

## WATER BEETLES: SPECIES DIVERSITY AND RESPONSE TO PHYSICO-CHEMICAL PARAMETERS IN DIFFERENT FRESHWATER HABITATS IN SAMTSE, BHUTAN

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### Abstract

Bhutan is located between the two zoogeographic regions which are assumed to be rich in diversity of water beetles. The study aimed to determine the diversity and distribution pattern of water beetles in different freshwater habitats in Namgaychholing under Samtse district, Bhutan. The study also analysed the association of water beetles with the physico-chemical parameters. The data were collected from four different habitats with sample plot of 3 m × 1 m for a standard time of 1 hour in every plot. A total of 30 species belonging to 7 families were recorded of which Dytiscidae (n=626, RA=51) was the most abundant family and *Lacconectus basalis* (n=416, RA=34.13) was the most abundant species. The overall Shannon diversity index of the study area was  $H' = 2.48$ . Amongst the different habitats, marshy area had the highest Shannon diversity index ( $H' = 2.45$ ) and stream had the lowest ( $H' = 1.82$ ). A Kruskal Wallis test on diversity among different habitats showed no significant difference ( $p < .05$ ). Through a cluster analysis, the species composition between the river and waterlogged showed 100% similarity index. Canonical correspondence analysis showed temperature as a main determining factor affecting the distribution of water beetles. With broad families encompassing the water beetles, similar study on water beetles in different regions of the country is recommended with particular emphasis on different families. Moreover, Bhutan falls in junction of Palearctic and Oriental Zoogeographic regions, which are known to be rich in diversity of water beetles. Hence, study in high-altitude lakes, which are assumed to be rich in diversity of water beetles are recommended despite social beliefs to keep lakes isolated and undisturbed.

Keywords: Marshy area; pH; Temperature; Water beetles

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## Introduction

Coleoptera is the largest order under the class Insecta (Jach and Balke, 2008). The aquatic beetles are found to inhabit in almost all kinds of aquatic habitats such as rivers, springs, lakes, ditches, puddles, phytotelma, seepages and ground water. They are known to survive even when trapped in ice and high saline water (Gerdes *et al.*, 1985). Beetles occur on all continents except Antarctica. Water beetles are found in all biogeographic regions and the highest diversity is found in the tropics. The smallest water beetles is less than 1 mm and the largest ones are more than 5 cm long. (Jack and Balke, 2008). Water beetles can either be herbivores, predators, or scavengers (Epler, 1996).

Water quality plays a key role in species richness of aquatic beetles; therefore, they could be considered as an indicator organism for ecosystem health of freshwater (Dong *et al.*, 2014; Ghannem *et al.*, 2018). The main physico-chemical factors that affect aquatic environments are temperature, discharge, dissolved oxygen (DO), pH, nutrients and electrical conductivity (Ngodhe *et al.*, 2014). There are decrease in trends for species richness, total abundance and diversity in water bodies affected by high values for chemical parameters in the water (Prenda and Gallardo-Mayenco 1996; Heino 2000).

Around the world, freshwater habitats are being subjected to increased levels of human disturbance (Saunders *et al.*, 2002). Globally, of all the inland water ecosystems, the most threatened river catchments are found in the Indian subcontinent (World Conservation Monitoring Centre [WCMC], 2000). In general, water beetle communities all around the world suffer from desertification, irrigation, eutrophication induced by livestock, man, and agriculture, as well as overall loss of primary habitats (Jach and Balke, 2008).

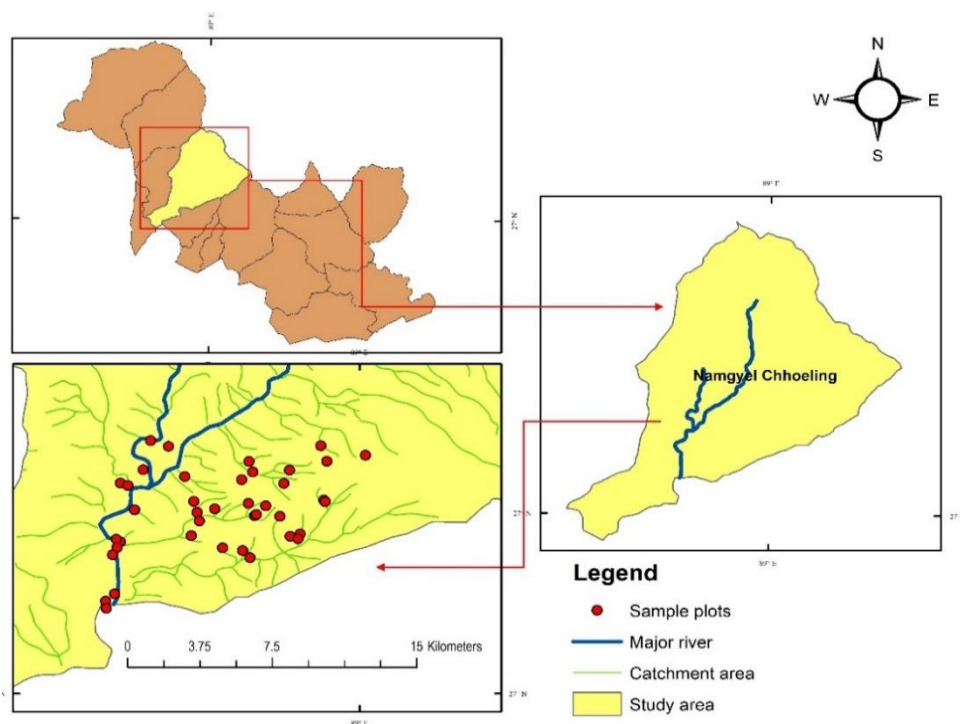
Water beetles in Himalayas is not much explored and the distribution of certain dytiscid genera in the Himalayas is still very incomplete (Brancucci and Wewelka, 2005). Bhutan is still regarded as one of the least explored countries in terms of aquatic entomology. Namgaychholing *Gewog* (block) under Samtse *Dzongkhag* (district) has a rich freshwater ecosystem encompassing different elevational gradient. Conversely, the study area is expected to have a high diversity of water beetles distributed across different habitats. This study aims to analyse the diversity and distribution of water beetles in different freshwater habitats and their response to physico-chemical parameters. The study will augment the scientific exploration in beetle diversity in Bhutan and especially, it will spearhead to form the baseline data for the water beetles in Bhutan as this is the first of its kind in the country.

## Materials and methods

### Study area

Namgaychholing *Gewog* (88°57'57.935"E and 27°5'33.421"N) is located in Samtse *Dzongkhag* to the North-West of *Dzongkhag* headquarter. It covers around 128.49 square kilometers of area (Dzongkhag Administration Samtse, 2018).

The *Gewog* experiences annual rainfall ranging from 1200 mm to 3000 mm. The *Gewog* has hot and humid summer, dry and cool winter with snowfall in the northern part of *Gewog* and falls in sub-tropical monsoon zone. The *Gewog* has red, alluvial and black types of soil. Namgaychholing *Gewog* consist of five *Chiwogs* (village) (Dzongkhag Administration Samtse, 2018).



**Figure 1:** Map showing the study area under Samtse Dzongkhag.

### Research Design

#### Sampling

The sampling were carried out following stratified random sampling with equal proportion (Williams *et al.*, 2007). Water beetles were collected from four different habitats (rivers, streams, water logged areas and marshy areas) of the gewog. Ten sample sites were selected from each habitat. Therefore, from four different habitat the total sampling site was 40 from different areas. The sampling unit for each habitat were selected through reconnaissance survey. In each sample site 1 hour was set as a standard time, including time spent sweeping and removing the debris and unwanted insects (Turic *et al.*, 2017). The sample size of 3m × 1 m

was adopted following Turic *et al.* (2017). Data collection was done during the pre-monsoon season before the water beetles were not affected by the monsoon vagaries.

### ***Data collection***

Water net and household sieve of various pore size was used to collect the specimens (Turner, 2007; Boukal *et al.*, 2007). In rivers, streams and water-logged areas, s kicking, brushing and rubbing the surface and then gently sweeping with the water net was done to collect the water beetles. The small water beetles that were seen floating on the surface of the water were collected by gently sweeping the surface with household sieve. The sampling techniques followed Lhundup and Dorji (2018). If the habitats were covered with woody debris and leaf litter, it was disturbed with poles and leg, and was swept with water net. The collected samples were washed thoroughly into the net and the water beetles were picked with forceps. In greater depth of water, it was disturbed with the help of poles. This was done to make sure that the water beetles that were inside the sand and soil are mixed with water and brought on the surface. In marshy areas with minimum water, it was disturbed with the sticks and household sieve was used to collect the specimens.

### ***Measurement of physico-chemical parameters***

All three physico-chemical parameters were measured in the field. The pH meter was used to measure water pH and pen type DO<sub>2</sub> meter to measure both water temperature and DO at every sampling unit before sampling.

### ***Fixation, preservation and identification***

The specimens were fixed using 10% formalin and preserved in 70% ethanol (Gaston *et al.*, 1996). Specimens which could be identified on the field were recorded, photographed and released back to their natural habitats. The collected samples were identified later in the laboratory using microscope, identification keys, monographs and assistance from taxonomic expert.

### ***Data analysis***

The species diversity was analysed using Shannon's Diversity Index. The taxon richness was computed using  $R = (S-1) / \log N$  (Wilson, 1992) and Evenness using Pielou evenness formula  $E = H' / H_{max}$  (McGinley, 2014). Berger-Parker's Dominance index (Das and Gupta, 2010) was used to find the dominance in a habitat. Relative abundance (Purkayastha and Gupta, 2012) was calculated for individual family and species. Kruskal Wallis test was done using R studio to compare the variations in diversity, richness and abundance among different habitats. Cluster analysis was done between four different habitats with their relative abundance (RA) using PC-ORD.

The Pearson's correlation was used to find the relationship between diversity indices and physico-chemical parameters. Furthermore, Canonical correspondence analysis was done using PC-ORD to find the distribution of water beetles according to physico-chemical parameters. Canonical correspondence analysis was done to find the distribution of water beetles according to the physico-chemical parameters Shannon-Wiener Index ( $H'$ ) (Konopinski, 2020)

$$H' = - \sum_{i=1}^S P_i \times \ln(P_i)$$

Where,  $P_i = ni/N$  (RA of each species, or proportion),

$ni$  = no. of individuals in species  $i$

$N$  = total number of all individuals

Berger-Parker Dominance ( $P_{i_{max}}$ )

$$\text{Dominance, } D = P_{i_{max}}$$

Where,  $ni$  = number of individuals of a species (of one plot)

$N$  = total number of individuals of all species (of one plot)

Relative Abundance

$$\text{Relative Abundance (RA)} = \frac{\text{No. of individual}}{\text{Total no. of individuals}} \times 100\%$$

## Results and discussion

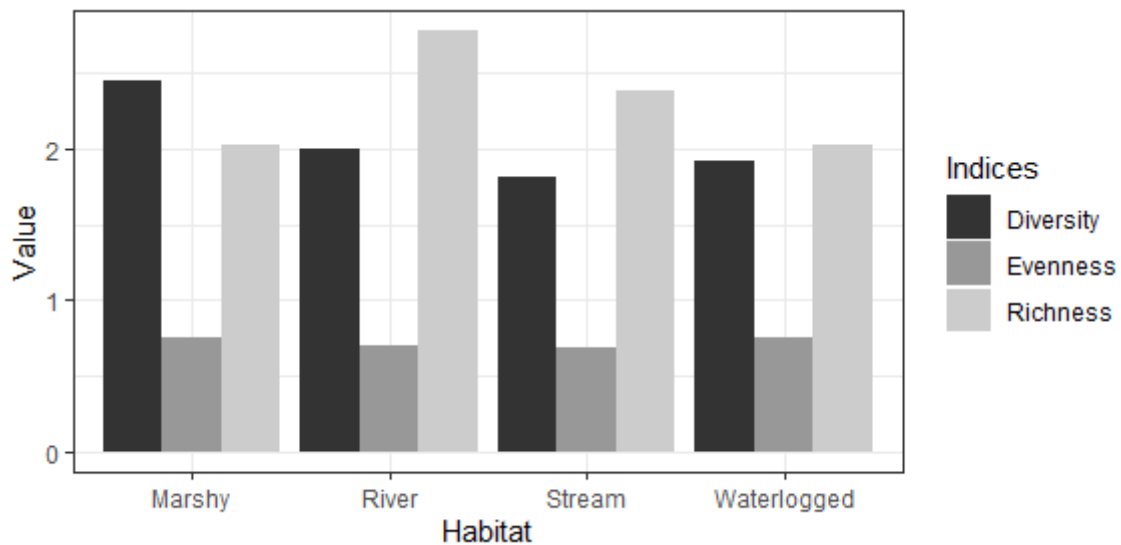
### *Diversity of water beetles*

A total of 1219 water beetles belonging to 7 families with 30 species (Table 1) were recorded. Family Dytiscidae ( $n=626$ ,  $RA=51$ ) was most abundant and family Sphaeriidae ( $n=3$ ,  $RA=0.25$ ) the least. Amongst all the species, *Lacconectus basalis* Sharp, 1882 ( $n=416$ ,  $RA=34.13$ ) of Dytiscidae family was most abundant and the least were *Cybister lateralimarginalis* De Geer, 1774 ( $n=1$ ,  $RA=0.08$ ) and *Aculomicrus alesi* Fikacek, 2010 ( $n=1$ ,  $RA=0.08$ ).

*Lacconectus basalis* was most abundant probably due to its adaptability to wide range of physico-chemical parameters (Table 2). The member of Dytiscidae family were the most abundant due to wide range of adaptability in different habitats (Freitag *et al.*, 2016) as compared to other families. The Dytiscidae family was found in all four habitats but Sphaeriidae was found only in streams and waterlogged areas. Dytiscidae family was also found to be the most abundant in Loktak Lake of Manipur, India (Devi *et al.*, 2014).

Overall, Shannon diversity index was  $H' = 2.48$ , evenness  $E_H = 0.73$ , taxon richness  $S_R = 4.08$  and dominance  $P_{i_{max}} = 0.34$ . Among the different habitats, marshy area had the highest Shannon diversity index

( $H' = 2.45$ ), evenness ( $E_H = 0.75$ ), taxon richness ( $S_R = 2.03$ ) and dominance ( $P_{imax} = 0.33$ ) followed by river with diversity ( $H' = 2.00$ ), evenness ( $E_H = 0.70$ ), taxon richness ( $S_R = 2.78$ ) and dominance ( $P_{imax} = 0.41$ ). Waterlogged area had diversity ( $H' = 1.92$ ), evenness ( $E_H = 0.75$ ), taxon richness ( $S_R = 2.02$ ) and dominance ( $P_{imax} = 0.39$ ) and the least was found in streams with diversity ( $H' = 1.82$ ), evenness ( $E_H = 0.69$ ), taxon richness ( $S_R = 2.38$ ) and dominance ( $P_{imax} = 0.275$ ) (Figure 3). The difference in these results among different habitats could be because, the diversity of macro invertebrates is directly influenced by the surrounding land use, which in turn influence water pH, temperature, salinity and DO (Dhakal, 2006).



**Figure 2:** Shannon Wiener Diversity, Evenness and Richness of water beetles in different habitats.

A Kruskal Wallis test showed no significant differences in water beetle diversity ( $H(3) = 1.901, p = .593$ ), richness ( $H(3) = 1.811, p = .612$ ), and abundance ( $H(3) = .279, p = .964$ ) among different habitats. Gimenez *et al.* (2015) also found no significant differences between urban and rural streams for the diversity and abundance of species. It was because the physical and chemical properties of the water were different between urban and rural streams. Consequently, the insect community responded to these differences and the species composition, diversity and abundance differed between streams.

Similarly, no significant difference among different habitats in current study maybe due to different habitat with different physical and chemical properties. This chemical and physical properties influenced Beetles community to respond to these differences which resulted in no significant differences among the habitat.

**Table 1:** Water beetles in the study area with family, species, number of individuals and relative abundance (RA).

Family	Species	Count	RA (Family)	RA (Species)
Dytiscidae	<i>Copelatus sibelaemontis</i> Balke, 2010	62	51	5.09
	<i>Cybister lateralimarginalis</i> De Geer, 1774	1		0.08
	<i>Lacconectus basalis</i> Sharp, 1882	416		34.13
	<i>Laccophilus chinensis</i> Boheman, 1858	129		10.58
	<i>Hydroglyphus geminus</i> (Fabricius, 1792)	7		0.57
	<i>Platynectes kashmiranus</i> Balfour-Browne, 1944	11		0.90
Elmidae	<i>Stenelmis sandersoni</i> Musgrave, 1940	25	2.05	2.05
Gyrinidae	<i>Patrus palawanensis</i> Regimbart, 1907	91	16.82	7.47
	<i>Dineutus spinosus</i> Fabricius, 1781	56		4.59
	<i>Dineutus unidentatus</i> Aube, 1838	14		1.15
	<i>Metagyrimus arrowi</i> (Regimbart, 1907)	44		3.61
Hydraenidae	<i>Hydraena rufipes</i> Curtis, 1830	60	9.68	4.92
	<i>Limnebius rubropiceus</i> Kuwert, 1890	9		0.74
	<i>Limnebius truncatellus</i> (Thunberg, 1794)	46		3.77
	<i>Ochthebius opacipennis</i> Champion, 1920	3		0.25
Hydrophilidae	<i>Aculomicrus alesii</i> Fikacek, 2010	1	16.08	0.08
	<i>Agraphydrus flavonotus</i> (Komarek, 2018)	2		0.16
	<i>Agraphydrus indicus</i> (Orchymont, 1932)	15		1.23
	<i>Agraphydrus kempii</i> (Orchymont, 1922)	26		2.13
	<i>Dactylosternum circumcinctum</i> Fikacek, 2010	2		0.16
	<i>Enochrus esuriens</i> (Walker, 1858)	80		6.56
	<i>Enochrus subsignatus</i> (Harold, 1877)	9		0.74
	<i>Helochares atropiceus</i> Regimbart, 1903	22		1.80

	<i>Helochaeres lentus</i> Sharp, 1890	2		0.16
	<i>Helochaeres taprobanicus</i> Sharp, 1890	6		0.49
	<i>Laccobius celsus</i> Gentili, 1989	15		1.23
	<i>Laccobius patruelis</i> Knisch, 1924	11		0.90
	<i>laccobius simulans</i> Orchymont, 1923	5		0.41
Noteridae	<i>Mesonoterus laevicollis</i> Sharp, 1882	46	3.77	3.77
Sphaeriusidae	<i>Sphaerius minutus</i> Liang, 2018	3	0.25	0.25
7	30	1219	100	100

### ***Species composition of water beetles***

The 7 families of water beetles found in the study area were Hydrophilidae, Dytiscidae, Gyrinidae, Hydraenidae, Noteridae, Sphaeriusidae and Elmidae. The family Hydrophilidae was found to have 13 different species making 43.3% (Table 1) of total species, followed by Dytiscidae with 6 species making 20%. The family Gyrinidae and Hydraenidae consist of 4 species contributing 14% by each family and the least was Noteridae, Sphaeriusidae and Elmidae with 1 species each making 3% of total species.

Though Dytiscidae was found abundantly in terms of number of species composition, Hydrophilidae had the high number of species. However, Dytiscidae had higher species composition than Hydrophilidae in Coleopteran Water Beetles in Kenyir Water Catchment of Terengganu, Malaysia (Abdullah, 2009). However, Freitag *et al.* (2016) reported that species composition was higher in family hydrophilidae than in Dytiscidae. The difference in findings could be because Abdullah (2009) studied in single habitat (Lake) but Freitag *et al.* (2016) did from both aquatic and riparian habitats. Similarly, this study area covers four different habits, hence species composition maybe higher in family Hydrophilidae than in Dytiscidae.

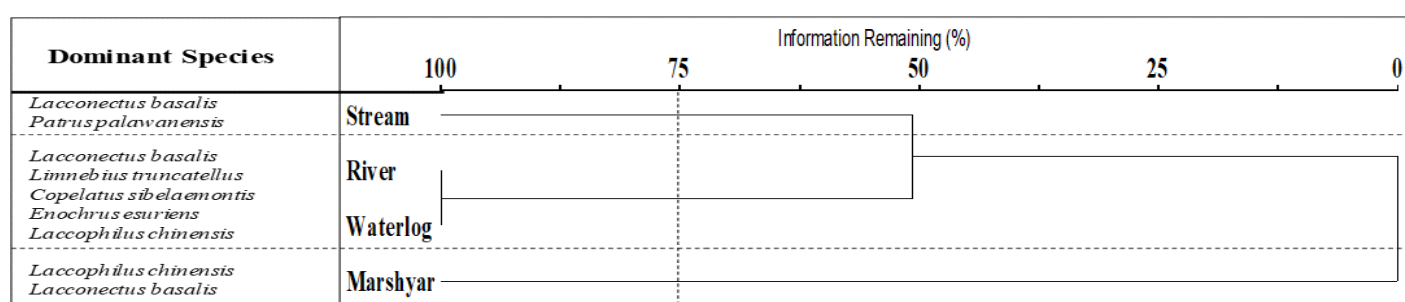
### ***Distribution of water beetles in different habitats***

The families Hydrophilidae, Dytiscidae, Gyrinidae and Hydraenidae was distributed in all four habitats (Figure 4). The species belonging to Noteridae ( $n=1$ ) were found each in waterlogged and in marshy habitat. In family Elmidae, species ( $n=1$ ) was found only in marshy habitat and Sphaeriusidae ( $n=1$ ) were encountered each in streams and waterlogged habitat but was of same species. The highest number of species ( $n=8$ ) of Hydrophilidae and ( $n=5$ ) of Dytiscidae were encountered in riverine habitat and lowest ( $n=5$ ) each in waterlogged and marshy habitats, and ( $n=2$ ) in streams respectively. However, highest number of species for Gyrinidae ( $n=3$ ) were recorded from streams and lowest number ( $n=2$ ) each in other three habitats. In



family Noteridae ( $n=1$ ) species was found each in waterlogged and marshy habitats. In Elmidae and Sphaeriusidae ( $n=1$ ) species was distributed in waterlogged habitat and streams respectively (Figure 6). The distribution of individual species with physico-chemical parameters in four different habitats is shown in Table 2. However, in some species there are only one value and no range for parameters because those species were found only in one plot.

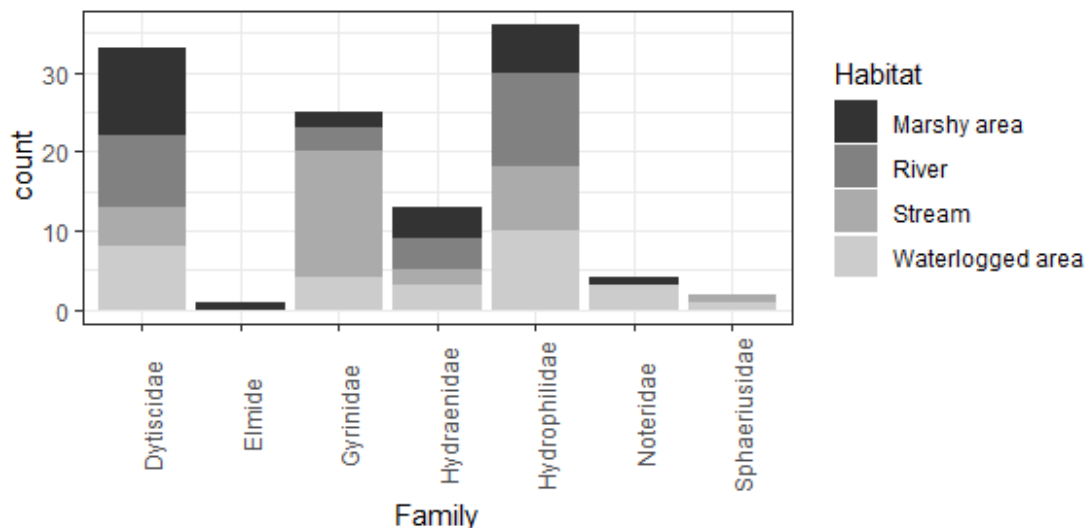
The cluster analysis based on the relative abundance of taxa grouped four different habitats into three major clades. The dendrogram showed that the habitat river and waterlogged area have similar species composition having closer, 100% similarity in dendrogram scale (Figure 4). The dominant species were *Lacconectus basalis*, *Limnebius truncatellus*, *Copelatus sibelaemontis*, *Enochrus esuriens* and *Laccophilus chinensis*. The similarity among these habitats could be because the plots in habitat rivers were sampled in riverine habitats, which moreover, are similar to waterlogged areas with similar physiochemical parameters.



**Figure 3:** Cluster analysis of different habitats using dendrogram based on relative abundance.

Similarly, river, waterlogged area and stream habitat were having 50% similarity (Figure 4). These indicate that these three habitats have similar water physiochemical parameters such as pH, water temperature and dissolved oxygen, which made the aquatic habitat suitable for that particular species of water beetle (Dorji, 2014).

The family Hydrophilidae, Dytiscidae, Gyrinidae and Hydraenidae are adapted to variety of freshwater habitats. The adults and larvae of most species of family Hydrophilidae lives in stagnant water, running water, seepages, numerous species are reportedly riparian or terrestrial (Jach and Balke, 2008). The species of family Dytiscidae inhabits stagnant water, running water, groundwater, seepages and phytotelmata (Khan and Ghosh, 2001). The family Gyrinidae are found commonly in fresh water ponds, lakes, open flowing streams and majority of the species are found in running water (Sharma *et al.*, 2019). The adults of Hydraenidae are aquatic and lives in stagnant water, running water, seepages any are riparian (Jach and Balke, 2008). The family Noteridae are commonly found in stagnant water between roots of water plants (Nilsson and Van, 2005). The most species of Elmidae occurs in well-aerated streams and rivers but some can be found in shores of lakes (Elliott, 2008). The Sphaeriusidae beetles are found in accumulations of gravel, sand and mud at the edges of streams and rivers (Lawrence and Slipinski, 2013).



**Figure 4:** Distribution of water beetle families in different habitats.

**Table 2:** Distribution of water beetle species with physico-chemical parameters in four habitats.

Species	Physico-chemical parameters			Habitats			
	pH	Temp (°C)	DO (ppm)	Stream	River	WA	MA
<i>Copelatus sibelaemontis</i>	9.1 - 10.08	13.6 - 18.1	0.73 - 6.34	-	-	+	+
<i>Cybister lateralimarginalis</i>	9.5	19.5	6.43	-	+	-	-
<i>Lacconectus basalus</i>	7.75 - 10.48	12.7 - 21.1	0.73 - 6.98	+	+	+	+
<i>Laccophilus chinensis</i>	8.41 - 9.96	19.5 - 25.1	2.29 - 6.43	+	+	+	+
<i>Hydroglyphus geminus</i>	8.98	26.5	5.66	-	+	-	-
<i>Platynectes kashmiranus</i>	9.07 - 9.77	14.7 - 19.5	4.39 - 6.43	-	+	-	+
<i>Stenelmis sandersoni</i>	9.78	12.7	5.61	-	-	-	+
<i>Patrus palawanensis</i>	8.87 - 11	12.7 - 22.8	5.03 - 6.29	+	+	+	+
<i>Dineutus spinosus</i>	8.57 - 11	14.2 - 23.3	2.45 - 6.19	+	-	+	+
<i>Dineutus unidentatus</i>	8.94 - 9.7	19.6 - 21.1	5.42 - 6.1	-	+	-	-
<i>Metagyrius arrowi</i>	8.87 - 9.96	12.7 - 21.1	5.03 - 6.29	+	-	-	+
<i>Hydraena rufipes</i>	9.07 - 9.84	12.7 - 18.1	0.73 - 6.29	+	-	+	+
<i>Limnebius rubropiceus</i>	9.77	16.9	4.39	-	-	-	+

<i>Limnebius truncatellus</i>	9.5 - 9.74	15.5 - 20.8	5.33 - 6.1	+	+	-	-
<i>Ochthebius opacipennis</i>	9.7	19.6	6.1	-	+	-	-
<i>Aculomicrus alesi</i>	10.48	14.2	5.82	+	-	-	-
<i>Agraphydrus flavonotus</i>	9.98	13.6	6.34	-	-	-	+
<i>Agraphydrus indicus</i>	9.5	19.5	6.43	-	+	-	-
<i>Agraphydrus kempi</i>	9.09 - 10.24	14.1 - 22.6	0.73 - 5.23	-	+	+	-
<i>Dactylosternum circumcinctum</i>	10.48	14.2	5.82	+	-	-	-
<i>Enochrus esuriens</i>	8.87 - 10.06	12.7 - 21.1	0.73 - 6.98	+	+	+	+
<i>Enochrus subsignatus</i>	9.09 - 9.31	19.6 - 22.6	5.02 - 6.1	-	+	+	-
<i>Helochaeres atropiceus</i>	8.87 - 9.77	16.9 - 19.5	4.39 - 6.43	+	+	-	+
<i>Helochaeres lentus</i>	8.98	26.5	5.66	-	+	-	-
<i>Helochaeres taprobanicus</i>	9.9 - 9.96	19.6 - 21.1	5.03 - 5.5	+	-	-	+
<i>Laccobius celsus</i>	9.31 - 10.45	12.7 - 24.8	4.95 - 6.34	+	-	+	+
<i>Laccobius patruelis</i>	9.5 - 10.24	14.1 - 19.5	4.82 - 6.43	-	+	+	-
<i>laccobius simulans</i>	8.98	26.5	5.66	-	+	-	-
<i>Mesonoterus laevicollis</i>	9.1 - 10.45	14.1 - 16.9	0.73 - 4.95	-	-	+	+
<i>Sphaerius minutus</i>	9.96 - 10.24	18.1 - 21.1	5.03 - 5.23	+	-	+	-

[Temp = Temperature, DO = Dissolved oxygen, WA = Waterlogged area, MA = Marshy area]

[+ Recorded, - Not recorded]

#### *Relationship between water beetles and physico-chemical parameters*

The highest mean for pH (9.93) was found in stream and lowest (9.43) in marshy areas. The mean water temperature for riverine (19.77) was highest and minimum mean (16.39) was recorded in streams, and the highest mean (5.81) for dissolved oxygen was recorded in riverine and lowest (4.45) in waterlogged areas (Table 3).

**Table 3:** Mean, standard error and (minimum and maximum values) of physico-chemical parameters of different habitats.

Habitat	pH	Temperature (°c)	Dissolved oxygen (ppm)
Stream	9.93 ± 0.19 (8.87-11.00)	16.39 ± 0.93 (12.70-21.10)	5.77 ± 0.15 (5.03-6.37)
River	9.50 ± 0.14 (8.94-10.42)	19.77 ± 1.09 (14.60-26.50)	5.81 ± 0.19 (5.08-6.98)
Waterlogged area	9.50 ± 0.29 (7.75-10.48)	18.20 ± 1.33 (13.50-25.10)	4.45 ± 0.51 (0.73-5.91)
Marshy area	9.43 ± 0.14 (8.57- 9.98)	17.86 ± 1.23 (12.70-24.80)	5.25 ± 0.36 (2.45-6.34)

A Pearson correlation test showed no significant association between diversity and physico-chemical parameters with pH ( $r_s = .217, p = .178$ ), temperature ( $r_s = -.297, p = .063$ ) and dissolved oxygen ( $r_s = -.009, p = .956$ ). There was also no significant correlation between taxon richness and pH ( $r_s = .269, p = .093$ ), dissolved oxygen ( $r_s = .031, p = .848$ ) and with temperature ( $r_s = -.261, p = .103$ ). The evenness with pH showed a significant moderate negative correlation ( $r_s = -.463, p = .003$ ) and weak positive correlation with temperature ( $r_s = -.370, p = .019$ ) but no significant correlation with dissolved oxygen ( $r_s = -.046, p = .778$ ). There was significant weak negative correlation between abundance and physico-chemical parameters with dissolved oxygen ( $r_s = -.330, p = .038$ ) but indicated no significant correlation with temperature ( $r_s = -.237, p = .141$ ) and pH ( $r_s = .107, p = .512$ ).

A pH range of 6.5 to 9.0 is the optimal for the life of freshwater bottom-dwelling macroinvertebrates (USEPA, 1986). There is a positive correlation between species richness and pH of aquatic insects (Dalal and Gupta, 2016). Similarly, the taxa richness of invertebrates and diversity increases with increases in pH (Scheibler *et al.*, 2014; Flores and Zafaralla, 2012).

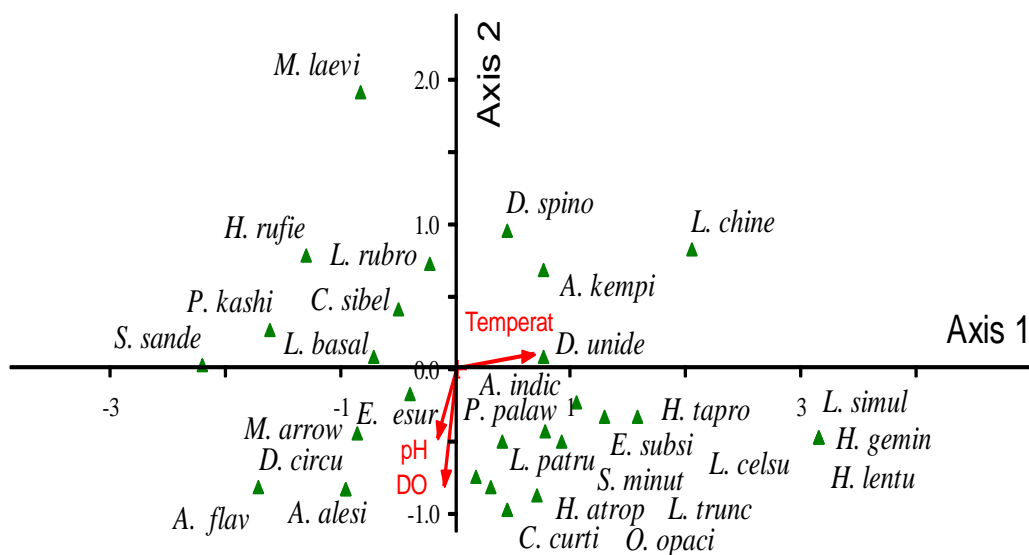
The negative correlation between diversity and richness with water temperature was recorded could mean that water beetles are less tolerant to warmer water. It is because when temperature increases dissolved oxygen and pH decreases making less favorable condition for water beetles. This is due to suspended particles in the water which absorb the heat resulting to increase the water temperature (Paaijmans *et al.*, 2008). When temperature increases, the dissolved oxygen in the water decreases because warm water holds less dissolved oxygen than the cold water (Mandal, 2014). According to Ngodhe *et al.* (2014), temperature negatively influences the species diversity, dominance and richness of a macroinvertebrates.

The dissolved oxygen was negatively correlated with diversity, evenness and abundance but was positively correlated with taxon richness in the study area. The concentration of dissolved oxygen (DO) is one of the most important physico-chemical parameters to determine water quality and to know the distribution of different aquatic insects (Wahizatul *et al.*, 2011). However, a study on aquatic insect biodiversity and water

quality parameters of streams in northern Thailand found that dissolved oxygen negatively influences the taxa richness (Prommi and Payakka, 2015).

### ***Distribution of water beetles in relation to physico-chemical parameters***

Canonical correspondence analysis used to find the distribution of water beetles in relation to the physico-chemical parameters (Figure 5) where the eigenvalues for axis 1 was 0.498, axis 2 was 0.247, and axis 3 was 0.183. The highest eigenvalue was found in axis 1 and the variable corresponded to temperature. Dissolved oxygen ( $r = -0.605$ ) was negatively correlated with axis 2 and pH ( $r = 0.430$ ) was positively correlated with Axis 3. Temperature ( $r = 0.760$ ) was positively correlated with Axis 1. Temperature was having the highest correlation with Axis 1 corroborating to be the main determining factor in distribution of water beetle. Water temperature is the most effective environmental variable in development rate in terrestrial and aquatic ectotherms and insects are affected from the changes of temperature (Ragland and Kingsolver, 2008; Chuche and Thiery, 2012).



**Figure 5:** Distribution of water beetle families in different habitats.

Distribution of *Dineutus unidentatus*, *Agraphydrus kempfi*, *Laccophilus chinensis*, and *Dineutus spinosus* were positively related with temperature. For distribution of species like *Dactylosternum circumcinctum*, *Aculomicrus alesi*, *Enochrus esuriens* and *Agraphydrus flavonotus* the parameters pH and dissolved oxygen are important factors as the direction of arrow is parallel towards the negative of axis 2, however, dissolved oxygen had more correlation compared to pH (Garca-Criadoet *al.*, 1999).

## **Limitation**

The study was conducted during pre-monsoon season only. All season data collection would yield more diversity of water beetles and help in better comparison of diversity, composition and distribution among different seasons of the year. Albeit there are many physico-chemical parameters that affects the aquatic environment, the unavailability of instrument for all the parameters had posed constraint to study only current parameters. Consideration of other physico-chemical parameters affecting the water beetles in the freshwater ecosystem would shape the better understanding of the variation of diversity, composition and distribution of water beetles in different habitats.

## **Conclusion**

A total of 1219 water beetles belonging to 7 different families with 30 species were recorded from the study area. The family Dytiscidae was the most common and the family Sphaeriidae was the least in the whole study area. The whole study area had Shannon diversity index of 2.48. while comparing in different habitats, marshy area had highest Shannon diversity index and lowest in streams. A Kruskal Wallis test on water beetle diversity among different habitats indicated no significant differences in water beetle diversity. It was because the sampling was conducted in four different habitat that have different physical and chemical properties. The families Hydrophilidae, Dytiscidae, Gyrinidae and Hydraenidae was distributed in all four habitats. The dendrogram showed that the habitat river and waterlogged area have similar species composition having very closer, 100% similarity in dendrogram scale.

Pearson's correlation indicated no significant correlation between diversity and taxon richness with all three physico-chemical parameters. The evenness with pH showed moderate negative correlation and weak positive correlation with temperature. Canonical correspondence analysis found temperature as a main determining factor in distribution of water beetle.

With broad families encompassing the water beetles, similar study on water beetles in different regions of the country is recommended with particular emphasis on different families. Moreover, Bhutan falls in junction of Palearctic and Oriental Zoogeographic regions, which are known to be rich in diversity of water beetles. Hence, study in high-altitude lakes, which are assumed to be rich in diversity of water beetles are recommended despite social beliefs to keep lakes isolated and undisturbed.

## **Conflict of interest**

The authors declare that they have no affiliations or involvement in any organization or entity with any financial or non-financial interests in the matter to the work reported in this paper.

### **Author contribution statements**

Mr. Tez Bdr. Ghalley conceived and designed the analysis. He was also actively involved in collection of data from the field. In addition, analyzed the data and wrote the first draft of manuscript.

Mr. Ugyen Dorji helped supervise the study. He helped in developing research framework and proposal since the initial stage. As a corresponding author, he also performed the analysis using statistical tools R and reviewed the manuscript after getting comments from reviewers.

Mr. Cheten Dorji with his knowledge in entomology helped in identification of specimens. He also gave critical inputs in formulating research framework and reviewed the manuscript.

Mr. Arjun Nepal and Mr. Namgay Shacha contributed to the interpretation of the results and provided critical feedback and helped shape the research, analysis and manuscript. Both were also involved in planning and supervising the field works.

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