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Research Article

Macroinvertebrate assemblages in mountain tributaries of glacial-fed and rain-fed rivers in eastern Nepal

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Abstract

While river macroinvertebrates are the most widely used form of bioindicators, their baseline information, although crucial, is scarce in Nepal. The main objective of this study was to assess the macroinvertebrate assemblages in mountain tributaries of the glacial-fed Tamor and rain-fed Kamala rivers. A total of eight sites were sampled during March 2015 (Spring), November 2015 (Autumn), January 2016 (Winter), and May 2016 (Summer). Altogether, 49 Families of macroinvertebrates belonging to 15 Orders were identified with 39 Families and 12 Orders in Tamor's tributaries, and 33 Families and 10 Orders in Kamala's tributaries. Non-metric multi-dimensional scaling (NMDS) revealed different assemblages between the two river systems. The most dominant Order in the Tamor was Ephemeroptera and it was Trichoptera in the Kamala. EPT (Ephemeroptera Plecoptera Trichoptera) assemblages were the most abundant in all four seasons for both the river systems and higher % EPT in Tamor's tributaries indicate better water quality than in the Kamala's tributaries. Typical cold water adapted Families such as Rhyacophilidae and Stenopsychidae were observed in Tamor's tributaries whereas in Kamala's tributaries, warm water adapted Families like Naididae and Thiaridae were found, reflecting a difference in the abiotic variables such as temperature, dissolved oxygen attributed to each of the catchments. This baseline data can serve as the foundation for further bioassessment including those of climate change impacts on aquatic biodiversity.

***Keywords*:** EPT-taxa, glacial-fed tributaries, macroinvertebrates, rain-fed tributaries

Introduction

Tributaries are important features of any river system serving a multitude of ecosystem services. Tributaries connect different rivers and watersheds thereby forming passageways for nutrient transport, organic and inorganic matter; provide unique habitats to aquatic biota and often act as spawning sites and refuges for these organisms (Rice et al., 2008). Mountain rivers and their tributaries exhibit tremendous variation across spatio-temporal scales attributed to differences in their origin, tectonics, watershed geology and size, landuse, connectivity, hydrology etc. (Wohl, 2010). These differences in turn result in a range of different river physical and chemical parameters and aquatic biota.

Nepal with its distinct physiographic zones has large numbers of rivers and streams and the country's river systems can be broadly classified into three types based on their origin (WECS, 2011) – those arising from the glaciers and snow-fed lakes in Himalaya and Trans-Himalaya; those arising from the Mahabharat ranges (1000 -3000 masl) and those arising from the Chure Hills (200 -1000 masl). Accordingly, the biotic communities are likely to vary in these lotic systems. For instance, the headwaters of glacial-fed and rain-fed rivers exhibit distinctive climatic, geologic and riparian conditions, affecting abiotic parameters like hydrology, temperature, food sources and water chemistry (Espinosa et al., 2020; Jacobsen, 2009; Laursen et al., 2015), which ultimately have an impact on the abundance and diversity of the different biotic assemblages present (Meyer et al., 2007). Rain-fed headwaters are often characterized by forested catchments and biological communities in such forested headwaters primarily comprise heterotrophic organisms because of the lower photosynthesis/respiration ratio attributed to the shading effect (Vannote et al., 1980).

Benthic macroinvertebrates encompassing a rich taxonomic diversity of Arthropods, Annelids, Molluscs, Nematodes and Turbellarians (Hauer & Resh, 2017; Heino, 2005) are important freshwater heterotrophic groups acting as linkages between the producers and higher level consumers (Wallace & Webster, 1996). Their community structures differ along the longitudinal gradient of lotic systems, reflecting the difference in abiotic variables such as temperature (Suren, 1994), flow (LeCraw & Mackereth, 2010), and available food sources (Mantyka-Pringle et al., 2014). In the upper reaches of forested mountain rivers, this group of organisms is often dominated by functional feeding group of shredders and collectors, primarily attributed to the presence of allochthonous coarse particulate organic matter (CPOM) which forms the principal food component (Cummins, 1974).

Macroinvertebrates are frequently used in the bioassessment of water bodies and watersheds because of their ability to reflect a range of environmental changes (Eriksen et al., 2021; Ofenböck et al., 2010). They have been used as bioindicators in a large number of studies to track changes in aquatic systems (Korte et al., 2010, Tamiru et al., 2017), particularly in streams and rivers (Buss et al., 2002; Patang et al., 2018). A number of macroinvertebrate-based indices and scores have been

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developed in many countries for water quality assessment and monitoring programmes (Metcalfe, 1989). Likewise, in Nepal, with the development of macroinvertebrate-based ecological assessment tool -NEPBIOS (Nepalese Biotic Score) (Sharma, 1996), these organisms have been frequently used to assess the impact of a range of stressors (Gurung et al., 2013; Sharma et al., 2005; Sharma et al., 2009). However, macroinvertebrate baseline studies in the country are still scarce and sporadic (Rundle et al., 1993; Suren, 1994), with a majority of these being concentrated in the western and central regions of Nepal (e.g., Gurung et al., 2013; Matangulu et al., 2017; Shah & Shah, 2012; Shah et al., 2020a, 2020b). Studies in the eastern region are scarce (Jha et al., 2015; Poudel et al., 2014) despite the region being a biodiversity hotspot (IUCN, 2016). Therefore, the main objective of this study is to generate baseline information on the macroinvertebrate assemblages of selected tributaries of the Tamor River (glacial-fed) and the Kamala River (rain-fed) of eastern Nepal.

MATERIALS AND METHODS

Study area

This study was conducted in selected tributaries of two major rivers - Tamor and Kamala - in Taplejung, Panchthar and Udaypur, eastern districts of Nepal (Fig. 1). The Tamor River originates from the Kanchenjunga range and is a perennial glacial-fed river (Negi, 1991; Shrestha et al., 2009). The Kamala River originates from the Chure Hills and is a rain/spring-fed river with minimal flow during dry seasons. The Mewa, the Maiwa and the Hewa are the Tamor's tributaries whereas the Tawa is a tributary of the Kamala and the Lalleri is a tributary of the Tawa (Fig. 1; Table 1). The Mewa *Khola* (Khola in Nepali means stream) originates in Sudu Pokhari at an elevation of 3800 masl and it confluences with the Tamor River at Dobhan (Shrestha et al., 2016).

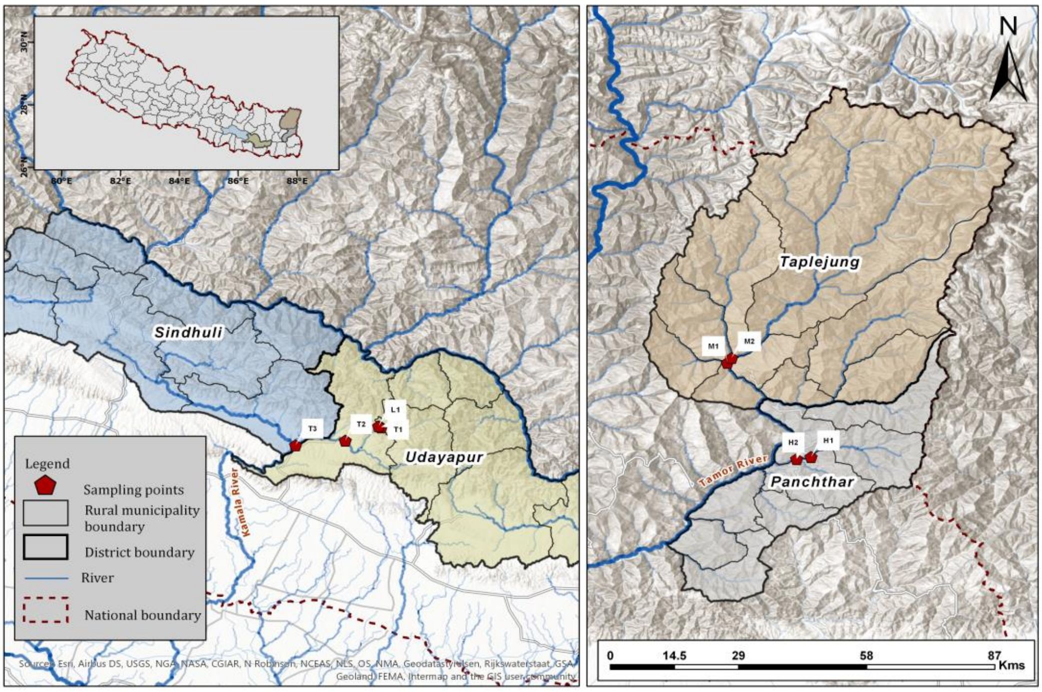

Figure 1 Map of Nepal showing the study area's location (in the inset) and the sampling sites.

Macroinvertebrate collection

A seasonal approach of sampling was adopted encompassing four seasons - March 2015 (Spring), November 2015 (Autumn), January 2016 (Winter), and May 2016 (Summer). Sites were chosen based on accessibility and proximity to the confluence in the case of the Tamor (Jha et al., 2018). Macroinvertebrates were sampled from around a hundred-meter river stretch with a hand net of mesh size 250 µm following Barbour et al. (1999). This method involves the inclusion of different microhabitats such as the riffles, pools, runs, and different substratum types. The collected organisms were preserved in 70 % ethanol in the field itself and samples were transferred to the laboratory for identification to Family level following standard literature (Merritt & Cummins, 1996; Nesemann et al., 2007). Selected water parameters viz. dissolved oxygen (DO), conductivity, temperature and pH were measured on-site using portable multi-parameter probe (LUTRON).

Data analysis

Percentage abundance of the different Orders of macroinvertebrates was calculated. Percentage EPT richness was also calculated for its comparison with the other Orders using the following formula:

% EPT = Number of Families belonging to Orders Ephemeroptera, Plecoptera and Trichoptera /Total number of Families found *100

Table 1 Sampling sites, geographical coordinates, dominant substratum types, surrounding land-use and river stressors. Modified from Jha et al. (2015; 2018).

River type	Site Name	Site Code	District	Geographical Co-ordinates	Elevation (masl)	Major Inorganic substrates	Land-use and river stressor
Glacial-fed (Tamor's tributaries)	Maiwa	M1	Taplejung	N 27°22.064' E 087°37.098'	664	Large rocks and boulders dominant	Electro fishing and washing, tractor crossing
	Mewa	M2	Taplejung	N 27°22.675' E 087°37.617'	666	Large rocks and boulders dominant	Electro fishing and occasional washing
	Hewa	H1	Panchthar	N 27°10.061' E 087°47.321'	629	Boulders and cobbles dominant	Upstream of the bridge of the Hewa Khola; hydropower construction
	Hewa	H2	Panchthar	N 27°09.802' E 087°45.560'	550	Boulders and cobbles dominant	Agriculture, cremation site, washing
Rain-fed (Kamala's tributaries)	Tawa	T1	Udaypur	N 26°59.211' E 086° 7.743'	330	Mostly cobbles	Agriculture
	Lalleri	L1	Udaypur	N 26°59.347' E 086°27.430'	327	Small stream, boulders and cobbles	Agriculture, road crossing
	Tawa	T2	Udaypur	N 26°57.512' E 086°23.361'	258	Pebbles	Agriculture
	Tawa	T3	Udaypur	N 26°56.925' E 086°17.291'	167	Pebbles	Agriculture, road crossing

Shannon-Wiener diversity index (up to Family level) was estimated following Magurran (2004). Non-Metric Multidimensional Scaling (NMDS) was performed using R-programming to assess site-wise similarity of macroinvertebrate assemblages. ANOSIM (Analysis of similarity) was performed to assess significant variation in assemblages between the Tamor and the Kamala systems. Spearman rank correlation coefficient was used to assess the correlation between % EPT, Shannon-Wiener diversity index (Family-based) and physico-chemical variables. T-test was conducted to assess significant differences in % EPT, Shannon-Wiener diversity index, and Family richness between river systems.

Results and Discussion

Macroinvertebrate assemblages

A total of 49 Families belonging to 15 Orders and 5 Classes of macroinvertebrates were identified from the two selected river systems (Fig. 2; Table 2) indicating a rich macroinvertebrate fauna.

Studies conducted by Füreder et al. (1998) and Shah et al. (2020b) found that the spring-fed systems had a greater number of macroinvertebrate Families. However, our study found more in the glacial-fed system. Pokharel (2013) found similar results where the glacial-fed system had a greater taxonomic richness due to less urban influence in such systems. This may be the case in our study as well. Although a number of Families were common to both rivers, some Families were observed exclusively in either the glacial-fed Tamor system or the rain-fed Kamala system. NMDS revealed two distinct assemblages for the glacial-fed and rain-fed tributaries (Fig. 3). ANOSIM revealed significant variation in assemblages between the Tamor and the Kamala systems ($R = 0.79$; $p < 0.05$).

Families Nemouridae, Rhyacophilidae and Stenopsychidae were found exclusively in the tributaries of the glacial-fed Tamor system. Nemouridae and Rhyacophilidae are typical of snow-fed and glacial-fed lotic systems and are well adapted to cold water mountain streams (Hilsenhoff, 2001; Hotaling et al., 2020; Saito et al., 2018) and have previously been reported from cold fast-flowing rivers and streams from Nepal as well (Sharma, 1996). In contrast, Naididae, Nepidae and Thiaridae were among the Families found exclusively in the tributaries of the Kamala. The presence of these taxa has been reported in warm lowland waters in Nepal by Nesemann (2006) and Khatri et al. (unpublished data) as well. These taxa are known to be pollution tolerant (Dodds & Whiles, 2010; Silva et al., 2010) and their presence points to human disturbances such as agricultural runoff, fish poisoning, cremation, washing and cattle farming (Thapa, 2015). A study by Akindele and Liadi (2014) have reported correlation of these taxa with nitrate in a tropical Nigerian stream as well. Agriculture was the most dominant landuse in the sampling sites at Tawa and Lalleri. These reasons coupled with lesser dissolved oxygen probably explain the presence of these organic pollution tolerant taxa in the Kamala system.

Table 2 Macroinvertebrate taxa observed in sampling sites

Macroinvertebrate taxa		Sites							
Order/Taxa	Family	M1	M2	H1	H2	T1	L1	T2	T3
Plecoptera	Perlidae	++	++	++	++	++	++	++	++
	Nemouridae	--	++	--	--	--	--	--	--
Ephemeroptera	Baetidae	++	++	++	++	++	++	++	++
	Caenidae	++	--	++	++	--	--	++	++
	Ephemerellidae	++	++	++	++	++	++	++	++
	Ephemeridae	++	++	--	--	++	--	--	--
	Heptageniidae	++	++	++	++	++	++	--	++
	Leptophlebiidae	--	--	--	++	++	++	++	++
Trichoptera	Hydropsychidae	++	++	++	++	++	++	++	++
	Glossosomatidae	++	++	++	--	--	--	--	--
	Philopotamidae	++	++	--	--	++	++	--	--
	Stenopsychidae	++	--	++	--	--	--	--	--
	Brachycentridae	--	++	--	--	--	--	--	--
	Limnocentropodidae	--	++	--	--	--	--	--	--
	Psychomyiidae	--	++	--	--	--	--	--	--
	Rhyacophilidae	--	++	--	--	--	--	--	--
	Uenoidae	--	++	++	--	--	--	--	--
	Trichoptera in. det.	--	++	++	--	--	--	--	--
Diptera	Blephariceridae	++	--	++	++	--	--	--	--
	Simuliidae	++	++	++	++	++	++	++	--
	Tabanidae	++	--	--	--	++	++	++	++
	Limoniidae	--	++	++	++	++	++	++	++
	Tipulidae	--	++	++	--	--	--	--	--
	Chironomidae	--	++	++	++	++	++	++	++
	Ceratopogonidae	--	--	--	--	--	--	++	--
	Dolichopodidae	--	--	--	++	++	++	--	--
	Diptera in. det.	--	--	++	--	--	--	--	--
Odonata	Gomphidae	++	++	++	++	++	++	++	++
	Euphaeidae	--	++	++	--	--	--	--	--
	Coenagrionidae	--	--	++	--	--	--	--	--
	Corduliidae	--	--	--	--	++	--	--	--
Hemiptera	Aphelocheiridae	++	--	++	--	--	--	--	++
	Naucoridae	--	++	--	--	--	--	--	--
	Gerridae	--	--	++	++	++	++	--	--
	Nepidae	--	--	--	--	++	++	--	++
	Micronectidae	--	--	--	--	--	--	++	--
Coleoptera	Gyrinidae	--	++	++	++	++	--	--	++
	Elmidae	--	++	++	++	--	--	++	++
	Psephenidae	--	--	--	++	++	--	++	--
Megaloptera	Corydalidae	--	++	--	++	++	++	++	--
Decapoda[1]	Potamidae	--	--	++	++	++	++	--	--
Crustacea[1#]	Palaemonidae	--	--	--	--	++	++	++	++
Oligochaeta[1†]	Hirudinidae	++	--	++	--	--	--	--	--
	Oligochaeta in. det.	--	--	++	--	--	--	--	--
	Naididae	--	--	++	--	--	--	++	--
Mollusca[1*]	Planorbidae	--	--	--	--	--	--	--	++
	Lymnaeidae	--	--	--	--	++		++	--
	Thiaridae	--	--	--	--	++	++	--	--
	Viviparidae	--	--	--	--	--	--	--	--

Note:1 means non-insect fauna; # means Class; † means Subclass; * means Phylum; ++ means present; -- means absent

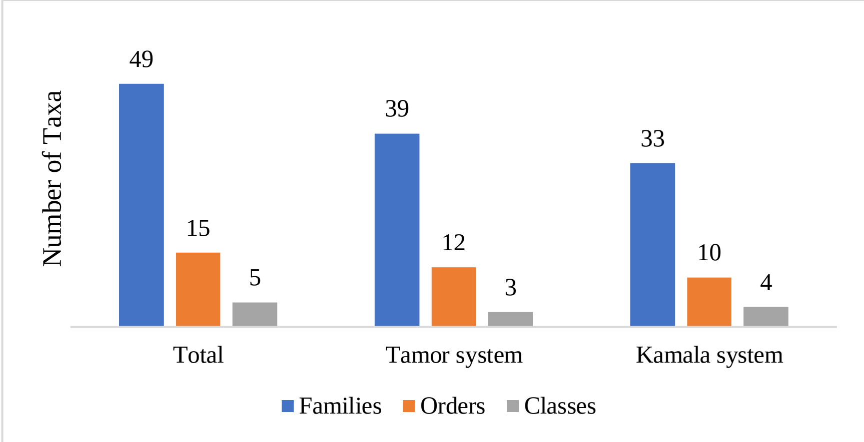

Figure 2 Total number of Families, Orders and Classes observed in the Tamor and Kamala systems.

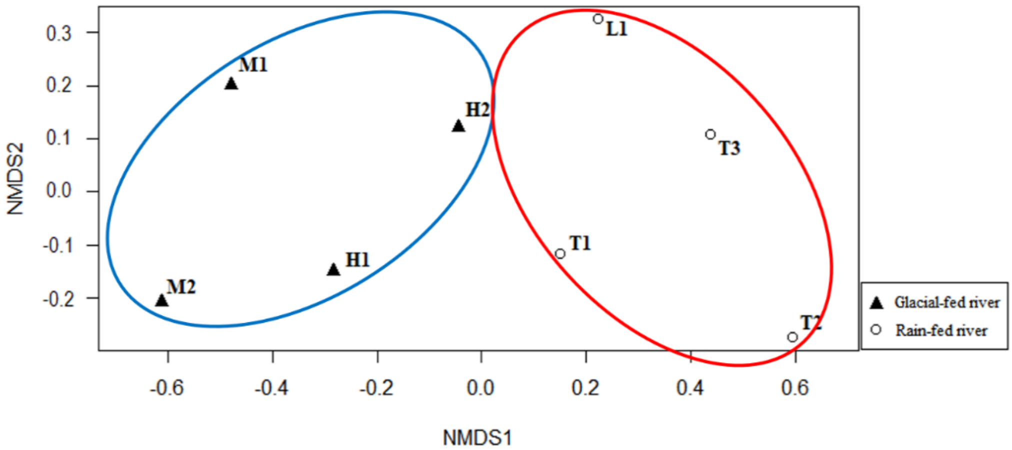

Figure 3 NMDS graph showing glacial-fed and rain-fed sites with two clusters

Figures 4a and 4b show the percentage composition of different macroinvertebrate Orders observed in the tributaries of the Tamor and the Kamala. The most dominant Order in Tamor's tributaries was Ephemeroptera, followed by Trichoptera and Diptera. In rain-fed systems, the Order Trichoptera was the most dominant, followed by Ephemeroptera, Diptera, and Odonata. The abundance of Plecoptera - a cold water taxon (Bouchard, 2004; Cui et al., 2019), was higher in the glacial-fed river. Similar findings were observed in the Patagonian Andean glacial streams and the Austrian Central Alps (Füreder et al., 1998; Martyniuk et al., 2019). The most dominant Family in the Tamor was Baetidae. This taxon is quite common in streams and the abundance of this Family has been reported in other glacial streams (Milner et al., 2001; Mishra et al., 2013) as well as in nutrient-rich streams (Harrington & Born, 2000). The Trichoptera Family Hydropsychidae is a cosmopolitan taxon commonly found in a range of lotic systems (Oliveira & Froehlich, 1996). This taxon represents one of the major insect groups in Southeast Asia (Uy et al., 2018) and has been reported from a large number of lotic systems across Nepal (Ormerod et al., 1994; Pokharel, 2013). This taxon was the most dominant Family in Kamala's tributaries. Jha et al. (2015) also reported abundant Hydropsychidae in the Kamala systems. This taxon is also referred to as net spinners and are filter feeders trapping fine particulate organic matter from running waters (Dudgeon, 1999). Their abundance in lowland Tawa is an indication of presence of suspended sediment and fine particulate organic matter in water.

The percentage of EPT (Ephemeroptera, Plecoptera and Trichoptera) was higher in Tamor's tributaries (Fig. 5). T-test further revealed that the observed sample means of %EPT differed significantly for the Tamor and Kamala river ($t(6) = -5.9$, $p = 0.001$).

Rundle et al. (1993), Ormerod et al. (1994), and Suren (1994) found EPT to be the abundant Orders in their studies from different lotic systems in Nepal. In this study also, EPT abundance was found to be greater than those of other Orders in both rivers in all the seasons (Figs. 6a and 6b). The overall EPT abundance in the glacial-fed river was 79%, whereas for the rain-fed river, it was 61%.

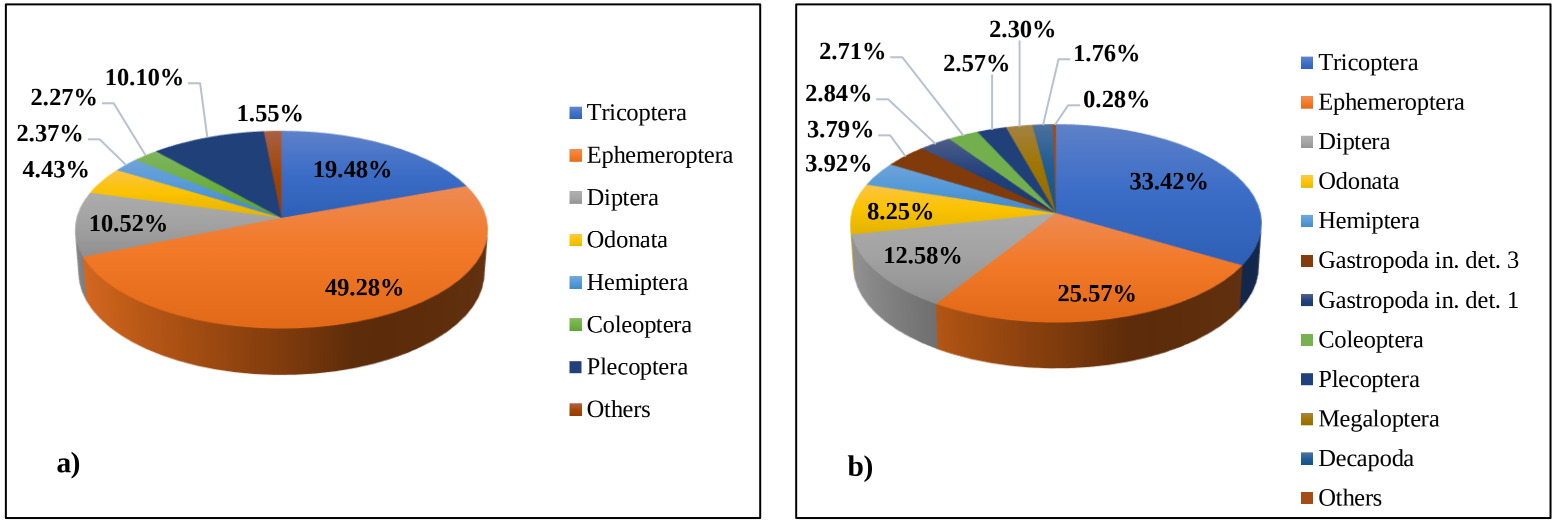

Figure 4 Percentage abundance of different macroinvertebrate Orders in (a) Tamor system and (b) Kamala system

Most of the EPT taxa are considered to be pollution sensitive and the % EPT is regarded as a simple and crude indicator of environmental water quality (Feld et al., 2010). Higher % EPT values in all seasons in sites M1, M2, H1 and H2 indicates better water quality in Tamor's tributaries. This finding is supported by the fact that Tamor's tributaries have higher levels of dissolved oxygen (Jha et al., 2018), fast-flowing waters, and less organic pollution. In contrast, although the overall proportion of % EPT was higher in all of the Kamala's tributaries, contributions from other Orders were also significant, most possibly reflecting agricultural runoff in the area.

Macroinvertebrate assemblages and seasonal variation

Figures 6a and 6b show the percentage abundance of EPT and other taxa in different seasons in the tributaries of the Tamor and Kamala respectively. The EPT dominated in all the seasons in both the systems. In the Tamor's tributaries, EPT was dominant throughout the seasons, and it was highest during the autumn. The highest EPT abundance in Kamala's tributaries was observed during the winter, but other taxa also showed significant contribution during other seasons.

Seasonal variation in macroinvertebrate assemblages have been reported by several authors (Brewin et al., 2000; Mesa, 2012). Seasons tend to affect a range of environmental variables, such as water temperature, discharge and habitat changes which in turn influence community characteristics like food availability (Mesa, 2012; Miserendino & Pizzolon, 2003). For instance, it has been shown that higher discharge during the wet season tend to dilute pollution and improve the quality of macroinvertebrate assemblages (Jacobsen, 1998; Jacobsen & Encalada 1998). Furthermore, seasons also affect the emergence time of macroinvertebrates (Baxter et al., 2017; Milner & Petts, 1994). Sweeney and Vannote (1982) and Malison and Baxter (2010) found that emergence was greatest during the summer season.

Macroinvertebrate assemblages and physico-chemical parameters

Table 3 shows the mean values of selected physico-chemical parameters and Tables 4a and 4b show the Spearman rank correlation of macroinvertebrate assemblages with physico-chemical parameters. All the sites were characterized by circum-neutral to slightly alkaline pH. As expected, rain-fed Kamala's tributaries had higher temperature than those of glacial-fed Tamor's tributaries. Conductivity was also higher in Kamala's tributaries (Table 3).

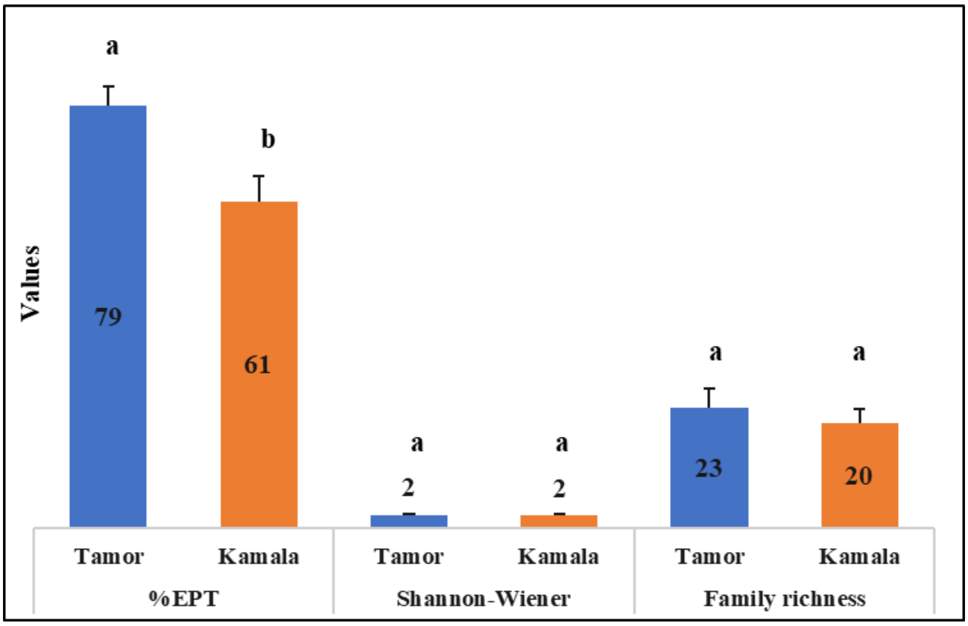

Figure 5 %EPT, Shannon-Wiener, Family richness difference between the headwater tributaries of the glacial–fed Tamor system and the rain-fed Kamala system. [Error bars with different letters denote significant difference (T-test; $p < 0.05$)].

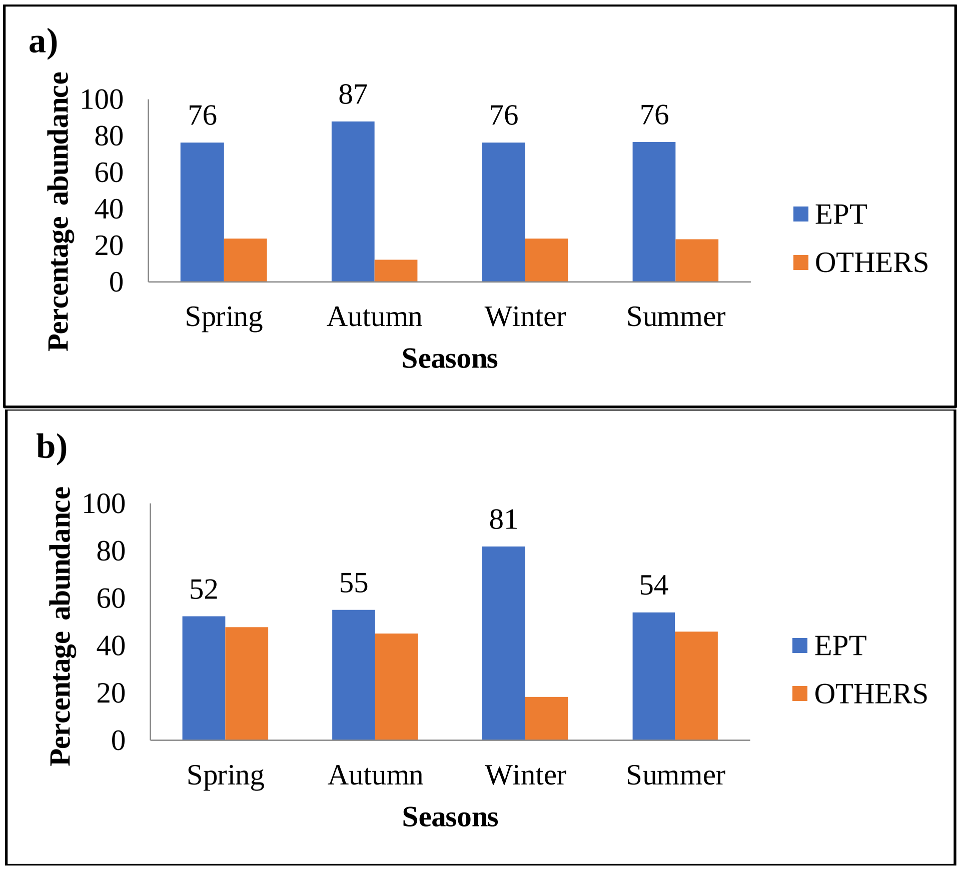

Figure 6 Seasonal comparison of EPT and other Orders (a) Tamor system (b) Kamala system

Table 3 Physico-chemical parameters of the sampling sites

River type	Site	Parameters			
		pH	Temperature (°C)	Dissolved oxygen (mgL^{-1})	Conductivity (μScm^{-1})
Glacial-fed (Tamor's tributaries)	M1	7.07±0.46	16.41±3.07	6.62±1.90	57.98±7.40
	M2	7.47±0.38	14.24±2.66	7.38±2.46	48.10±6.48
	H1	7.23±0.16	17.28±5.50	6.29±1.65	50.26±11.22
	H2	7.51±0.26	16.73±3.49	6.30±2.49	62.63±6.05
	Average	**7.32±0.37**	**16.17±3.89**	**6.65±2.11**	**54.74±9.78**
Rain-fed (Kamala's tributaries)	T1	7.99±0.53	23.89±3.72	6.03±1.70	241.15±58.14
	L1	8.12±0.63	24.95±4.94	5.45±2.57	283.52±26.24
	T2	8.22±0.54	25.18±6.20	5.41±2.04	286.50±43.03
	T3	7.62±0.92	24.02±6.86	5.46±3.36	354.18±29.73
	Average	**7.99±0.69**	**24.51±5.41**	**5.60±2.40**	**293.83±60.10**

Source: Jha et al. (2018)

Table 4a Spearman rank's correlation coefficient for physico-chemical parameters and macroinvertebrate assemblages attributes in Tamor's tributaries

	pH	**Temperature**	**DO**	**Conductivity**	**Taxa Richness**	**% EPT**	**Shannon Diversity (H')**
pH	1						
Temperature	0.325	1					
DO	.662*	0.294	1				
Conductivity	0.364	0.465	.643*	1			
Taxa Richness	0.188	-.507*	0.014	-0.142	1		
% EPT	-0.036	-.651**	0.257	-0.085	.762**	1	
Shannon Diversity (H')	0.050	-0.302	0.021	-0.129	.870**	.647**	1

Table 4b Spearman rank's correlation coefficient for physico-chemical parameters and macroinvertebrate assemblages attributes in Kamala's tributaries

	pH	**Temperature**	**DO**	**Conductivity**	**Taxa Richness**	**% EPT**	**Shannon Diversity (H')**
pH	1						
Temperature	-0.168	1					
DO	-0.455	0.294	1				
Conductivity	-0.385	0.394	0.203	1			
Taxa Richness	-0.129	-0.138	-0.169	-0.067	1		
% EPT taxa	-0.101	-0.096	-0.431	-0.099	.734**	1	
Shannon Diversity (H')	-0.253	0.371	0.287	0.335	.650**	0.203	1

In both the river systems, % EPT and Shannon Wiener diversity showed positive correlation ($p < 0.01$) with taxa richness. In Tamor's tributaries, taxa richness and % EPT showed significant negative correlation ($p < 0.01$) whereas in Kamala's tributaries although these parameters showed negative correlation, significant variations were not observed. Temperature and oxygen are crucial parameters in shaping up macroinvertebrate communities in many aquatic systems (Fumetti et al., 2017; Jacobsen, 2008) along with substrate types, hydrological regimes (Beauger et al., 2006). Being ectotherms, for most macroinvertebrate assemblages, an increase in temperature may lead to their decline (Durance & Ormerod, 2007; Woodward et al., 2010). Tamiru et al. (2017) found that low dissolved oxygen levels caused disease and slow growth rates, resulting in a drop in the percentage of EPT. Studies have found that macroinvertebrate abundance and richness decreased with a decrease in pH (Baldigo et al., 2009; Duggan et al., 2007; Gaskill, 2014) particularly the Ephemeroptera (Courtney & Clements, 1998).

Conclusion

This study was conducted to generate baseline information on macroinvertebrate assemblages of the glacial-fed Tamor's and rain-fed Kamala's tributaries in eastern Nepal. A total of 49 macroinvertebrate Families were observed indicating rich macroinvertebrate diversity. The macroinvertebrate assemblages differed in the two systems. The most dominant Order was Ephemeroptera and Trichoptera in the Tamor's tributaries and Kamala's tributaries respectively. Certain taxa like Rhyacophilidae and Stenopsychidae were observed exclusively in glacial-fed Tamor's tributaries whereas warm water taxa were characteristics of rain-fed systems, clearly reflecting a difference in the abiotic variables attributed to glacial-fed and rain-fed catchments. The presence of taxa like Naididae and Thiaridae were observed in Kamala's tributaries and indicate organic pollution in the rivers. This baseline data can serve as the foundation for further bioassessment including climate change impacts on aquatic biodiversity.

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Author Contributions: SG: Conceptualization, sampling, analysis, manuscript preparation; RS and BW: manuscript preparation and statistical analyses; BRJ: Logistics and sampling; KK: Sampling; DJ: MS revision.

Conflict of Interest: The authors declare no conflict of interest.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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