

## **Comparative study on macro-invertebrates as bio indicator from the Rivers Mawa and Ratuwa, Nepal**

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### **Abstract**

This study investigates the diversity of freshwater macroinvertebrates and their correlation with physicochemical parameters in the Ratuwa and Mawa Rivers in Nepal. These rivers, which flow through both hilly and plain regions, are significantly impacted by anthropogenic activities, including urban pollution and cultural practices. Macroinvertebrates serve as bioindicators of aquatic health, making their assessment crucial for evaluating water quality. A total of 7 species from the Mawa River and 8 species from the Ratuwa River were recorded, with Arthropoda being the dominant phylum in both rivers, followed by Mollusca and Annelida. Water samples and macroinvertebrates were collected from five stations along each river from January 2024 to March 2024. Physicochemical parameters were analyzed, including Temperature, pH, Turbidity, Conductivity, Dissolved Oxygen (DO), Total alkalinity, total hardness and Biological Oxygen Demand (BOD). Statistical analyses such as ANOVA, MANOVA, and Canonical Correspondence Analysis (CCA) revealed that Temperature, Turbidity, DO and BOD were major influencing factors in the Mawa River, whereas conductivity, alkalinity, DO, and BOD were more significant in the Ratuwa River. The analysis of similarity (ANOSIM) indicated a moderate similarity between the two rivers ( $R = 0.184$ ). Comparative assessments showed that Ratuwa had a slightly higher macroinvertebrate diversity than Mawa, with variations in species distribution influenced by environmental conditions. The study highlights that macroinvertebrate diversity is affected by pollution, habitat loss due to sand mining, and industrial waste discharge. Conservation measures such as regulating sand mining, reducing pollution, and implementing sustainable management practices are

recommended. This research provides baseline data for future studies and underscores the need for policy interventions to protect aquatic biodiversity in the region.

**Keywords:** macro-invertebrates, bioindicators, physicochemical parameters, Ratuwa river, Mawa river, urban pollution, aquatic biodiversity

## Introduction

Macroinvertebrates play a crucial role in freshwater ecosystems serving as key indicators of water quality and ecosystem health, as their presence, diversity, and abundance reflect environmental conditions. Their study provides valuable insights into ecological balance, nutrient cycling, and the overall functioning of freshwater ecosystems. Malla et al. (1978) investigated aquatic insects in various water bodies within the Kathmandu Valley, documenting a total of 59 species. Yadav et al. (1983) studied the biological parameters of Taudah Lake, Kathmandu, recording 36 species of macroinvertebrates, including Oligochaeta, Ephemeroptera, Chironomida, and Mollusca as major groups. Mahato and Yadav (1984) reported 31 species of macroinvertebrates from two ponds in the Mahottary district, Nepal. Loeb and Spacie (1994) acknowledged the significance of macroinvertebrates in evaluating environmental quality. Sinha et al. (1994) investigated the physicochemical properties of Kavar Lake in North Bihar from November 1991 to October 1993, reporting pH levels between 6.3 and 7.23, dissolved oxygen ranging from 2.15 to 7.6 mg/L, and free carbon dioxide levels between 2.8 and 12.75 mg/L. Additionally, they analyzed the physicochemical conditions of the Sone River at Dalmianagar, Bihar. Singh (1995) observed increased water transparency during winter, while Rawat et al. (1995) studied the morphometry and physicochemical characteristics of Deoria Tal, a high-altitude lake in the Garhwal Himalayas. Sharma (1996) reported 18 species of gastropods and 10 species of bivalves from the Koshi River basin belt of Nepal-India (Northern Bihar). Subba and Ghosh (2000) presented a list of mollusks collected from nine districts and reported 25 species. Yu & Lee (2002) demonstrated the impact of conductivity on aquatic ecosystems, while Magalhaes et al. (2002) highlighted the effects of stream width and altitude. Vlach et al. (2005) and Kadye et al. (2008) examined depth, distance from the source, water temperature, and substrate composition as influencing factors on aquatic organisms. Menni et al. (2005) studied the role of climate, and Niroula et al. (2010) documented seasonal fluctuations in water quality parameters. Lucadamo et al. (2008) recognized freshwater macroinvertebrates as valuable indicators of ecosystem health and reliable tools for biomonitoring. Kamal et al. (2007) examined the physicochemical characteristics of the Mouri River in Khulna, Bangladesh. Carter et al. (2017) highlighted the role of aquatic

organisms in assessing environmental quality and human-induced stressors at multiple biological levels. Shrestha & Basnet (2018) analyzed multiple water parameters in the Ratuwa River to evaluate pollution levels and raise awareness among local communities. Pokharel et al. (2018) linked urbanization to increased conductivity, turbidity, free CO<sub>2</sub>, nitrates, and phosphates, along with decreased transparency, dissolved oxygen, and pH. Bhandari et al. (2019) conducted a study on benthic macroinvertebrates in five tributaries of the Budhiganga River in western Nepal. Pal et al. (2016) and Pal and Talukdar (2019) investigated hydro-ecological shifts due to damming in the Tangon, Ganga-Padma, and Atreyee rivers across India and Bangladesh. Roy et al. (2020) assessed water quality in the Tangon River, while Mondal et al. (2020) observed a negative correlation between water temperature and pH in Mirik Lake, Darjeeling.

## **Materials and Methods**

### **Study area**

The Ratuwa River, which flows through parts of the Ilam and Jhapa districts (Figure 1). Originating from the Chure range near Saptami Bazar in Ilam, the river eventually joins the Kankai River in Bihar, India, before ultimately flowing into the Bay of Bengal. As the river progresses into the flatter terrain of the lower Terai region, its gradient gradually decreases.

The second study area was Mawa River (Figure 2), originates from Chure hills of Dare Gauda, a border between Ilam and Panchthar. The river flows through Mangsebung, Chulachuli towards east and Miklajung towards west in the Chure range while it is the border of Jhapa and Morang districts in the plain region. It merges with Ratuwa River in the southern part of Damak.

In both rivers, the substrata primarily result from erosion and weathering of sandstones, consisting of materials ranging from boulders, pebbles, cobbles, gravel, and sand. Along the elevation gradient (600–1853 m), *Alnus nepalensis* dominates as the primary natural vegetation, with the riverbed composed mainly of large boulders, cobbles, pebbles, and minimal sand. In contrast, at lower elevations (600–70 m), the dominant vegetation includes *Schima wallichii*, *Shorea robusta*, and various shrubs, with the riverbed featuring sand, cobbles, pebbles, and gravel. Both rivers benefit from tropical and temperate climates, fostering rich biological diversity.

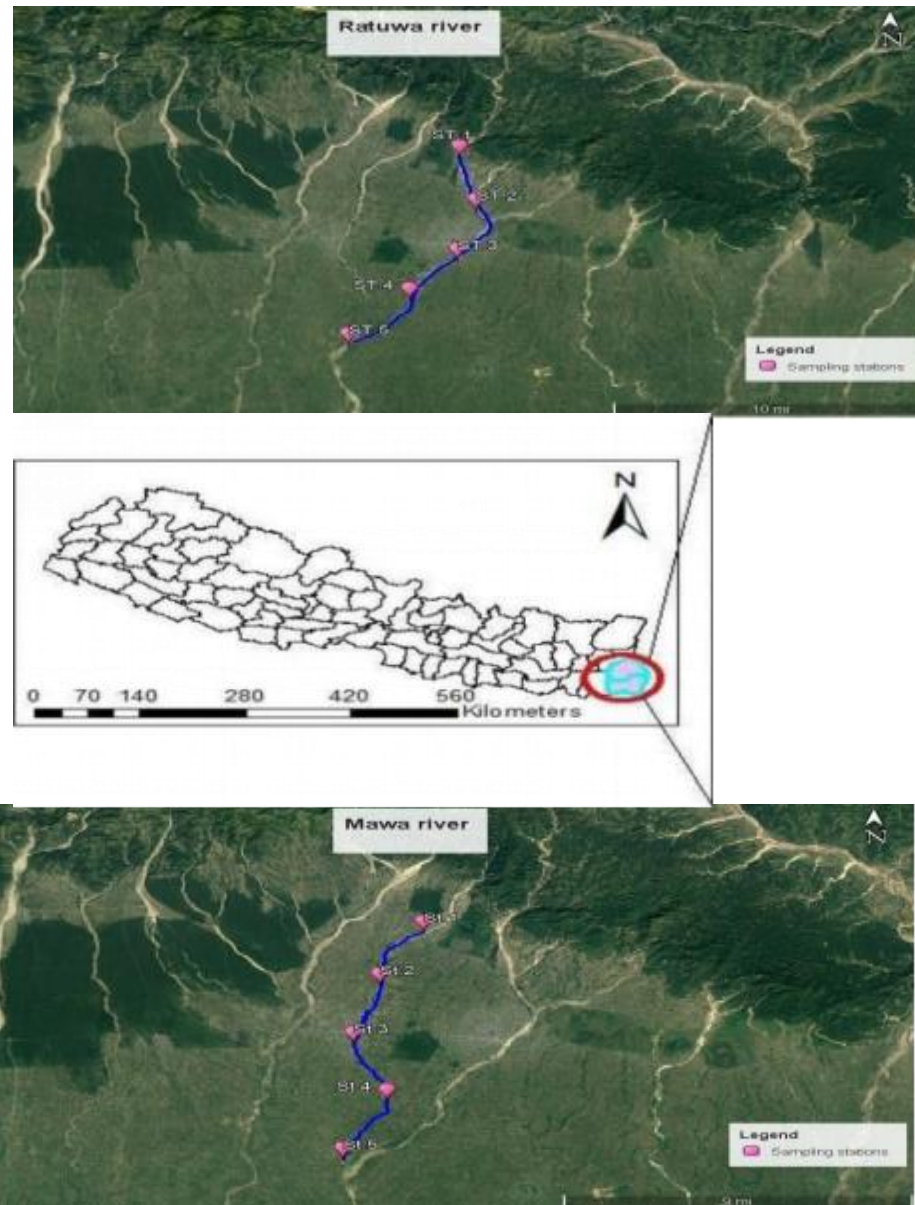


Figure1. Samplig stations of the Rivers Ratuwa and Mawa

### Data collection

The study area was divided into 5 sampling stations based on different habitat types for both rivers Ratuwa and Mawa (Figure 1 and Figure 2). The sampling sites of Rauwa were Chapeti, Peltimari, Baluwatar, Sanobaraghare, and Jharkaha. On the other hand, The stations of Mawa were Madhumalla, Golatar, Tarabari, Sanobaraghare, and Mawa-Ratuwa confluence. Macro-invertebrate were collected based on habitat representativeness from January 2024 to March 2024.

### **Physico-chemical characteristics**

The physicochemical parameters of the water were analyzed at Batabaraniya Sewa Kendra, a fully equipped laboratory in Biratnagar, using standardized methods outlined by the American Public Health Association (APHA, 2012).

#### **1. Temperature (°C)**

The air and water temperature was recorded by using standard thermometer (HI98501, HANNA)

#### **2. Hydrogen Ion Concentration (pH)**

The pH was measured with a portable pH meter (HI 98107, HANNA).

#### **3. Turbidity (NTU)**

Turbidity, or water clarity, was measured using a Nephelometer (HI93703, HANNA).

#### **4. Electrical Conductivity (µS/cm)**

An electrical conductivity meter (EC meter) was employed to measure the conductivity of the river water. (HI99300, HANNA)

#### **5. Dissolved Oxygen (DO mg/l)**

Dissolved oxygen levels were measured using a DO meter (HI9147, HANNA)

#### **6. Total Alkalinity (mg/l)**

Alkalinity was measured by titration using 0.1N hydrochloric acid, with phenolphthalein and methyl orange serving as indicators.

#### **7. Total Hardness (mg/l)**

Total hardness was assessed using two methods: calcium was measured via the EDTA titrimetric method, while magnesium was determined using the gravimetric method.

#### **8. Biological Oxygen Demand (BOD mg/l)**

BOD was determined using the Azide modification method (APHA-5240 B) along with the APHA-4500 Ascorbic Acid method.

### **Macro-invertebrates**

Aquatic Dip Net of 500 µm Mesh size, gloves, and forceps were used to collect macro-invertebrates. The samples were preserved in plastic jars and finally released to their natural habitat after the study. The identification of the species was done with the help of literatures like Adoni et al. (1985), Baker (1928), Datta Munshi and Datta Munshi (1995) and Pennak. (1953).

### **Data analysis**

Statistical analysis including one-way analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA), were used to compare different water parameters. Similarity percentage (SIMPER) (Clarke, 1993) and analysis of similarities (ANOSIM) were also performed to observe the similarities between the Rivers Mawa and

Ratuwa. The Pearson correlation coefficient matrices (Anderson, 2006) were calculated to assess the relationships among the environmental variables. The softwares like Excell, PAST and Originlab were used for statistical analysis while Arcgis and Google Earth used for mapping.

## Results

The present study of macroinvertebrates involved a total of 7 species from the Mawa River and 8 species from the Ratuwa River. For the Mawa River, the highest number of species recorded belonged to the phylum Arthropoda, with a total of 4 species, followed by Mollusca (2 species) and Annelida (1 species), as outlined below.

Table 1. Coding of the Arthropod by class, order and species of the River Mawa

Class	Order	Code	Species	IUCN red list
Crustacea	Decapoda	C1	<i>Neocaridinadavidi</i> (Bouvier, 1904)	LC
Insecta	Hemiptera	C2	<i>Nepa cinerea</i> (Linnaeus, 1758)	NE
Insecta	Hemiptera	C3	<i>Ranatra linearis</i> (Linnaeus, 1758)	NE
Insecta	Psocodea	C4	<i>Trogium pulsatorium</i> (Linnaeus, 1758)	NE

Table 2. Coding of the Molluscs by class, order and species in the River Mawa

Class	Order	Code	Species	IUCN redlist
Gastropoda	Architaenioglossa	C5	<i>Pila globosa</i> (Swainson, 1822)	LC
Bivalvia	Unionida	C6	<i>Lamellidens marginalis</i> (Lamarck, 1819)	LC

Table 3. Coding of the Annelids by class, order and species

Class	Order	Code	Species	IUCN redlist
Oligochaeta	Opisthopora	C7	<i>Pheretima Posthuma</i> (Valliant, 1868)	NE

The maximum numbers of macroinvertebrate species were collected from the stations 2 and 5 followed by station 4, while minimum species were observed in stations 1 and 3 given in Figure 2 below.

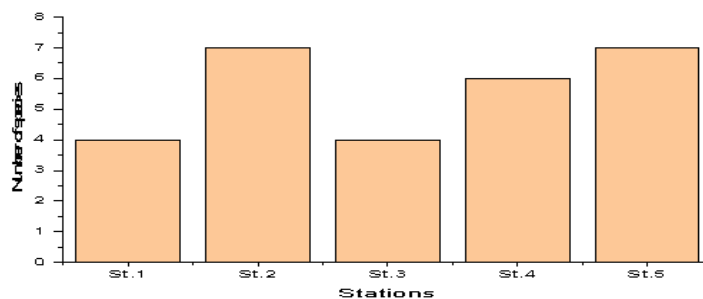


Figure 2. Station-wise distribution of macroinvertebrate species in the River Mawa

The water parameters and their characters are given in the table 4 and 5 respectively.

Table 4. Water parameters of the River Mawa

Stations	Tempr	pH	Turbidity	Conduct	DO	Alkalinity	Hardness
St. 1	27.4	7.6	0.95	203	17	106	90
St. 2	27.4	7.8	0.71	220	15	127	105
St. 3	27.5	7.9	1.45	240	13	139	137
St. 4	27.5	8	1.82	255	8	130	130
St. 5	27.6	8.5	1.41	200	7	110	94

Table 5. Characters of variables of the River Mawa

Parameters	Mean	SD	Sum	Min	Max
Temperature	27.48	0.08367	137.4	27.4	27.6
pH	7.96	0.33615	39.8	7.6	8.5
Turbidity	1.268	0.43888	6.34	0.71	1.82
Conductivity	223.6	23.7129	1118	200	255
DO	12	4.3589	60	7	17
Alkalinity	122.4	13.9392	612	106	139
Hardness	111.2	21.2297	556	90	137
BOD	15.4	11.2161	77	6	33

Pearson's linear correlation of water parameters in the River Mawa shows that highest positive correlation found between conductivity and temperature while Dissolved Dxygen and temperauture got the highest negative correlation.

Table 6. Pearson's linear Correlation of the water parameters in the River Mawa

Parameters	Tempr	pH	Turbidity	Conduct	DO	Alkalinity	Hardness	BOD
Tempr		0.016522	0.19444	0.99037	0.042391	0.98362	0.81784	0.31005
pH	0.94223		0.39749	0.82169	0.044693	0.84954	0.93491	0.15507
Turbidity	0.69309	0.49413		0.27142	0.11646	0.58618	0.27198	0.54706
Conduct	0.007561	-0.14051	0.61318		0.68016	0.067654	0.020688	0.67088
DO	-0.89116	-0.88721	-0.78409	-0.25396		0.89534	0.70018	0.11781
Alkalinity	-0.01286	-0.11845	0.33117	0.85074	-0.08229		0.020914	0.64489
Hardness	0.14356	-0.05115	0.61262	0.93283	-0.23774	0.93233		0.73671
BOD	-0.57545	-0.73734	-0.36394	-0.2615	0.78237	-0.28271	-0.2083	

The Canonical Correspondance Analysis indicated that species C4 is positively correlated with conductivity but negatively correlated with BOD and DO. Similarly, C3 and C6 are positively correlated with turbidity, temperature and total hardness of water but negatively correlated with total alkalinity. Species C2 and C5 show a positive

correlation with Dissolved Oxygen and Biological Oxygen Demand but a negative correlation with conductivity. In contrast, species C7 is positively correlated with alkalinity but negatively correlated with temperature, turbidity, and total hardness. The Canonical Correspondence Analysis (CCA) plot highlights that Dissolved Oxygen, Biological Oxygen Demand, temperature, and turbidity are the primary factors influencing the Mawa River. Additionally, the analysis of variance (ANOVA) revealed that the physicochemical parameters in the Mawa River exhibited significant differences ( $p < 0.05$ ).

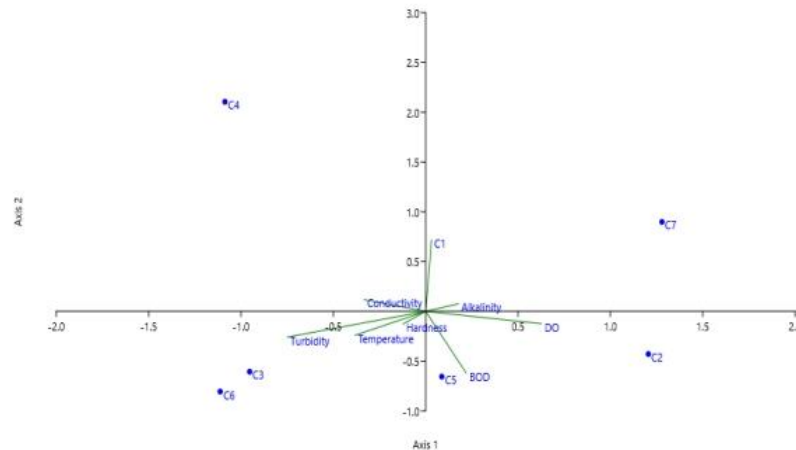


Figure3. CCA plot of water parameters vs macroinvertebrate species of the River Mawa  
In the Ratuwa River, the highest number of species documented belonged to the phylum Arthropoda, with a total of 5 species, followed by Mollusca (2 species) and Annelida (1 species), as detailed below.

Table 7. Coding of the Arthropod by class, order and species

Class	Order	Code	Specie	IUCN red list
Crustacea	Decapoda,	C1	<i>Macrobrachium lamarrei</i> (Edwards, 1837)	LC
Crustacea	Decapoda	C2	<i>Neocaridinadavidi</i> (Bouvier, 1904)	LC
Insecta	Hemiptera	C3	<i>Nepa cinerea</i> (Linnaeus, 1758)	NE
Insecta	Hemiptera	C4	<i>Ranatra linearis</i> (Linnaeus, 1758)	NE
Insecta	Psocopter	C5	<i>Trogiumpulsatorium</i> (Linnaeus, 1758)	NE

Table 8. Coding of the Molluscs by class, order and species in the River Mawa

Class	Order	Code	Species	IUCNredlist
Gastropoda	Architaenioglosa	C6	<i>Pilaglobosa</i> (Swainson, 1822)	LC
Bivalvia	Unionida	C7	<i>Lamellidensmarginalis</i> (Lamarck,1819)	LC

Table9.CodingoftheAnnelidsbyclass,orderandspecies

Class	Order	Code	Species	
Oligochaeta	Opisthopora	C8	<i>Pheretima Posthuma</i> (Vailliant,1868)	NE



The maximum numbers of macroinvertebrate species were collected from the station 4 followed by station 5 and station 2 while minimum species were observed in stations 1 and 3 given in Figure 3 below.

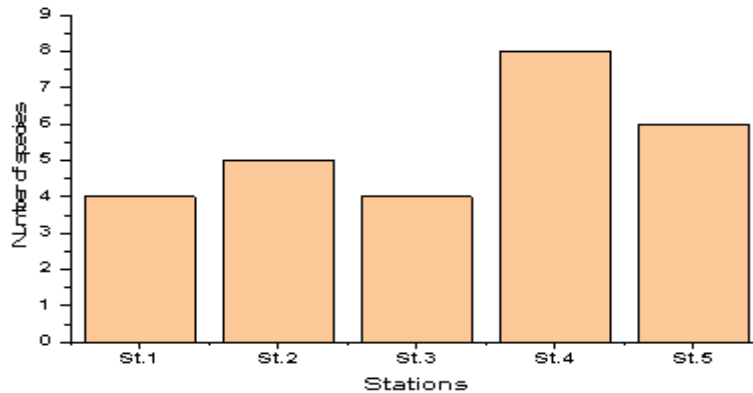


Figure 3. Station-wise distribution of macro invertebrate species in the River Ratuwa

The water parameters and their major characters are given in Tables10 and 11 respectively.

Table 10. Water parameters of the River Ratuwa

Stations	Temperature	pH	Turbidity	Conductivity	DO	Alkalinity	Hardness	BOD
<b>St. 1</b>	27	7.27	0.45	190	16	107	74	8
<b>St. 2</b>	27.2	7.35	0.68	207	16.2	116	90	9
<b>St. 3</b>	27.3	7.44	2.05	220	14.8	114	88	7
<b>St. 4</b>	27.3	7.48	4.01	230	15.3	113	84	7
<b>St. 5</b>	27.4	8.01	4.43	200	16.2	96	73	9

Table 11. Characters of water parameters of the River Ratuwa

Parameters	Mean	SD	Sum	Min	Max
<b>Temperature</b>	27.24	0.15166	136.2	27	27.4
<b>pH</b>	7.51	0.29112	37.55	7.27	8.01
<b>Turbidity</b>	1.9025	1.82704	7.61	0.45	4.43
<b>Conductivity</b>	209.4	15.8682	1047	190	230
<b>DO</b>	15.7	0.6245	78.5	14.8	16.2
<b>Alkalinity</b>	109.2	8.10555	546	96	116
<b>Hardness</b>	81.8	7.8867	409	73	90
<b>BOD</b>	8	1	40	7	9

Pearson's linear correlation analysis of water parameters in the RatuwaRiver revealed the strongest positive correlation between temperature and BOD, whereas the highest negative correlation was observed between total alkalinity and hydrogen ion concentration (pH).

Table 12. Pearson's linear Correlation of the water parameters in the River Ratuwa

Parameters	Temper	pH	Turbidity	Conductivity	DO	Alkalinity	Hardness	BOD
Temper		0.11857	0.075747	0.35623	0.70039	0.65736	0.83019	1
pH	0.78142		0.09749	0.92975	0.69999	0.099343	0.42152	0.53061
Turbidity	0.83886	0.80872		0.44966	0.7909	0.3927	0.6668	0.83626
Conductivity	0.53189	-0.0552	0.44767		0.14918	0.3438	0.23504	0.20893
DO	-0.23757	0.23789	-0.16498	-0.74422		0.41424	0.43352	0.026467
Alkalinity	-0.27252	-0.80625	-0.49846	0.54346	-0.47907		0.043923	0.43269
Hardness	0.13377	-0.47257	-0.26482	0.65003	-0.46191	0.88853		0.64175
BOD	-5.86E-15	0.37785	-0.12896	-0.67746	0.92074	-0.46265	-0.28529	

The Canonical Correspondence Analysis revealed that species C1, C3, and C5 have a positive association with total alkalinity and total hardness but a negative relationship with turbidity. Species C7 is positively linked to dissolved oxygen and biological oxygen demand, while it negatively correlates with conductivity and temperature. Likewise, species C2, C4, and C8 exhibit a positive correlation with turbidity but a negative association with total hardness and total alkalinity. Species C6, on the other hand, shows a positive correlation with conductivity and temperature but a negative correlation with dissolved oxygen and biological oxygen demand. The CCA plot emphasizes that conductivity, total alkalinity, dissolved oxygen, and biological oxygen demand are the key factors influencing macroinvertebrate distribution. Additionally, the analysis of variance (ANOVA) revealed that the physicochemical parameters in the Ratuwa River are significantly different ( $p < 0.05$ ).

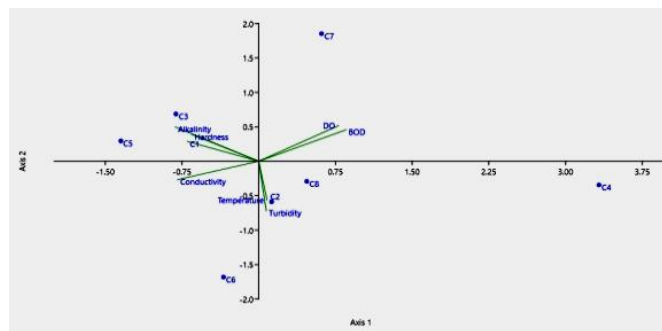


Figure5. CCA plot of water parameters vs macroinvertebrate species of the River Ratuwa

### Comparative study of the Rivers Mawa and Ratuwa

The water parameters from both of the Rivers Mawa and Ratuwa were studied through a multivariate analysis of variance (MANOVA) that revealed that the parameters were significant ( $p > 0.05$ ) to each other. The value of R was found to 0.184 from the analysis of similarity (ANOSIM) suggesting quite similarity in the physicochemical parameters

of the rivers Mawa and Ratuwa. On the other hand, Table 15 shows the station-wise distribution of macro-invertebrates suggesting more number of species and individuals found in the River Ratuwa than Mawa.

Table 13. Station-wise distribution of macro-invertebrates

Ratuwa River			Mawa River		
Stations	Total individuals	Species no.	Stations	Total individuals	Species no.
St.1	17	4	St.1	18	4
St.2	20	5	St.2	16	7
St.3	10	4	St.3	13	4
St.4	15	8	St.4	23	6
St.5	13	6	St.5	20	7

When various diversity indices were compared from the Rivers Mawa and Ratuwa, then it was found that the value of Simpson index and Margalef index were found higher in Mawa but Dominance and Shannon index were observed higher in the River Ratuwa as shown in table 14 below.

Table 14. Species diversity index

Indices	Mawa	Ratuwa
Dominance	0.1489	0.1577
Simpson	0.8511	0.8423
Shannon	1.926	1.949
Margalef	1.147	1.279

The Comparative study of similarity of percentage (SIMPER) for the Rivers Mawa and Ratuwa showed that total hardness got highest value of dissimilarity while temperature was found to have the least average dissimilarity. But, the cumulative percentage was highest for Temperature and lowest for hydrogen ion concentration (pH).

Table 15. Similarity of percentage (SIMPER) for the Rivers Mawa and Ratuwa

Parameters	Av. Dissimilarity	Contribution %	Cumulative %
Temperature	0.02468	0.2925	100
pH	0.05255	0.6229	99.71
Turbidity	0.1657	1.965	99.08
Conductivity	2.344	27.78	62.45
DO	0.4355	5.161	97.12
Alkalinity	1.632	19.34	81.79
Hardness	2.925	34.67	34.67
BOD	0.8579	10.17	91.96

## Discussion

It was found that Dissolved Oxygen, Biological Oxygen Demand, temperature and turbidity were the major influencing factors in the River Mawa while conductivity, total alkalinity, Dissolved Oxygen and Biological Oxygen Demand were found to be the principal influencing factors in the distribution of macroinvertebrates in the River Ratuwa. The analysis of variance (ANOVA) indicated that the physicochemical parameters were significantly different ( $p < 0.05$ ) in both the Mawa and Ratuwa Rivers, showing similar trends. Water quality varied across different sampling stations in these rivers, consistent with findings by Basnet (2013) in the Bagmati River. Macroinvertebrates exhibited both positive and negative associations with the physicochemical parameters of the Mawa and Ratuwa Rivers. Biological monitoring using macroinvertebrates is considered accurate and advantageous due to their high sensitivity to organic pollutants, widespread distribution, and cost-effective sampling methods. Dense riparian vegetation along the riverbanks likely provides a rich nutrient source for macroinvertebrates (Patang et al., 2018). The dominance of Gastropoda can be attributed to their tolerance to intermediate pollution levels, commonly found in lowland areas and ponds (Nasemann, 2007). During periods of low water levels, reduced macrophyte diversity decreases the physical complexity of aquatic habitats, creating stressful conditions for algae and macroinvertebrates (Choi et al., 2014), potentially leading to lower diversity. The Seti River, with 10 families and 8 orders dominated by Plecoptera, and the Kali River, with 10 families and 5 orders dominated by Diptera, showed the highest diversity, while the Kotre River, with only 3 families and 3 orders, had the lowest diversity among the studied rivers. The Nepidae family was frequently found at various stations along the Mawa and Ratuwa Rivers, consistent with the observations of Upadhyay (2020) in the Setikhola watershed. These results were further supported by Shrestha (2008). According to Dévai (1990), the larvae of Diptera, Hemiptera, and Plecoptera play a crucial role in nutrient cycling in lakes and reservoirs, potentially influencing the rate of eutrophication through their feeding activities. These species are also known for their adaptability and tolerance to a wide range of environmental conditions, explaining their dominance in the study areas.

## Conclusion

The study of macroinvertebrates involved a total of 7 species from Mawa River and 8 species from Ratuwa River. In the case of the Mawa River, a maximum of 4 species were recorded belonging to phylum Arthropoda followed by Mollusca (2 species) and Annelida (1 species). In Ratuwa River, a maximum of 5 species were recorded belonging to

phylum Arthropoda, followed by Mollusca (2species) and Annelida (1species). The multivariate analysis of variance (MANOVA) revealed no significant variation ( $p > 0.05$ ) in the water parameters between the Mawa and Ratuwa Rivers. Macro-invertebrates were affected by activities such as water pollution, sand mining which resulted in the loss of habitat, causing the species to become vulnerable. It is imperative to minimize, monitor, and, if necessary, prohibit activities like bouldering, direct discharge of industrial waste into water bodies, overfishing, and sand mining to safeguard the aquatic flora, fauna, and natural balance of the river. The present study will be a baseline for further study in the field of macroinvertebrates and water parameters in the Rivers Mawa and Ratuwa.

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