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# Diversity of benthic macroinvertebrates in White-bellied Heron landscape Punatsangchhu and Mangdechhu River, Bhutan

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## Abstract
Freshwater biodiversity contributes diverse ecosystem services. However, it is also the most vulnerable to degradation and extinction due to anthropogenic activities and climate change. There is limited information on benthic macroinvertebrate diversity in major rivers of Bhutan although it is an integral component of freshwater ecosystems. We assessed two major rivers of Bhutan Punatsangchhu and Mangdechhu to establish baseline information on benthic macroinvertebrates. The samples were collected from the littoral zone of the river using a modified D-frame net. The study recorded 3375 individuals belonging to 10 orders and 39 families. The dominant order was Ephemeroptera followed by Trichoptera. There was no significant difference of diversity indices between post and pre-monsoon season ($p>0.05$) but significant difference of abundance ($P<0.05$). The physico-chemical parameters of water are within the permissible limit which is favorable for macroinvertebrates to thrive with no major indication of water pollution.

**Keywords:** Punatsangchhu, Mangdechhu, Bhutan, benthic macroinvertebrates, richness, abundance

## Introduction
The rapid economic growth and hydropower developments are major threats to the freshwater ecosystem. Freshwater systems occupy less than 1% of the earth's surface, yet it is known to harbor 5% of all known biological species of the world [16, 20]. Pristine water bodies usually harbor a great variety of aquatic life, representing a natural state of freshwater ecosystem [34]. Freshwater biodiversity contributes diverse ecosystem services. However, it is most affected ecosystems [30] and the degradation of freshwater ecosystems is attributed to anthropogenic activities which is further exacerbated by climate change [14, 40, 43].

Benthic macroinvertebrates are one of the important components of freshwater biodiversity in head water streams and river ecosystems [10]. They play a significant role in the circulation and recirculation of nutrients in aquatic ecosystems and expedite the decomposition of organic matter into simpler inorganic forms [17, 1, 8, 7]. Most macroinvertebrates feed on debris that settles on bottom of the river and in turn serve a key food source for other freshwater vertebrates such as amphibians and fishes [24]. Which is the main diet for critically endangered, White-bellied Heron [32]. In Addition, benthic macroinvertebrates also served as main food source for shorebirds [36].

In Bhutan, the documentation of freshwater biodiversity is still in its rudimentary state [14, 21, 22]. The first documentation was initiated by the National Environment Commission in collaboration with Hindu Kush Himalayan experts in 2004 [39]. With lack of experts within the country, the identifications of macroinvertebrates were done only up to order or family level and hardly to genus and species level. Recently several studies in Wangchuck Centennial Park [38], Bumthang [41], Trongsa and Thimphu [13], tributary of Punatsangchhu river Toeberongchhu [22], Mangdechhu river [40] and Phobjikha valley [39] has reported the taxa but no comprehensive study done in the major rivers. Therefore, this study focuses on documentation of benthic macroinvertebrates and physico-chemical properties of water in non-wadeable river Punatsangchhu and Mangdechhu. Which is the prime habitat for critically endangered, White-bellied Heron (WBH) in Bhutan [33].

## Materials and Methods

### Study area

The study was conducted in two major rivers: Punatsangchhu and Mangdechhu (Figure 1). Punatsangchhu flows across western region through six districts: Gasa, Punakha, Wangduephodrang, Tsirang and Dagana. Phochhu and Mochhu are the two major tributaries. The elevation ranges from 250-1500 m with dominant vegetation of warm broadleaved forest at lower elevation of 250-500 m, mixed vegetation of chir pine trees and broadleaved trees at elevation between 500-1000 m and dry chir pine forest between 1000m-1500 m. Mangdechhu flows through the central region of Bhutan through three districts; Wangduephodrang, Trongsa and Zhemgang. The elevation of ranges from 250 to 1000 m with dominant vegetation of warm broadleaved forest at lower elevation of 250-500 m and mixed vegetation of both chirpine and broadleaved trees at high elevation of 500-1000m.

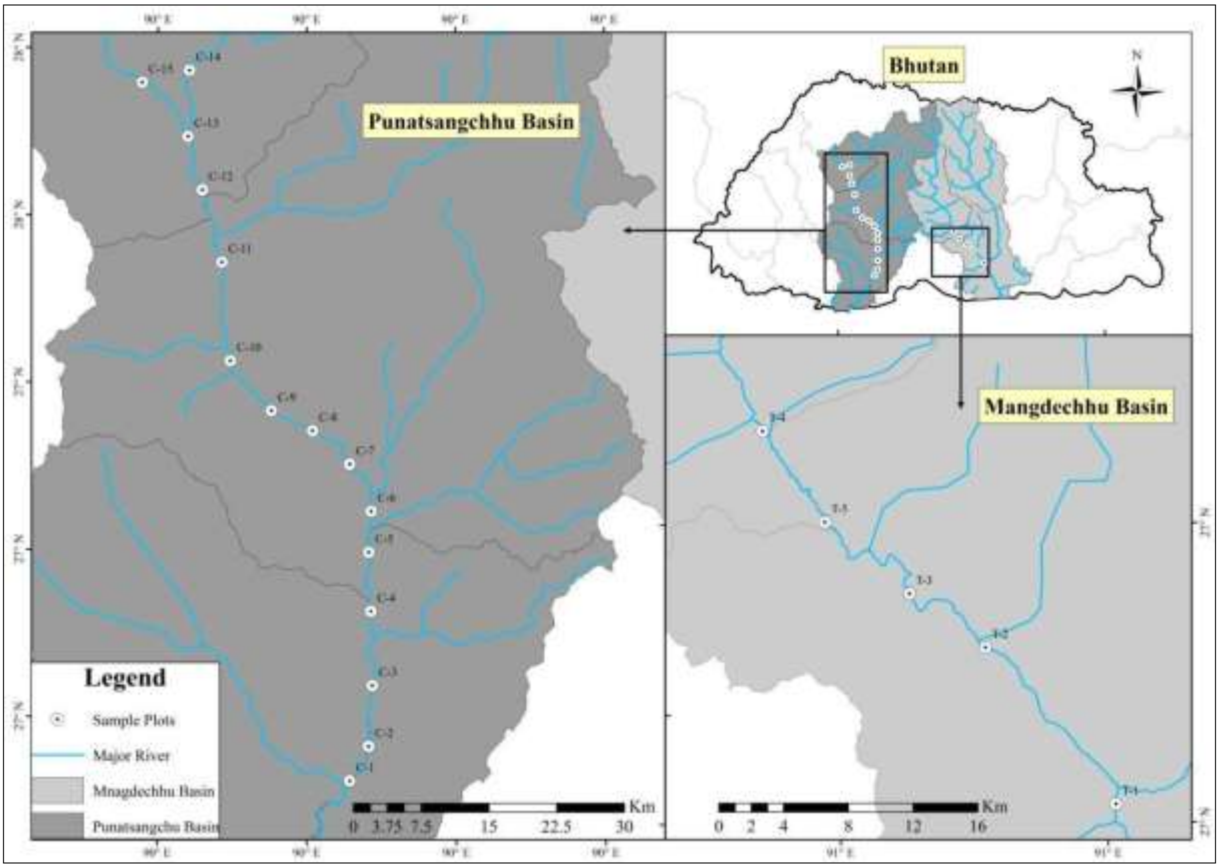


**Fig 1:** Study area with Macroinvertebrates Sample Plots

### Sampling Design

A systematic random sampling was done in Arcgis 10.8 to generate the sample plot's location along the river with a minimum plot distance of 6.5km. A total of 20 sample plots were generated in Arcgis that is 15 plots in Punatsangchhu and 5 plots in Mangdechhu with the plot size 3*6 m. The inaccessible plots were shifted a maximum of 500m either upstream or downstream from the initial location and two rounds of survey was done; post-monsoon (September and October, 2021) and pre-monsoon (April and May, 2022)

### Field method and Specimen identification

The sampling of benthic macroinvertebrates in non-wadeable rivers is usually restricted by depth and strong current of the river and only littoral zones with sufficient current and depth can be sampled [9]. In this study the sample plots were laid at littoral zone with sufficient current and maximum depth of 115 cm to sample the macroinvertebrates and measure Physico-chemical properties of the river. The sample collections were done using a modified D-frame kick net with a 250 µm mesh size. The D-frame net was placed against the river current, and substrates were disturbed for 3-4 min to dislodge the macroinvertebrates to wash away by running water into the net.

The macroinvertebrates were carefully picked from the net with the help of forceps and put it in the white color tray filled with water before segregating into different taxa. The specimens were euthanized using ethyl alcohol and were preserved in 70% alcohols for identification. The repeated samples were released back to the river after taxa counting and recording had finished. The physicochemical properties (Salinity, Temperature, Total dissolved solute (TDS), pH, Electrical Conductivity) of water were measured using multi-parameter meters (HANNA HI98194) at each sampling site. The river subtract type was also recorded and it was classified into five classes based on size (Sand<5 mm, pebbles 5mm-5cm, small pebbles 5-50 cm, large stone 50-100cm, Boulders<100 cm).

The specimens collected were assessed using Trinocular stereo microscope (Amscope SM-1TS/BS) and specimens were identified to family level using multiple existing keys from Hindu Kush Himalayan region and other regions that is Aquatic Invertebrate Families of Mongolia[3], Key to the larval stages of common Odonata of Hindu Kush Himalaya, with short notes on habitats and ecology [28], Family-level keys to freshwater fly (Diptera) larvae [15], Aquatic Invertebrates of Alberta and North America[11].

**Data analysis**

The data collected were analyzed following Magurran (2004) [26] to calculate family diversity indices and composition of benthic macroinvertebrate in the study area. Data cleaning was done in Microsoft Excel 2010 and the family diversity indices; Shannon-Weaver index (*H*) [35] and Pielou's index (*J*) [31] was calculated in R using the "Vegan" package[44]. Both descriptive and inferential statistical analysis was also done in R-software and family dominance analysis was done in PC-ORD 5. The Shapiro-Wilk test was performed to check the data normality. Paired-sample t-test and Wilcoxon sign rank test was performed to compare the diversity indices, abundance and physico-chemical parameters of post and pre-monsoon season. Spearman's correlation was performed to measure the relationship between diversity indices and abundance with physico-chemical parameters and environmental variables.

## Result and Discussion

**Benthic macroinvertebrates Diversity in WBH Landscape**

The study recorded a total of 3375 individuals of benthic macroinvertebrates that belong to 39 families and 10 orders. Of 40 sampling units, 20 represent post-monsoon of 2021 and 20 represent pre-monsoon of 2022 from Punatsangchhu and Mangdechhu river. Post monsoon recorded 2048 individuals that belong to 33 families and nine orders while pre-monsoon recorded 1327 individuals that belong to 32 families and 10 orders. The highest richness in family level was Trichoptera (*S* =10) that comprises 25.6% of total families recorded followed by Diptera (*S* = 8) with 20.5% (Figure 2.a). The most dominant family was Baetidae (*n* = 1050) followed by Heptageniidae (*n* = 928) and Perilidae (*n*= 171) (Figure 2.b & Figure 3). The least dominant family was Gyrinidae, Hydroptilidae, Chrysomelidae, Leptophlebiidae, Athericidae and Chironomidae (*n* = 1) (Figure 2.b).

Similarly, a study done in Nepal [25] has also reported Baetidae as abundant taxa in the Bheri river systems during autumn (post-monsoon) and spring (Pre-monsoon) season. The dominance of Baetidae species might be due to the presence of stony substrate (Figure 4) as most Baetidae species inhibited stony substrate [4]. Moreover, Baetidae were common taxa known to occur in almost all freshwater habitats including fast flowing riffle, pools, runs and wetlands [12].

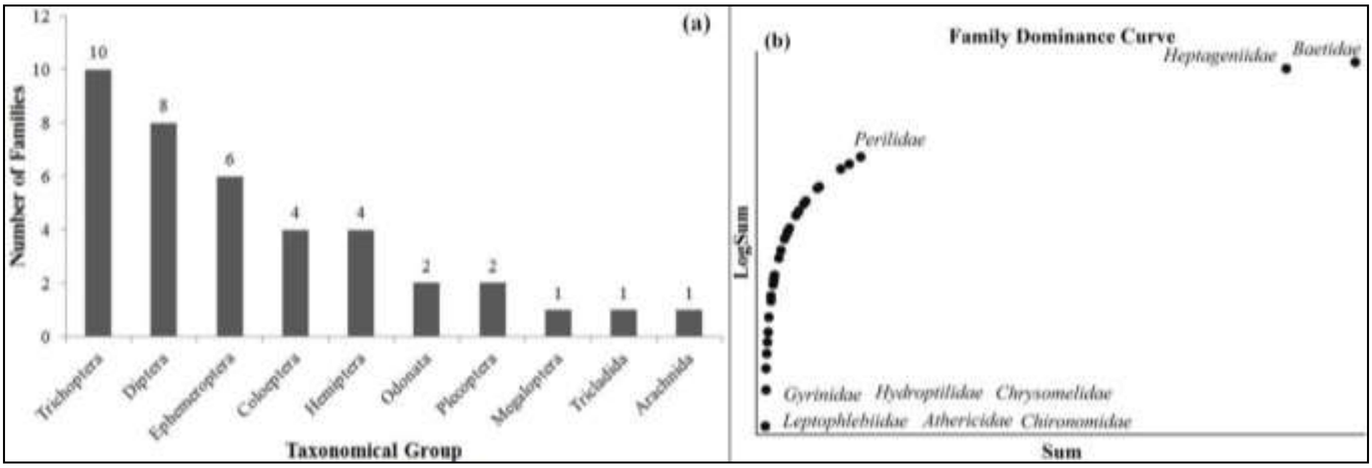


**Fig 2:** a) Overall Family richness under different order, b) Overall Family dominance


**Fig 3:** Most abundant species during sampling a) Baetidae b) Heptageniidae c) Perilidae

The overall mean diversity index (*H*) of benthic macroinvertebrates in the study area was 1.56 (±0.32 SD) and evenness index (*J*) of 0.73 (±0.11 SD). These diversity indices show that the freshwater system in WBH landscape harbors a high number of benthic macroinvertebrates. Family based Shannon-diversity (H) value > 1 indicates poor water quality, *H* value of 1 or < 1> 3 indicates moderate quality and *H* > 3 indicates good water quality [42]. Most of the diversity value in the study area was 1 or < 1> 3 indicating moderate water quality. The results are further limited as the taxa were identified till family level and the diversity would have substantially increased if the taxa were identified till genus and species level.

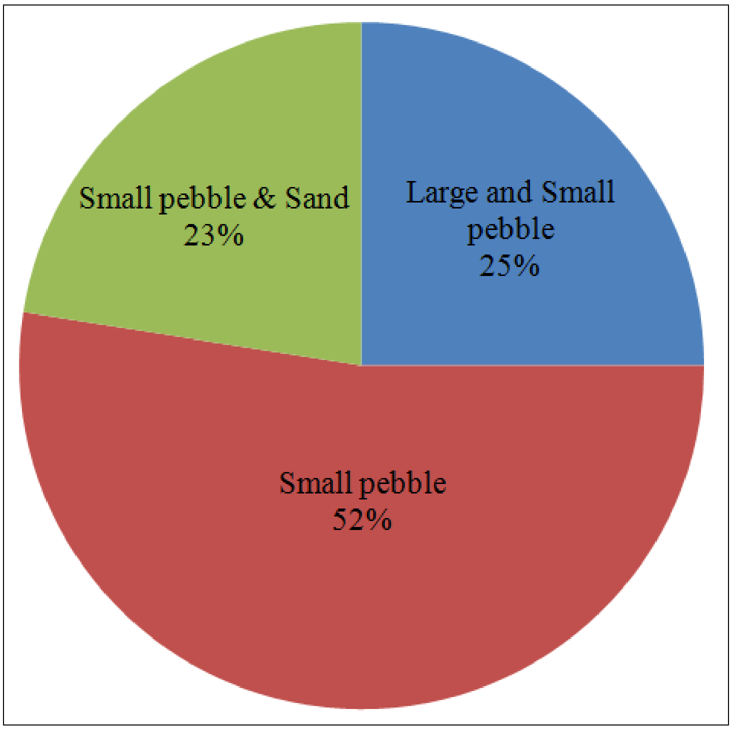


**Fig 4:** Substrate composition

**Composition of benthic macroinvertebrates assemblage in WBH Landscape**

In WBH landscape, overall composition of benthic macroinvertebrates was dominated by order Ephemeroptera (63.7% *n* = 2151) followed by Trichoptera (16.9% *n* = 572). For both post and Pre-monsoon season, the order Ephemeroptera was recorded as the dominant order (Pose-monsoon- 61.13%, *n* = 1252 and Pre-monsoon- 67.75%, *n* = 899) (Table 1). A similar finding was reported by Gurng and Dorji [22] and it might be due to the wide range of habitat preferred by Ephemeroptera [37]. Three individuals belonging to order Arachnida (Table 1) were recorded only during Pre-monsoon. Hamid and Rawi (2014) [23] reported that Ephemeroptera and Trichoptera are very sensitive to environmental perturbation and are found to inhibit a well-oxygenated and clean environment. Moreover, Ephemeroptera are called a bio-indicator of good water quality [2] and their presence as a dominant order in the study area indicates that there is no major pollution in the river.

**Table 1:** Order composition in WBH landscape

| Order | Count (*n*) | % Composition post-monsoon | Count (*n*) | % Composition pre- monsoon |
|---|---|---|---|---|
| Coleoptera | 48 | 2.34 | 13 | 0.98 |
| Diptera | 97 | 4.74 | 40 | 3.01 |
| Ephemeroptera | 1252 | 61.13 | 899 | 67.75 |
| Hemiptera | 137 | 6.69 | 76 | 5.73 |
| Megaloptera | 9 | 0.44 | 2 | 0.15 |
| Odonata | 27 | 1.32 | 9 | 0.68 |
| Plecoptera | 114 | 5.57 | 65 | 4.90 |
| Trichoptera | 355 | 17.33 | 217 | 16.35 |
| Tricladida | 9 | 0.44 | 3 | 0.23 |
| Arachnida | 0 | 0 | 3 | 0.23 |

**Diversity indices and abundance between post and pre-monsoon season**

The overall mean diversity index (*H*) of benthic macroinvertebrates for post and pre-monsoon season in the study area was 1.56 (±0.31 SD) and 1.51 (±0.35 SD) respectively (Table 2 & 3). The evenness index (*J*) was 0.75 (±0.11 SD) and 0.71 (±0.35 SD) for post and pre-monsoon respectively (Table 2 & 3). A paired sample t-test was used to determine the differences between the diversity indices of post and pre-monsoon season. The test revealed no significant difference in the diversity index of post-monsoon ($m$=1.56, $SD$=0.31) and pre-monsoon ($m$ =1.51, SD =0.35) with test value $t$ (19) = 0.01, $p$>0.05 and a similar finding was reported in studies done in Threlpang and Kawajangsa freshwater stream [13] and Phobjikha valley [39]. Macroinvertebrate assemblages mainly depend on water quality [27]. No significant difference in diversity index between post and pre-monsoon season might be due to minimal change in water quality in post and pre-monsoon season [27]. Baetidae, Heptageniidae and Aphelocheiridae dominated the post-monsoon season (Table 4) and Heptageniidae, Baetidae and Perlidae dominated the pre-monsoon season (Table 5).

Similarly, there was no significant difference in the evenness index of post-monsoon ($m$= 0.75, $SD$=0.11) and pre-monsoon ($m$ =0.71, $SD$ =0.35) with test value $t$ (19) = 1.034, $p$>0.05. The abundance showed significant difference between post-monsoon monsoon ($m$ = 102.4, $SD$ = 29.9) and pre-monsoon ($m$ = 66.4, $SD$ = 28.3), $t$ (19) = 4.47, $P$< 0.05 (Figure 5**)** and the past study conducted in Phobjikha valley also reported similar result with high density of benthic macroinvertebrates during post-monsoon than pre-monsoon season.

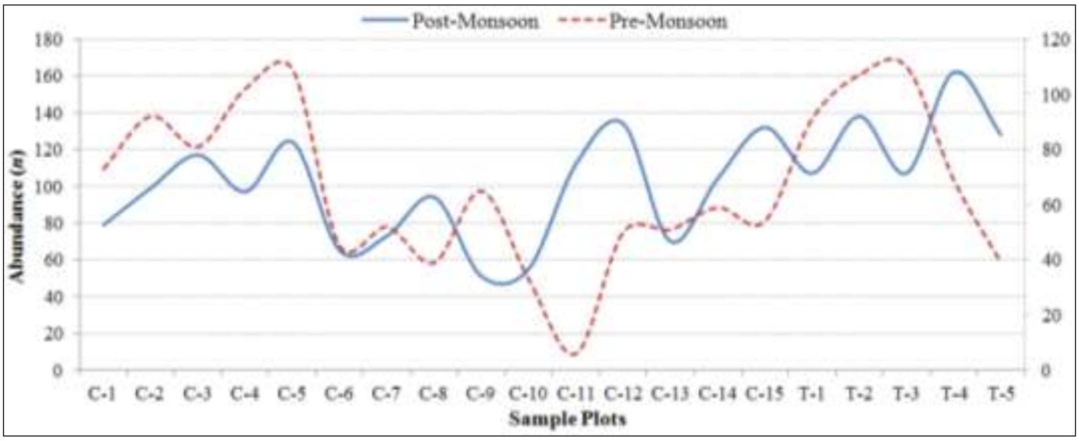


**Fig 5:** Benthic macroinvertebrates abundance between post and pre-monsoon season

**Table 2:** Diversity indices and Taxa count for post-monsoon season.

| Plot ID | Order | Family | Count (*n*) | Diversity Index | Evenness Index |
|---|---|---|---|---|---|
| C-1 | 6 | 7 | 79 | 1.20 | 0.62 |
| C-2 | 3 | 7 | 99 | 1.47 | 0.75 |
| C-3 | 7 | 11 | 117 | 1.62 | 0.68 |
| C-4 | 4 | 7 | 97 | 1.70 | 0.87 |
| C-5 | 5 | 10 | 124 | 1.80 | 0.78 |
| C-6 | 3 | 5 | 65 | 0.88 | 0.54 |
| C-7 | 6 | 7 | 73 | 1.57 | 0.81 |
| C-8 | 5 | 7 | 94 | 1.64 | 0.84 |
| C-9 | 4 | 5 | 51 | 0.99 | 0.62 |
| C-10 | 5 | 7 | 55 | 1.75 | 0.90 |
| C-11 | 5 | 8 | 112 | 1.55 | 0.75 |
| C-12 | 5 | 9 | 134 | 1.35 | 0.61 |
| C-13 | 5 | 7 | 70 | 1.72 | 0.88 |
| C-14 | 4 | 9 | 104 | 1.65 | 0.75 |
| C-15 | 6 | 8 | 132 | 1.15 | 0.55 |
| T-1 | 6 | 10 | 107 | 1.88 | 0.82 |
| T-2 | 8 | 11 | 138 | 2.02 | 0.84 |
| T-3 | 4 | 9 | 107 | 1.87 | 0.85 |
| T-4 | 6 | 11 | 162 | 1.85 | 0.77 |
| T-5 | 6 | 8 | 128 | 1.56 | 0.75 |
| Mean | 5.15 | 8.15 | 102.4 | 1.56 | 0.75 |
| SD | 1.26 | 1.81 | 29.93 | 0.31 | 0.11 |

**Table 3:** Diversity indices and Taxa count for pre-monsoon season.

| Plot ID | Order | Family | Count (*n*) | Diversity Index | Evenness Index |
|---|---|---|---|---|---|
| C-1 | 6 | 12 | 73 | 1.74 | 0.70 |
| C-2 | 7 | 12 | 92 | 1.73 | 0.70 |
| C-3 | 5 | 11 | 81 | 1.84 | 0.77 |
| C-4 | 6 | 16 | 102 | 2.46 | 0.89 |
| C-5 | 4 | 8 | 109 | 1.37 | 0.66 |
| C-6 | 3 | 6 | 45 | 1.14 | 0.64 |
| C-7 | 3 | 8 | 52 | 1.56 | 0.75 |
| C-8 | 3 | 7 | 39 | 1.65 | 0.85 |
| C-9 | 4 | 8 | 65 | 1.37 | 0.66 |
| C-10 | 3 | 7 | 33 | 1.04 | 0.54 |
| C-11 | 2 | 3 | 6 | 1.01 | 0.92 |
| C-12 | 5 | 7 | 50 | 1.19 | 0.61 |
| C-13 | 3 | 8 | 51 | 1.68 | 0.81 |
| C-14 | 4 | 9 | 59 | 1.34 | 0.61 |
| C-15 | 6 | 10 | 54 | 1.52 | 0.66 |
| T-1 | 7 | 10 | 91 | 1.62 | 0.71 |
| T-2 | 6 | 10 | 107 | 1.32 | 0.57 |
| T-3 | 5 | 14 | 110 | 1.83 | 0.69 |
| T-4 | 6 | 11 | 69 | 1.93 | 0.81 |

| | | | | | |
|---|---|---|---|---|---|
| T-5 | 5 | 9 | 39 | 1.83 | 0.83 |
| Mean | 4.65 | 9.3 | 66.35 | 1.51 | 0.71 |
| SD | 1.49 | 2.92 | 28.84 | 0.35 | 0.35 |

**Table 4:** Total number of orders, Family, Individual counts, and percentage composition for post-monsoon season

| Order | Family | Total Count (*n*) | % Composition |
|---|---|---|---|
| Coleoptera | Elmidae | 27 | 1.32 |
| | Gyrinidae | 1 | 0.05 |
| | Psephenidae | 4 | 0.20 |
| | Unknown | 16 | 0.78 |
| Diptera | Blephariceridae | 2 | 0.10 |
| | Ceratopogonidae | 2 | 0.10 |
| | Limoniidae | 6 | 0.29 |
| | Simuliidae | 57 | 2.78 |
| | Tabanidae | 1 | 0.05 |
| | Tanyderidae | 29 | 1.42 |
| Ephemeroptera | Baetidae | 670 | 32.71 |
| | Caenidae | 41 | 2.00 |
| | Ephemerellidae | 8 | 0.39 |
| | Heptageniidae | 465 | 22.71 |
| | Siphlonuridae | 68 | 3.32 |
| Hemiptera | Aphelocheiridae | 118 | 5.76 |
| | Corixidae | 17 | 0.83 |
| | Gerridae | 2 | 0.10 |
| Megaloptera | Corydalidae | 9 | 0.44 |
| Odonata | Euphaeidae | 4 | 0.20 |
| | Gomphidae | 23 | 1.12 |
| Plecoptera | Peltoperlidae | 7 | 0.34 |
| | Perlidae | 107 | 5.22 |
| Trichoptera | Brachycentridae | 47 | 2.29 |
| | Glossosomatidae | 14 | 0.68 |
| | Hydropsychidae | 98 | 4.79 |
| | Lepidostomatidae | 72 | 3.52 |
| | Odontoceridae | 8 | 0.39 |
| | Philopotamidae | 13 | 0.63 |
| | Rhyacophilidae | 2 | 0.10 |
| | Sericostomatidae | 94 | 4.59 |
| | Stenopsychidae | 7 | 0.34 |
| Tricladida | Planariidae | 9 | 0.44 |
| Total count | | 2048 | 100 |

**Table 5:** Total number of orders, Family, Individual counts, and percentage composition for pre-monsoon season

| Order | Family | Total Count (*n*) | % Composition |
|---|---|---|---|
| Coleoptera | Chrysomelidae | 1 | 0.08 |
| | Coleoptera | 3 | 0.23 |
| | Elmidae | 9 | 0.68 |
| Diptera | Athericidae | 1 | 0.08 |
| | Blephariceridae | 3 | 0.23 |
| | Ceratopogonidae | 1 | 0.08 |
| | Chironomidae | 1 | 0.08 |
| | Limoniidae | 12 | 0.90 |
| | Simuliidae | 17 | 1.28 |
| | Tabanidae | 5 | 0.38 |
| Ephemeroptera | Baetidae | 380 | 28.64 |
| | Ephemerellidae | 53 | 3.99 |
| | Heptageniidae | 463 | 34.89 |
| | leptophlebiidae | 1 | 0.08 |
| | Siphlonuridae | 2 | 0.15 |
| Hemiptera | Aphelocheiridae | 32 | 2.41 |
| | Notonectidae | 44 | 3.32 |
| Megaloptera | Corydalidae | 2 | 0.15 |
| Odonata | Euphaeidae | 7 | 0.53 |
| | Gomphidae | 2 | 0.15 |
| Plecoptera | Peltoperlidae | 1 | 0.08 |
| | Perlidae | 64 | 4.82 |
| Trichoptera | Brachycentridae | 59 | 4.45 |

| | | | |
|---|---|---|---|
| | Glossosomatidae | 1 | 0.08 |
| | Hydropsychidae | 38 | 2.86 |
| | Hydroptilidae | 1 | 0.08 |
| | Lepidostomatidae | 25 | 1.88 |
| | Odontoceridae | 32 | 2.41 |
| | Philopotamidae | 12 | 0.90 |
| | Stenopsychidae | 49 | 3.69 |
| Tricladida | Planariidae | 3 | 0.23 |
| Arachnida | Hydrachnidae | 3 | 0.23 |
| Total count | | 1327 | 100.00 |

**Benthic macroinvertebrates diversity in Punatsangchhu and Mangdechhu river**

In Punatsangchhu, a total of 2317 individuals were recorded that belong to nine orders and 36 families. The dominant order was Ephemeroptera (66.2% *n* = 1535) followed by Trichoptera (17.2% *n* = 399) and the least dominant order was Arachnida (0.13% *n* = 3). Post-monsoon season recorded 1456 individuals belonging to eight orders with 24 families and pre-monsoon recorded 911 individuals with nine orders and 27 families. The Order Arachnida (freshwater mites) with family Hydrachnidae was recorded only in pre-monsoon. The mean diversity index for post and pre-monsoon was 1.47 (± 0.29 *SD*) and 1.51 (± 0.37*SD*) respectively with evenness index 0.73 (± 0.12 *SD*) for post monsoon and 0.71 (±0.11*SD*) for pre-monsoon. The family Baetidae, Heptageniidae, Brachycentridae, Sericosomatidae and Hydropsychidae were dominant for both post and pre-monsoon season.

In Mangdechhu, a total of 1058 individuals were recorded with eight orders and 25 families. The dominant order was Ephemeroptera (58.2% *n* = 616) followed by Trichoptera (16.4% *n* = 173) and least dominant was Coleoptera (1.02% *n* = 11). The Post-monsoon season recorded 642 individuals that belong to eight orders and 18 families, and the Pre-monsoon season recorded 416 individuals with seven orders and 22 families. Baetidae, Heptageniidae and Aphelocheiridae dominate both the post and Pre-monsoon season. The mean diversity index for post and pre-monsoon season was 1.84 (±0.17*SD*) and 1.71(±0.12*SD*) respectively with evenness index of 0.8 (±0.04*SD*) for post and 0.72 (±0.1*SD*) for pre-monsoon season.

**Physico-chemical variables of Punatsangchhu and Mangdechhu**

Benthic macroinvertebrate communities in rivers or streams are affected by physico-chemical parameters [19] and these parameters are influenced by a range of natural and anthropogenic activities including seasonal changes in water flow regime [18]. The mean pH of Punatsangchhu river during post-monsoon was 7.65 (±0.29*SD*) and Pre-monsoon was 7.84 (±0.15*SD*), and the conductivity of 125.27 (±31.37*SD*) for post and 105.35(± 19.8*SD*) for pre-monsoon. The mean TDS was 62.73 (±15.8*SD*) for post and 52.85 (±10.01*SD*) pre-monsoon season and the Temperature of 17.09 (±1.53*SD*) for post and 16.7 (±1.47*SD*) for pre-monsoon season. Similarly, for Mangdechhu river the mean was pH 7.65 (±0.31*SD*) for post-monsoon and 7.63 (±0.24*SD*) for pre-monsoon and the conductivity of 104.6 (±17.44*SD*) for post and 103.6 (±14.01*SD*) for pre-monsoon. The mean TDS was 52 (±8.72*SD*) for post-monsoon and 51.6 (±7.02*SD*) pre-monsoon and the Temperature of 17.99 (±0.98*SD*) for post and 17.6 (± 1.5) for pre-monsoon season.

Overall, the Wilcoxon sum rank test shows that there was insignificant difference of pH, Salinity, and Temperature between post and pre-monsoon season but significant difference of conductivity and TDS between post and Pre-monsoon season (Table 6). The difference in conductivity and TDS might be due to seasonal change in river flow regime [18]. The pH of both rivers was within the permissible limit (6.5-8) neutral to slightly alkaline [29] and within optimum range (6-8) preferred by aquatic organisms [5]. The electrical conductivity of the water shows the ionic nature of the water. In the study area, the electrical conductivity was far below the permissible limit (500µS/cm) indicating the water has less dissolved solutes. The total dissolved solute (TDS) determines the taste of the water, and it is the measure of organic salt, inorganic materials, and other materials in the water [2]. In this study the TDS of both the rivers was within the permissible limit (500 mg/L). The salinity of water is the measure of the dissolved salts, and the mean salinity of the study area was very less indicating very less dissolved salts in the river.

**Table 6:** Physico-chemical parameters between post and Pre-monsoon season

| | pH | Conductivity | TDS | Salinity | Temperature |
|---|---|---|---|---|---|
| Z | -1.13 | -2.18 | -2.39 | -0.71 | -1.53 |
| Asymp. Sig. (2-tailed) | .257 | .029 | .017 | .478 | .126 |

**Relationship between diversity indices with environmental and Physico-chemical variables**

The health of the environment is necessary for the organisms to live and survive. Since life depends on continuous exchange of essential substances and energies between organisms and its surroundings. Water in pure state has a specific range of physico-chemical parameters that supports diverse living organisms. The correlation analysis showed no significant association between diversity index, evenness, and richness with physico-chemical variables like pH, conductivity, TDS, and salinity. In contrast, Study done in Tropical stream in Kenya [27] reported that there is significant association with pH, conductivity, and TDS. There was significant positive association between diversity index and temperature ($r_s$= 0.34, $p$<0.05) and negative association with altitude ($r_s$= -0.33, $p$<0.05). Similarly, there was significant association between richness and temperature ($r_s$= 0.32, $p$<0.05) and negative association with altitude ($r_s$= -0.33, $p$<0.05) (Table 7) indicating the species diversity and richness decreases with increases in altitude and increases with increases in temperature. The study done by Castro *et al.* (2019) [6] confirmed that macroinvertebrate family richness and abundance decrease with increase in altitude in freshwater mountain ecosystems [6].

**Table 7:** Relationship between diversity indices with Physico-chemical and Environmental variables

| | pH | stm (ohm) | TDS (mg/L) | Sali (PSU) | Tem (ºC) | Alt (m) | *S* | *H* | *J* |
|---|---|---|---|---|---|---|---|---|---|
| pH | 1.00 | -0.24 | -0.25 | -0.10 | -0.43 | .46** | -0.13 | -0.30 | -0.17 |
| stm | | 1.00 | .99** | .79** | 0.29 | -0.47 | -0.13 | 0.07 | 0.16 |
| TDS | | | 1.00 | .79** | 0.30 | -0.47 | -0.14 | 0.05 | 0.14 |
| PSU | | | | 1.00 | 0.14 | -0.24 | -0.29 | -0.13 | -0.03 |
| Tem | | | | | 1.00 | -0.81 | 0.33* | 0.34* | 0.13 |
| Alt | | | | | | 1.00 | -0.32* | -0.33* | -0.11 |
| *S* | | | | | | | 1.00 | 0.66** | 0.06 |
| *H* | | | | | | | | 1.00 | 0.70** |
| *J* | | | | | | | | | 1.00 |

** Significant at 0.01 * Significant at 0.05

**Note:** WD = Water Depth, TDS= Total Dissolved Solute, *S* = Richness, *H*= Diversity, *J* = Evenness, Alt = Altitude, Tem= Temperature, Sali= Salinity, stm = Electrical Conductivity

## Conclusion

The study was a steppingstone to explore and document benthic macroinvertebrate diversity in the major rivers of Bhutan. The study found that major rivers in White-bellied Heron landscape in Bhutan harbor high diversity of macroinvertebrates. The pH and TDS of both the river was within the permissible (pH = 6.5-8.5; TDS = 500 mg/L). All these physicochemical parameters, acidity, conductivity, TDS, and salinity analysis shows that the major rivers in WBH landscape are of good quality to support diverse living organisms with no major pollution. Therefore, it is not too late to start conserving freshwater river systems in Bhutan. The findings of this study might be limited due to many factors including human resource experts in macroinvertebrates particularly in identification of taxa, inadequate sampling, and time. Thus, we suggest the need in collaboration with regional experts to document the macroinvertebrates taxa and include a sampling period for whole seasons of the year.


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