and morphometric parameters on surface water temperature in Polish Lakes. Water 10 (5): 580. DOI: 10.3390/w10050580.

Rastegar-Pouyani N, Gholamifard A, Karamiani R, Bahmani Z, Mobaraki A, Abtin E, Faizi H, Heidari N, Takesh M, Sayyadi F. 2015. Sustainable management of the Herpetofauna of the Iranian plateau and coastal Iran. Amphib Reptile Conserv 9: 1-15.

Ren J, Chen J, Xu C, van de Koppel J, Thomsen MS, Qiu S, He Q. 2021. An invasive species erodes the performance of coastal wetland protected areas. Sci Adv 7: eabi8943. DOI: 10.1126/sciadv.abi8943.

Rittenhouse TAG, Semlitsch RD. 2007. Distribution of amphibians in terrestrial habitat surrounding wetlands. Wetlands 27: 153-161. DOI: 10.1672/0277-5212(2007)27[153:Doaith]2.0.Co;2.

Riyanto A, Rahmadi C. 2021. Amphibian and reptile diversity of Peleng Island, Banggai Kepulauan, Central Sulawesi, Indonesia. Biodiversitas 22: 2930-2939. DOI: 10.13057/biodiv/d220558.

R-Studio Team. 2022. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.

Salman NA. 2019. A Review of Southern Iraq Herpetofauna. Biol Appl Environ Res 3: 61-71.

Serrano L, Diaz-Paniagua C, Gomez-Rodriguez C, Florencio M, Marchand MA, Roelofs JG, Lucassen EC. 2016. Susceptibility to acidification of groundwater-dependent wetlands affected by water level declines, and potential risk to an early-breeding amphibian species. Sci Total Environ 571: 1253-1261. DOI: 10.1016/j.scitotenv.2016.07.156.

Shah KB, Tiwari S 2004. Herpetofauna of Nepal: A Conservation Companion. IUCN Nepal.

Shaikh K, Gachal GS, Memon SQ, Ahmed N, Sodho M, Yusuf S. 2014. Assessment of amphibian environment through physicochemical analysis in Pakistan. J Biodivers Environ Sci 5: 255-261. DOI: 10.1.1.657.1086.

Singh VB, Ramanathan AL, Mandal A. 2016. Hydrogeochemistry of high-altitude lake: A case study of the Chandra Tal, Western Himalaya, India. Arabian J Geosci 9: 308. DOI: 10.1007/s12517-016-2358-1.

Skei JK, Dolmen D. 2006. Effects of pH, aluminium, and soft water on larvae of the amphibians *Bufo bufo* and *Triturus vulgaris*. Canad J Zool 84: 1668-1677. DOI: 10.1139/z06-166.

Skelly DK, Freidenburg LK, Kiesecker JM. 2002. Forest canopy and the performance of larval amphibians. Ecology 83: 983-992. DOI: 10.1890/0012-9658(2002)083[0983:Fcatpo]2.0.Co;2.

Sparling DW 2010. Water-quality criteria for amphibians. In: Dodd Jr. (eds.) Amphibian Ecology and Conservation: A Handbook of Techniques. Oxford University Press, New York.

Stiffler DF. 1993. Amphibian calcium metabolism. J Exp Biol 184: 47-61. DOI: 10.1242/jeb.184.1.47.

Szklarek S, Gorecka A, Wojtal-Frankiewicz A. 2022. The effects of road salt on freshwater ecosystems and solutions for mitigating chloride pollution - A review. Sci Total Environ 805: 150289. DOI: 10.1016/j.scitotenv.2021.150289.

Taylor M, Elliott HA, Navitsky LO. 2018. Relationship between total dissolved solids and electrical conductivity in Marcellus hydraulic fracturing fluids. Water Sci Technol 77: 1998-2004. DOI: 10.2166/wst.2018.092.

Thakuri S, Pokhrel GK. 2017. Herpetofaunal diversity in Manaslu Conservation Area, Nepal. Our Nat 14: 99-106. DOI: 10.3126/on.v14i1.16448.

Trombulak SC, Frissell CA. 2000. Review of ecological effects of roads on terrestrial and aquatic communities. Conserv Biol 14: 18-30. DOI: 10.1046/j.1523-1739.2000.99084.x.

Wheeler CA, Bettaso JB, Ashton DT, Welsh HH. 2015. Effects of water temperature on breeding phenology, growth, and metamorphosis of foothill Yellow-Legged Frogs (*Rana boylii*): A case study of the regulated mainstem and unregulated tributaries of California's trinity River. River Res Appl 31: 1276-1286. DOI: 10.1002/rra.2820.

Wu T, Zhu G, Zhu M, Xu H, Zhang Y, Qin B. 2020. Use of conductivity to indicate long-term changes in pollution processes in Lake Taihu, a large shallow lake. Environ Sci Pollut Res Intl 27: 21376-21385. DOI: 10.1007/s11356-020-08590-x.

Yang K, Yu Z, Luo Y, Zhou X, Shang C. 2019. Spatial-temporal variation of lake surface water temperature and its driving factors in Yunnan-Guizhou Plateau. Water Resour Res 55: 4688-4703. DOI: 10.1029/2019wr025316.

Zhao Q, Zhang Y, Guo F, Leigh C, Jia X. 2021. Increasing anthropogenic salinisation leads to declines in community diversity, functional diversity and trophic links in mountain streams. Chemosphere 263: 127994. DOI: 10.1016/j.chemosphere.2020.127994.