



# Seasonal Variation of Zooplankton Assemblage in the Lakes of Pokhara Valley, Nepal

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## Abstract

Zooplankton studies and observation are useful for managing the physio-chemical and biological environment of the aquatic ecosystems, thus playing a vital role in fisheries. Seasonal variations affect the physio-chemical variables thus causing variation in abundance and diversity of plankton. Zooplankton samples were collected from Phewa, Begnas, Rupa, Khaste, Dipang, Neureni, Maldi and Gunde lakes in the month of November 2023 (Post- monsoon) and April 2024 (Pre-monsoon). A total of 33 species of zooplankton with 5 groups, comprising Rotifer (23), Copepod (4), Cladocera (3), Protozoa (2), and Cnidarians (1) were recorded from the lakes of Pokhara Valley. Comparatively, the mean zooplankton abundance was significantly higher ( $p < 0.05$ ) in Rupa Lake and was with the highest species richness in post-monsoon. The value of the Shannon-Wiener Index ( $H'$ ) ranged from 1.26 - 2.75 indicated moderate pollution in lakes, whereas Pielou's Species Evenness ( $J$ ) less than 0.5 indicated the presence of ecological stress in all lakes except in Begnas Lake. Rotifers were abundant in all lakes over both seasons, indicating changing lake's condition. It is important to prevent nutritional load on water quality parameters during the urbanization process and agrochemicals to increase zooplankton stability and diversity in these lakes.

**Keywords:** Abundance, diversity, richness, Rotifer

## Introduction

Zooplankton is a secondary producer which serves as the intermediate link between phytoplankton and fish in aquatic environment. Zooplankton is critical indicators of pressures impacting freshwater ecosystems (Goździejewska et al., 2024). Zooplankton studies and observations are crucial for managing aquatic ecosystems and fisheries (Mahaseth, 2013), as they facilitate energy fluxes from primary producers like phytoplankton and microorganisms to consumer levels of the food chain (Al et al., 2018). In addition to supporting the ecosystem's relative stability, zooplankton diversity serves as a water quality indicator (Tang et al., 2019). Factors like habitat types, physico-chemical characteristics, climate change, and biotic factors influence zooplankton's occurrence and dispersion (Cottenie et al., 2001; Ahmad et al., 2011; Khanna et al., 2019). Zooplanktons significantly impact ecosystem dynamics (Bruce et al., 2006) by feeding on algae and bacteria, providing nitrogen and phosphorus, and completing a perfect cycle of nutrient recycling (Hudson et al., 1999). Certain zooplankton species have been recognized as bio-indicators that are responsive to changes in their native environments, due to this, they are referred to as "sentinels of environmental changes and pressures" (Richardson, 2008; Beaugrand et al., 2010).

Numerous research on the dynamics of zooplanktons in the lakes of the Pokhara valley have been conducted (Swar & Fernando, 1980; Swar, 1981; Mulmi & Rai,

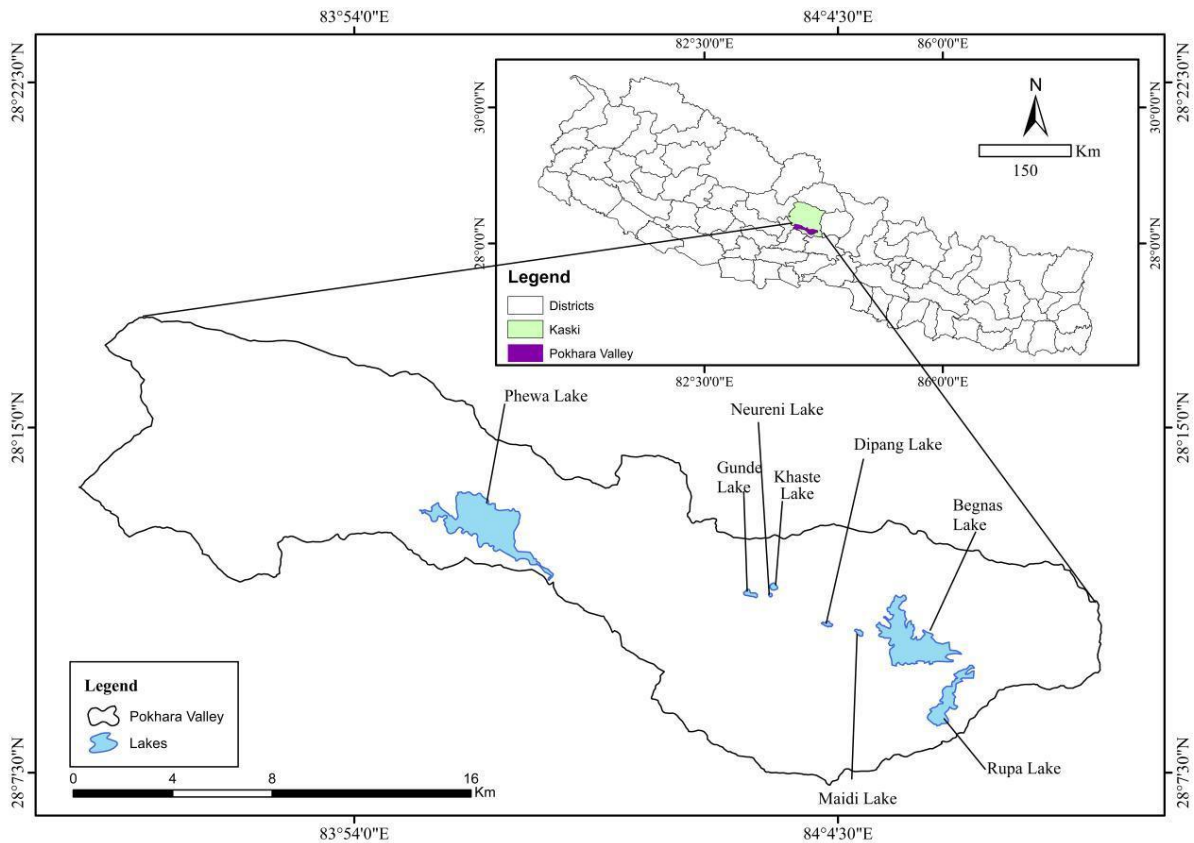
1988; Rai, 2000; Husen & Dhakal, 2009; Gautam et al., 2016). The zooplankton of Dipang, Neureni, Maldi, and Gunde Lakes has not yet been the subject of any research. Thus, the current study aimed to investigate the species' diversity, richness, and abundance of zooplankton in Dipang, Neureni, Maldi, and Gunde Lakes, as well as of major Lakes (Phewa, Begnas, Rupa), and Khaste before and during the monsoon.

## Materials and Methods

### Study Area

The Pokhara Valley Lakes Cluster, Nepal's largest Ramsar site, is located in Kaski District, Gandaki Province (MoFE, 2018a). The catchment area is 261.1 square kilometers, which includes water bodies, agricultural land, woods, populated areas, and other built-up areas. Agricultural land predominates the catchment area, followed by the woods and water bodies (Gauli, 2016). These lakes and their surroundings provide vital habitats for a diverse range of plant and animal species, contributing to both aquatic and terrestrial biodiversity (Bhujju et al., 2007). However, human activities like urban growth, agricultural runoff, pollution, land encroachment, and the introduction of invasive species are putting a strain on the area's ecological balance (Husen et al., 2024). The water bodies of Phewa, Begnas, Rupa, Khaste, Neureni, Dipang, Maldi, and Gunde are 4.33 km<sup>2</sup>, 3.13 km<sup>2</sup>, 1.11 km<sup>2</sup>, 0.13 km<sup>2</sup>, 0.027 km<sup>2</sup>, 0.14 km<sup>2</sup>, 0.007 km<sup>2</sup> and 0.08 km<sup>2</sup> respectively (MoFE, 2018b).

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**Figure 1** Map of Nepal showing study area (Kaski district). The lower map shows the locations of studied lakes in the southern part of the Kaski district.

### Sample Collection

Water samples were collected early in the morning (8:00 am - 10:00 am) from three distinct locations with varying depths in each lake during the post-monsoon (November 2023) and pre-monsoon (April 2024). For bigger lakes (Phewa, Begnas) water samples were collected from different depths i.e. 0 m, 2.5 m, 5 m, 7.5 m, and 10 m. For Rupa, Khaste and Dipang Lake samples were taken from 0 m, 2.5 m and 3.5 m. While for Neureni and Maldi samples were collected from 0 m and 2.5 m and for Gunde from 0 m and 1.5 m.

Ten liters of water sample were taken from each depth by using Vandorn water sampler of 3-liter capacity. To ensure accuracy and reliability, three replicated samples were collected per depth. Collected water sample was filtered through plankton net of mesh size  $75\mu\text{m}$  with a diameter of 20cm and concentrated to 100 mL and samples were anesthetized with 3% procaine and preserved in 5 % formalin following the methods described by Gannon (1971). Identification of zooplankton was done by Balcer (1984) and Johnson and Allen (2012). Samples were identified and enumerated under a compound microscope using Sedgwick rafter. Cell numbers were expressed in

number per liter ( $\text{No.L}^{-1}$ ). The community structure of the zooplankton was assessed by using the Shannon-Wiener Index ( $H'$ ) for species diversity (Shannon & Weaver, 1949). The evenness ( $J$ ) was calculated as proposed by Pielou (Pielou, 1966) to prevent the weighting of the  $H$  index by rare species and lastly the Margalef's index ( $d$ ) for species richness (Margalef, 1969).

A one-way analysis of variance (ANOVA) was used to evaluate whether there were significant variations in the abundance of zooplankton between the eight lakes. Prior to the ANOVA, the assumption of variance homogeneity was tested using Levene's Test. IBM SPSS statistics var. 31 was used for all statistical analyses, with a significance threshold ( $\alpha$ ) of 0.05.

### Results and Discussion

#### Spatial and Temporal Variation of Zooplanktons

A total of 33 species of zooplankton were recorded from 8 Lakes throughout the study period with 5 Groups, comprising 23 species of Rotifer, 4 species of Copepoda, 3 species of Cladocera, 2 species of Protozoa, and 1 species of Cnidarian (Table 1).

**Table 1** Taxonomic composition and seasonal distribution of the zooplankton community

Zooplankton Species	Rupa		Phewa		Begnag		Dipang		Khaste		Neureni		Maidi		Gunde	
	Ps	Pr	Ps	Pr	Ps	Pr	Ps	Pr	Ps	Pr	Ps	Pr	Ps	Pr	Ps	Pr
<b>Cladocera</b>																
<i>Ceriodaphnia dubia</i> (Richard, 1894)	++	+	-	-	++	+	+	-	-	-	-	-	-	-	-	-
<i>Bosmina longirostris</i> (De Melo & Hebert, 1994)	++	+	++	+	++	+	-	+	++	-	++	-	++	-	++	++
<i>Alona quadrangularis</i> (O.F.Müller, 1776)	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-
<b>Rotifers</b>																
<i>Brachionus forficula</i> (Wierzejski, 1891)	+	+	++	+	+	+	-	+	++	-	-	+	-	+	-	-
<i>Brachionus falcatus</i> (Zacharias, 1898)	+	-	++	-	+	-	+	+	+	+	+	-	-	-	-	-
<i>Brachionus diversicornis</i> (Daday, 1883)	++	-	++	-	++	-	++	+	++	*	+	*	+	+	+	++
<i>Brachionus angularis</i> (Gosse, 1851)	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-
<i>Brachionus donneri</i> (Brehm, 1951)	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
<i>Brachionus calyciflorus</i> (Pallas, 1776)	-	+	-	-	+	-	-	-	-	-	-	++	-	-	-	-
<i>Brachionus caudatus</i> (Barroisand Daday, 1894)	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-
<i>Asplanchna priodonta</i> (Gosse, 1850)	++	-	+	-	++	+	-	+	++	++	+	+	+	-	-	-
<i>Trichocera similis</i> (Wierzejski 1893)	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
<i>Trichocera cylindrical</i> (Imhof, 1891)	++	+	++	+	++	+	*	*	++	+	++	++	++	*	++	++
<i>Keratella tropica</i> (Apstein, 1907)	+	-	+	+	-	++	-	-	-	+	+	-	-	-	+	-
<i>Keratella tecta</i> (Gosse, 1851)	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
<i>Keratella quadrata</i> (Müller, 1786)	-	++	-	+	-	++	-	-	-	-	-	-	-	-	-	-
<i>Keratella cochlearis</i> (Gosse, 1851)	++	++	++	*	++	++	++	-	-	+	+	-	+	-	++	-
<i>Conochiloides natans</i> (Seligo, 1900)	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
<i>Conochilus unicornis</i> (Rousselet, 1892)	-	++	-	*	+	+	+	++	-	+	-	-	-	+	-	+
<i>Collotheca ornate</i> (Dobie, 1849)	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
<i>Anuraeopsis fissa</i> (Gosse, 1851)	-	-	-	-	+	-	-	+	-	-	-	+	-	+	-	-
<i>Filinia longiseta</i> (Ehrenberg, 1834)	+	-	-	-	+	-	-	+	+	+	+	-	-	-	-	-
<i>Synchaeta stylata</i> (Wierzejski, 1893)	+	-	-	-	-	-	-	+	+	+	-	+	-	+	-	-
<i>Polyarthra vulgaris</i> (Carlin, 1943)	+	+	+	-	+	+	++	+	+	-	-	-	-	+	+	-
<i>Monostyla lunaris</i> (Ehrenberg, 1832)	+	-	+	-	+	+	-	-	-	+	-	+	-	+	-	-
<i>Hexarthra mira</i> (Hudson, 1871)	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-
<b>Copepoda</b>																
<i>Microcyclops varicans</i> (Sars G.O., 1863)	++	-	+	-	++	-	++	-	++	-	++	-	++	-	++	-

<i>Cyclops vicinus</i> (Uljanin, 1875)	-	++	++	++	-	+	-	+	++	+	-	+	-	+	-	+
<i>Mesocyclops leuckarti</i> (Claus, 1857)	-	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-
<i>Diaptomus spp.</i>	++	+	++	++	++	+	+	+	-	-	++	+	+	+	+	+
<b>Protozoa</b>																
<i>Difflugia sp.</i>	+	++	+	+	++	+	+	+	-	+	+	+	-	+	-	-
<i>Centropycsis aculeate</i> (Ehrenberg, 1832)	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
<b>Cnidarian</b>																
Actinula larvae	-	+	-	-	-	++	-	-	-	-	-	+	-	-	-	-
- Nauplis	++	+	++	*	++	*	++	++	++	++	++	++	+	++	+	*

Absent: -, (10-100) number per liter: +, (100-300) number per liter: ++, >300 number per liter: \*, Ps: Post-Monsoon, Pr: Pre-Monsoon

### Total Number of Species of Zooplankton of Eight Lakes

The total number of zooplankton species in post-monsoon and pre-monsoon is presented in Fig.2. In overall, Rupa Lake was with the highest number of zooplankton species followed by Phewa, Begnas, Neureni, Dipang, Khaste, Maida and Gude with 26, 24, 23, 20, 19, 18, 17 and 11 numbers of species respectively. Husen and Dhakal (2009) reported 20 species of zooplanktons from Phewa Lake out of which 10 species were from class Rotifer, 6 species from class Cladocera and 4 species belong to class Copepods. According to Husen et al. (2007), a total of 13 species of zooplankton were recorded from Begnas Lake comprising of 6 species of Rotifer, 3 species of Cladocera and 4 species of Copepoda. Husen et al.

(2011) reported a total of 17 species of zooplanktons, including Rotifers (8), Cladocera (5), and Copepoda (4) from Rupa Lake and noted the fluctuations of plankton diversity in different seasons and Gautam et al. (2016) recorded 17 species of zooplanktons from three classes namely Rotifera, Cladocera and Copepoda. Bastakoti and Timilsina (2020) recorded 11 species of zooplankton from Khaste Lake from which 3 genera were from Rotifer, 6 genera from Cladocera and 1 genus from Copepod. Protozoa is new to this lake with the Genus *Difflugia*. In addition, *Trichocerca cylindrica*, *Conochilus unicornis*, *Filinia longiseta*, *Synchaeta stylata*, *Polyarthra vulgaris*, *Monostyla lunaris* are the new species found from this lake. Excluding Phewa and Rupa, higher numbers of species were recorded in pre-monsoon than in post-monsoon.

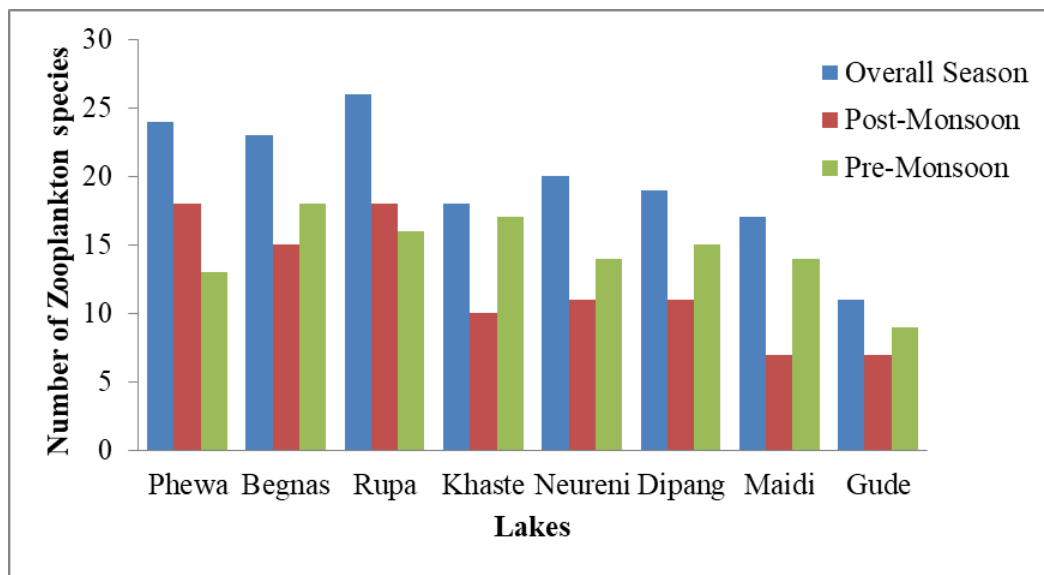


Figure 2 Total number of zooplankton species recorded from eight lakes

### Seasonal Zooplankton Abundance across Different Lakes

#### Post-Monsoon

Prior to the ANOVA, Levene's Test revealed that the variances were homogeneous ( $F_{7,16} = 0.791$ ,  $p = .606$ ).

The one-way ANOVA revealed a significant variation in species abundance among lakes ( $F_{7,16} = 796.58$ ,  $p < .001$ ). The high eta-squared value ( $\eta^2 = 0.997$ ) suggests that differences between lakes account for 99.7% of the variance in species richness.

Tukey's HSD post-hoc tests indicated significant differences between lakes. There was no significant difference in mean abundances between the lakes Dipang and Phewa. Rupa Lake had the highest mean abundance, whilst Maldi Lake had the lowest.

### Pre-Monsoon

The assumption of homogeneity of variances was validated by Levene's Test, which was conducted prior to performing an ANOVA ( $F(7,16)=0.634, p=.722$ ). A statistically significant difference in species abundance between the lakes was revealed by the one-way ANOVA ( $F(7,16)=730.441, p<.001$ ). A high eta-

squared value ( $\eta^2=0.997$ ) further substantiated this large effect, showing that the variations across the lakes account for around 99.7% of the variance in species richness.

Tukey's HSD post-hoc tests revealed significant variations in abundance across a number of lake pairs throughout the pre-monsoon season. There was no significant difference in mean abundance between Lakes Neureni, Dipang and Khaste. Phewa Lake showed the highest mean abundance, Maldi Lake had a considerably higher abundance, and Gunde Lake had the lowest mean abundance of any lake.

**Table 2** One way ANOVA and post hoc comparison of species abundance among 8 Lakes

Lake	Mean $\pm$ S.D.		F (df1, df2)		p-value		Post-Hoc Groupings (Tukey's HSD)	
	Post-Monsoon	Pre-Monsoon	Post-Monsoon	Pre-Monsoon	Post-Monsoon	Pre-Monsoon	Post-Monsoon	Pre-Monsoon
Phewa	1821 $\pm$ 49	4080 $\pm$ 98.06					E	F
Begnas	2011 $\pm$ 18.52	1998 $\pm$ 28.62					F	D
Rupa	2105 $\pm$ 9.64	1412 $\pm$ 39.85					G	B
Khaste	1582 $\pm$ 22.07	1783 $\pm$ 57.11					D	C
Neureni	1393 $\pm$ 23.64	1731 $\pm$ 51.64					C	C
Dipang	1773 $\pm$ 28.62	1733 $\pm$ 53.25					E	C
Gunde	989 $\pm$ 21	1035 $\pm$ 87.54					B	A
Maldi	878 $\pm$ 32	3106 $\pm$ 63.53					A	E
ANOVA (Between and within Groups)			796.58 (7,16)	730.44 (7, 16)	<.001	<.001		

### Percentage Contribution of Zooplankton Groups in Pre-Monsoon and Post-Monsoon

The percentage contribution of zooplankton groups in pre-monsoon and post-monsoon is presented in Fig. 3 and Fig. 4. In both seasons, the community was dominated by Rotifers contributing more than 45% of total zooplankton abundance followed by Copepods and Cladocera. According to Rai (2000), Rotifera dominated Phewa and Rupa Lake, whereas Begnas Lake was dominated by Cladocera and *Keratella* spp. and *Synchaeta stylata* were major species of Rotifera in Phewa Lake, *Synchaeta stylata*, *Keratella* spp., and *Polyarthra* spp. were major species of Rotifera in Rupa Lake and *Bosmina longirostris* and *Ceriodaphnia* spp. in Begnas Lake. Gautam et al. (2016) also reported Rotifera as the most dominant group in Rupa Lake. Along with this, *Brachionus* spp., *Keratella* spp., *Trichocerca* spp. were seen dominant in most of the lakes and even *Conochilus unicornis* and Nauplius were seen dominant in some of the lakes.

### Diversity Indices

The Shannon Wiener Index ( $H'$ ), Pielou's Species Evenness ( $J$ ), and Margalef's Species Richness are presented in Figures 5, 6, and 7 respectively. The value

of Shannon Wiener diversity Index ( $H'$ ) usually varies from 1.5 to 3.5 (Shannon & Weaver, 1949). The values of diversity indices of zooplankton were higher in post-monsoon than in pre-monsoon. The values of the ( $H'$ ) recorded in post-monsoon were highest in Rupa (2.7) and lowest in Gunde (2.11) Lake. Similarly, in pre-monsoon, ( $H'$ ) value was highest in Begnas (2.75) and lowest in Maldi Lake (1.26). According to the species diversity scale of Wilhm and Dorris (1968) ( $H' > 3 =$  clean water,  $H' = 1-3 =$  moderately contaminated,  $H' < 1 =$  heavily polluted during both seasons, the value of  $H'$  ranged from 1.75 to 2.75 which indicates lakes to be moderately polluted.

The value of the Pielou's evenness index ( $J$ ) of zooplankton of all lakes was higher in Post-monsoon than that in pre-monsoon. The  $J$  value of Phewa, Rupa and Begnas was more than 0.50 in post-monsoon, while, in pre-monsoon, Begnas was the only lake to have  $J$  value higher than 0.50. According to Frutos et al., (2009) and Ismail and Zaidin (2015), if the Pielou Index is less than 0.5, it could be the indication of the presence of ecological stress.

The value of Margalef's species richness index ( $d$ ) of zooplankton of all lakes was higher in pre-monsoon

except Rupa and Phewa. Rupa Lake showed the highest d value with 3.83 while Maidu showed the lowest d

value with 0.02 in post-monsoon. In pre-monsoon, the value of d varied from 1.96 (Gude) to 3.39 (Begnas).

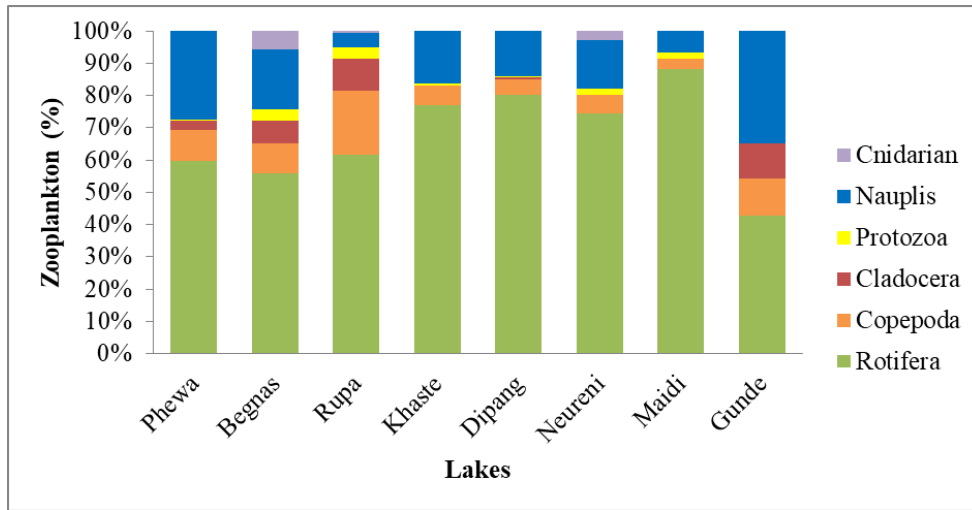


Figure 3 Percentage contribution of zooplankton in pre-monsoon season

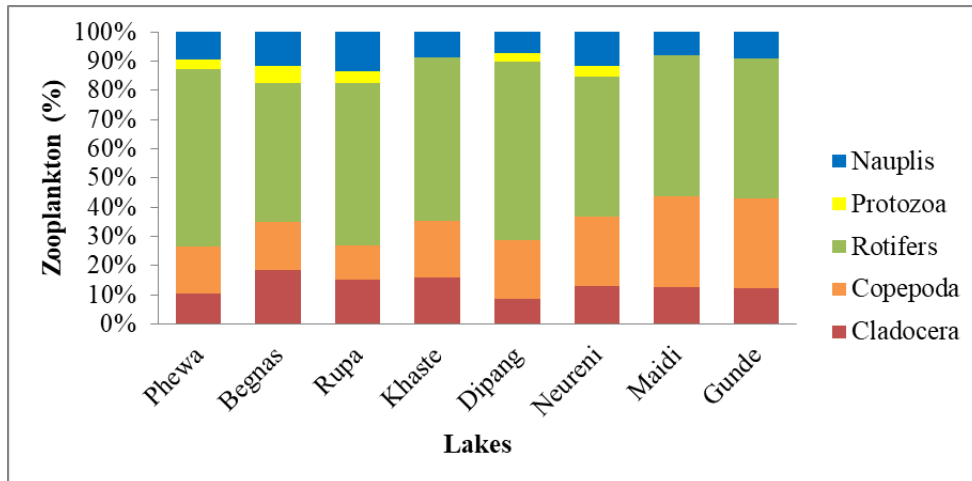


Figure 4 Percentage contribution of zooplankton in post-monsoon season

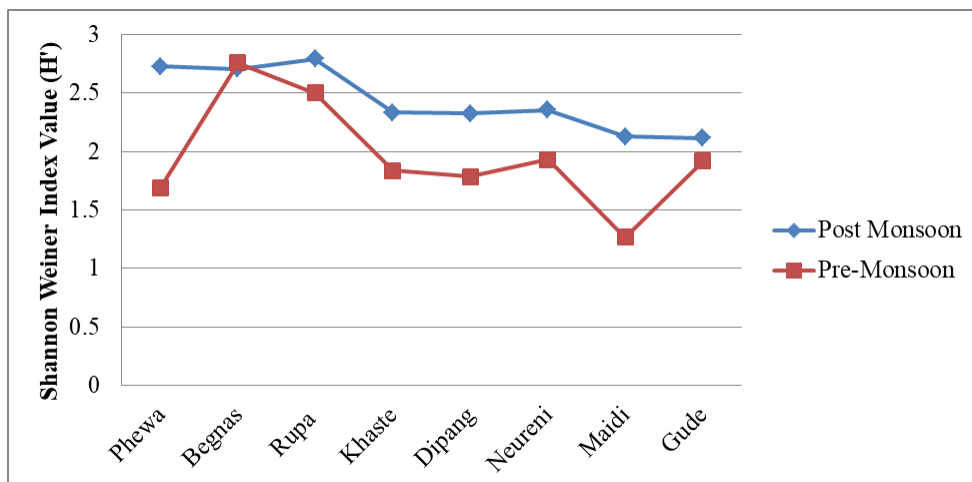


Figure 5 Range of Shannon Weiner Diversity Index

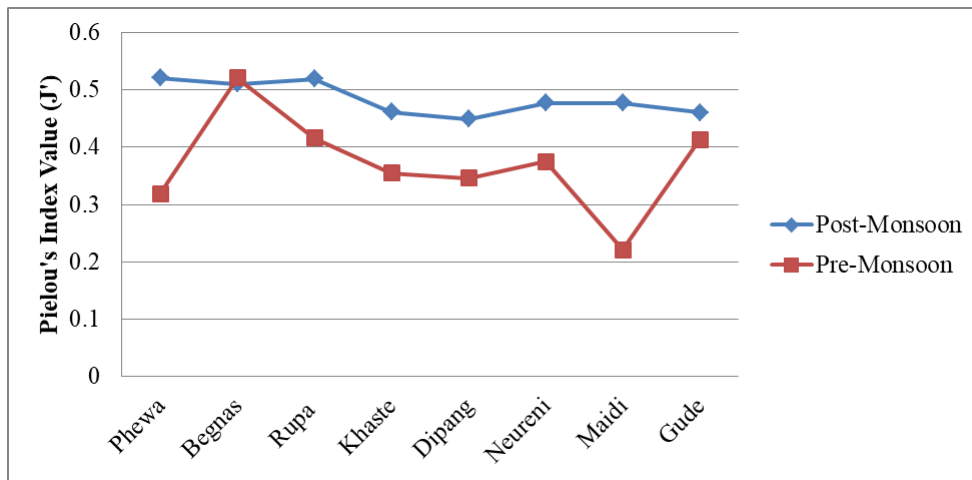


Figure 6 Range of Pielou's Evenness Index

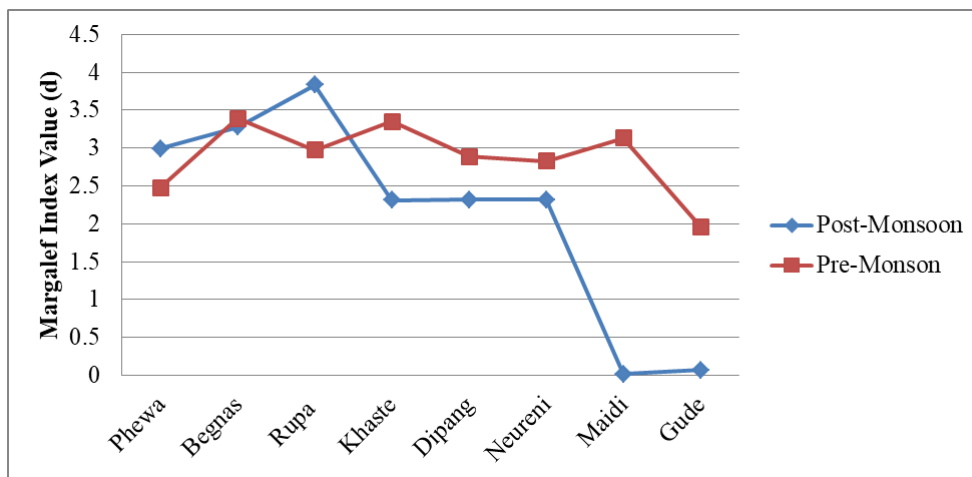


Figure 7 Range of Margalef Species Richness Index

In freshwater ecosystems, Rotifers are generally the primary contributors to zooplankton diversity and density (Beyene et al., 2022). Our data supports this assertion, showing that Rotifers accounted for over 45% of the overall zooplankton population seen in practically all lakes. Generally small-bodied zooplankton such as Rotifers, especially from the genera *Keratella*, *Brachionus*, and *Trichocerca*, tend to dominate plankton communities in highly eutrophic conditions (Sampaio et al. 2002; Kumari et al. 2023). Dadhich et al. (1999) showed that among the other genera, *Brachionus* and *Trichocerca* are reported to exist in eutrophic environments. The researchers de Attayde and Bozelli (1998) pointed out that Rotifers *Asplanchna*, *Brachionus* and *Polarthra* were good indicators of eutrophic conditions. Some Rotifer species recorded during the present study were *B. angularis*, *B. calyciflorus*, *B. diversicornis*, *K. cochlearis*, *F. longiseta*, which are considered as indicators of higher trophic status by many researchers (Gannon and Stemberger, 1978; Miura, 1990; Baloch et al., 2000; Sampaio et al., 2002). Some Protozoa species such *Centropyxis* sp., *Diffugia* sp. have been regarded as markers of nutrient rich waters

by various authors (Wanganeo and Wanganeo, 2006; Kumar et al. 2010) which were found in almost all lakes. Matsumura-Tundisi et al. (1990) reported that *Conochilus unicornis* is an indicator of eutrophication in the reservoir at Barra Bonita on the River Tietê, SP. Similar to this, *Conochilus unicornis* was abundant in Phewa Lake in pre-monsoon. Further, as per Sampaio et al. (2002), Dulić et al. (2006) and Sousa et al. (2008), *Brachionus* spp. is regarded to tolerate polluted waters. Valencia-vargas et al. (2022) reported that cyclopoids can feed on rotifers and the high density of cyclopoids in eutrophic lakes might be connected with the high rotifer densities in those conditions. Similar to this, in almost all the lakes, Copepoda were the second leading group. Dominating rotifers in all the lakes can be said that the lakes are leading towards eutrophication, and the excessive proliferation of plankton is a certain sign that the aquatic environment is becoming more eutrophic (Prescott, 1961).

### Conclusions

The present study revealed the presence of five taxonomic groups of zooplankton, i.e., Rotifers (23),

Cladocera (3), Copepoda (4), Protozoa (2), and Cnidarians (1) from the eight lakes of Pokhara valley. Rotifers predominated over copepods in all lakes during both the pre- and post-monsoon seasons, indicating eutrophication. Similarly, low Shannon diversity values indicate that lakes are substantially contaminated and have high anthropogenic activities. Effective mitigations measures should be taken to promote these lakes for sustainable fisheries.

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**Author Contributions:** MAH: Conceptualized and designed the study, formally analyzed it, guided and supervised throughout the research and proof reading; NK, AB and RS: Involved in sampling, data collection, laboratory work, manuscript preparation, statistical analysis, and contributed to interpretation of results. All authors reviewed and approved the completed manuscript.

**Conflict of interest:** The authors declare that they have no conflict of interest.

**Data availability:** The data of the current study is accessible upon reasonable request from the corresponding author.

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