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DIVERSITY OF AQUATIC BEETLES (Dytiscidae) ALONG THE ALTITUDINAL GRADIENT AND ITS RELATIONSHIP WITH PHYSICO-CHEMICAL PARAMETERS OF WATER AT TASHIDING, TSENDAGANG AND GOZHI GEWOGS, DAGANA, BHUTAN

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Abstract

Dytiscidae plays a vital role in the ecosystem; purifies water and serv es as the prey on many organisms. This family of aquatic beetles as predators feeds on small organisms. They are sensitive to environmental changes, it is used as a biodiversity indicator and also a tool for conservation assessment. This study assessed the diversity of Dytiscidae in relation to physico-chemical parameters of water using stratified random sampling at three Geogs in Dagana. Furthermore, the relationship of Dytiscid diversity and taxon richness with the physico-chemical parameters of the lentic and the lotic water bodies along the altitudinal gradient were determined and collected specimens. The total 664 individuals were collected in which five subfamilies of Dytiscidae, 12 genera and 17 species were identified. Pearson's correlation showed moderately negative correlation between the species diversity and taxon richness based on altitude and pH ($p < 0.05$). Temperature had moderate positive correlation with species diversity of Dytiscidae. Canonical correspondence analysis (CCA) showed temperature had an effective determining factor for distribution of Dytiscidae. Kruskal Wallis test among different habitats and altitudinal strata showed significant difference in Dytiscids diversity and taxon richness ($p < 0.05$). The diversity and richness showed a decreasing trend when the altitude increased. The highest diversity was found in marshy area ($H = 2.36$) and second altitude stratum (800 – 1200 masl) ($H = 2.07$) in the current study area. Therefore, the best conservation strategy of Dytiscidae could be to protect these areas before they are degraded.

Keywords: Coleoptera, Diversity, Dytiscidae, Elevation, Physiochemical Parameter.

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1. Introduction

Coleopteran is the most specious order of insect in animal kingdom occurring all over the world except Antarctica (Bouchard et al., 2011). There are approximately 4,00,000 species described in 170 families out of which 12,600 species; 30 families are aquatic representatives (Jach and Balke, 2008; Archangelsky et al., 2009). They play a vital role in the ecosystem; purifying the water and serving as the prey for many organisms such as fishes, birds and reptiles (Dettner, 2014). They are sensitive to changes in the environment. They are considered as the indicator for biodiversity and use for assessment on conservation in New Zealand (Ward, 2004; Pawson et al., 2008; Leschen et al., 2012).

Variety of macro invertebrates which includes Coleopteran species occupy different spaces depending upon on the natural variables of the rivers, such as depth, width, width, type of substrate, water speed and physicochemical variables (such as dissolved oxygen, total dissolved solid, pH, temperature, electrical conductivity etc.), which are often altered by human and natural intervention (Kantzaris et al., 2002; Wangyel, 2013). And their diversity too differs along the altitudinal gradients. For instance, Dytiscids were found to be most diverse between 1400 to 1600 masl as in the case of Mongolia by Enkhnasan and Boldgi (2019). Water beetles can be either predators, scavengers or herbivores. Herbivores feed on phyto-planktons such as algae or leaves. Scavengers consume decomposed organic materials such as aquatic vegetation and other small organisms. The great diving beetle belonging to Dytiscidae family is a predator, which feeds on small organisms such as tadpoles, worms or fingerlings (Epler, 1996).

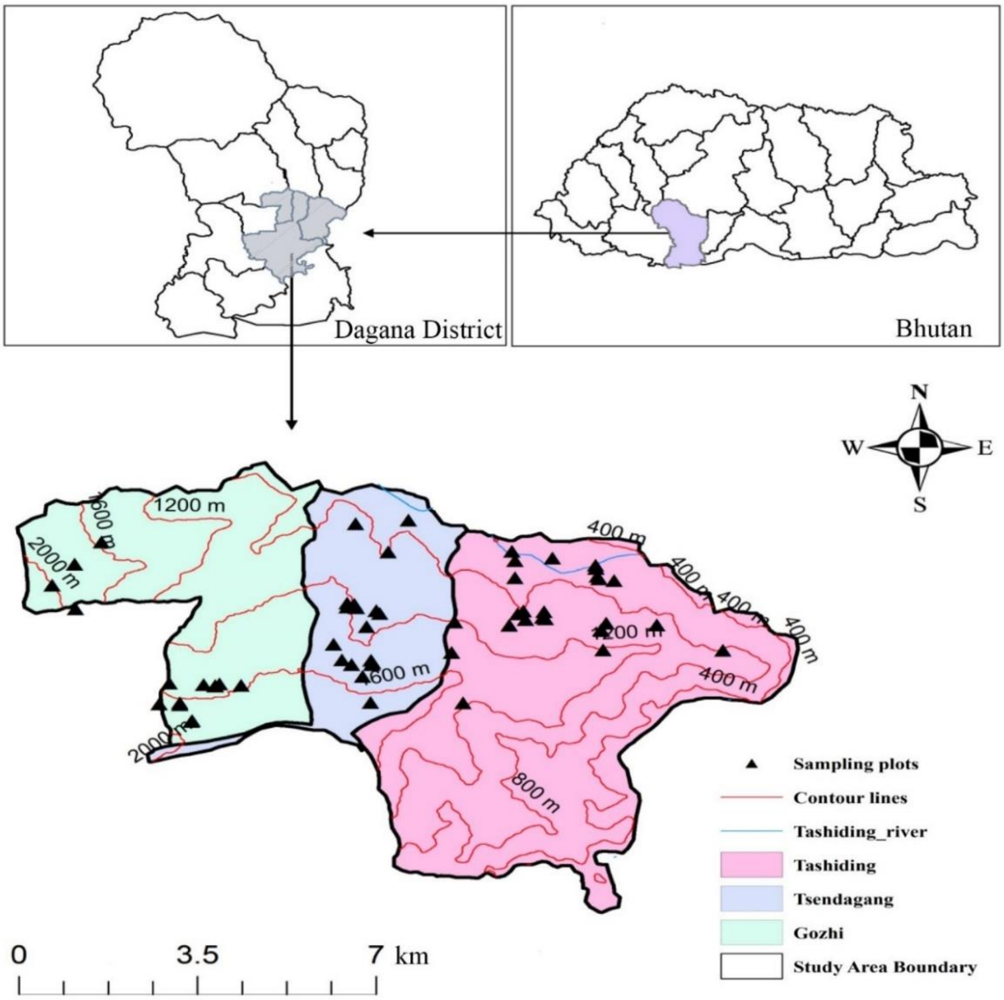
At present, the Dytiscidae family has more than 4,000 described species in numerous genera. The 17 species of Dytiscidae (Wewelka, 1975) were recorded in Bhutan, and in 2005, 24 species of Dytiscidae were reported according to a study conducted by Brancucci and Wewelka (2005). This shows that only a few studies have been carried out in Bhutan because of inadequate knowledge on the water beetles and other freshwater biodiversity in the country (Gurung et al., 2013; Gurung and Thoni, 2015; Dorji, 2016).

Countries such as China, Japan and Taiwan, the *Cybister* sp. are being consumed (Jach, 2003; Flores and Zafaralla, 2012; Lawrence and Slipinski, 2014). Besides those countries, it is also consumed by few local people in the study area as a salad and it helps in blood circulation (P. Lethro, personal communication,10th February, 2021). Therefore, there is a chance of extinction of Dytiscidae because it will be exported to the above-mentioned countries or heavily harvested for self-consumption or its medical value. Thus, conservation is essential. However, no specific study was carried out on altitudinal diversity pattern and effect of water physico-chemical parameters on the diversity of Dytiscidae is conducted in the country. The main objectives of the study are (i) to determine the most effective water parameters that affect the diversity of Dytiscidae, and (ii) to determine the favorable altitude range for Dytiscidae growth.

2. Materials and methods

2.1. Study area

Dagana district has a total area of 1,723.893 square kilometers located with the altitudinal range of 1,300 to 3,000 meters above sea level in the south western part of Bhutan (Forest Resources Management Division [FRMD], 2017). The study was conducted at different places such as Tashiding, which is located within the



coordinates of 26º55'00''N latitude and 90º00'00''E

Figure 1: Map of study area

longitude, and Tsendagang which is within the coordinates of 26º52'30''N latitude and 89º55'00''E longitude. The study has been extended to Gozhi Geog as the stream habitat at higher altitude was found dried in the former two Geogs (Figure 1). Dagana district has a warm and temperate climate. The average annual rainfall of the study areas is 1316.7 mm. The temperature ranges from 13.2 – 22.4^{0} C (National Center for Hydrology and meteorology, 2020). The relative humidity of the atmosphere is 69.79% (Dagana Dzongkhag Administration, 2018). The soil type is mostly sandy loam with slightly acidic in nature (Tshering et al., 2020).

2.2. Sampling methods

Stratified random sampling was applied for the survey. The number of dytiscid species were collected from streams, ponds and marshy areas along the altitudinal gradient. The altitude was further stratified into four ranges (400 m – 800 m, 800 m – 1,200 m, 1,200m – 1,600 m, 1,600m – 2,000 m). Five sampling plots (standardized) were randomly laid out for each type of freshwater habitat in each altitudinal range. The sample plot size of 3 m by 1 m as recommended by Turic et al. (2017) was adopted. Therefore, a total of 60 sample plots were marked and collected data.

Samples were collected using a water net (250 µm - 1 mm mesh – mesh sizes sufficiently small to catch all aquatic beetles) as recommended by Boukal et al. (2011) and Turic et al. (2017). Field sample specimens were euthanized using 10% formalin solution, then stored in 70% ethanol. It was identified using standard keys to identification of water beetle families of Bhutan which is excerpted from Jach and Balke (2008) with that of Beutal and Raffaini (2003).

2.3. Data collection

The pH, temperature, total dissolved solid (TDS), dissolved Oxygen (DO), salinity, resistivity and electrical conductivity (EC) of the water samples were measured using a water testing device. The collected specimens were euthanized with ethyl acetate and preserved in 70% ethanol for identification. Then samples were identified in the laboratory using a light microscope, the identified samples were compared based on the physical appearance from internet resources and seeking help from the taxonomist.

2.4. Data analysis

The species diversity of dytiscids were analyzed using Shannon's diversity index. The taxon richness was calculated using R= (S-1)/LogN (Wilson, 1992) and Shannon evenness using the formula $EH = H'/\text{Hm}$. Relative abundance was calculated for individual subfamilies and the species. Berger-Parker's dominance index (Das and Gupta, 2010) were used to determine the species dominance in a habitat and an altitudinal stratum. The graphs were plotted using Microsoft excel. The Kruskal Wallis test was performed using the statistical software IBM SPSS statistics version 23 to compare the variations in species diversity, richness and evenness among different altitude strata and habitats. Pearson's correlation was performed using statistical software IBM SPSS statistics to assess the relationship between indices and the physico-chemical parameters.

Canonical correspondence analysis (CCA) was followed using PC-ORD software to find the dytiscids distribution according to the physico-chemical parameters.

3. Results and discussion

3.1. Species composition of dytiscids in whole study area

From the study area**,** a total of 664 dytiscids, belonging to five different subfamilies with 17 species (Table 1) were found. The subfamily of Dytiscidae, i.e., Dytiscinae (*n*= 272, *RA*= 40.36) was the most abundant, and the least was Agabinae (*n*= 3, *RA*= 0.452). Amongst all the species *Platynectes kashmiranus* J. Balfour Browne (*n*=192, *RA*=28.92) belonging to the subfamily Dytiscinae was found to be the most abundant and the least was *Agabus bipustulatus* Linnaeus, (*n*= 3, *RA*= 0.452) belonging to the subfamily Agabinae. *Platynectes kashmiranus* J. Balfour Browne was most abundant because it adapts to different habitats compared to *Agabus bipustulatus* Linnaeus. It may be because of its adaptability to a wide range of physico-chemical parameters. The five subfamilies of Dytiscidae found in the study area were Colymbetinae, Dytiscinae, Hydroporinae, Agabinae
and Laccophilinae. The family Dytiscinae had five species, which makes 29% of the total species, followed by Laccophilinae with four species (24%).

The Hydroporinae consists of four species which makes 23% of the total species, followed by Colymbetinae with three species making 18% of total species and Agabinae with one species making 6% of the total observed species (Figure 2). Lawrence and Slipinski (2013) and Yee (2014) had found Hydroporinae and Agabinae are the largest dytiscid subfamilies on earth and the dominant groups in most habitats. However, the current study indicates Dytiscinae was the most abundant due to small sample area/size in three regions. *Agabus conspersus* and *Agabus bipustulatus* (Taher and Heydarnejad, 2019) are the most common insects belonging to species *Agabus*. However, the species ranking and Berger parker's dominance for all the species in the study area resulted that *Platynectes kashmiranus* (relative abundance = 28.96 %) belonging to subfamily Dytiscinae was the most dominant, and the *Agabus bipustulatus* (relative abundance = 0.45%) belonging to Agabinae was the least dominant species (Figure 3 and 4). This may be because the study area has the factors essential for distribution more suitable to *Platynectes kashmiranus* than *Agabus bipustulatus*.

Table.1: Dytiscidae in the study area with subfamily, species, number of individuals and relative abundance

SN.	***Subfamily***	***Species***	***Count***	***RA (Species)***
1	Colymbetinae	*Rhantus exosletus* Foster, 1771	20	3.012
		Colymbetes paykulli Erichson, 1837	40	6.024
		Rhantus articolor Aube, 1838	13	1.958
2	Agabinae	*Agabus bipustulatus* Linnaeus, 1767	3	0.452
3	Dytiscinae	*Hydaticus bipunctatus bipunctatus* Wehncke, 1876	9	1.355
		Platynectes kashmiranus J. Balfour Browne, 1944	192	28.916
		Thermonectus basillaris Harris, 1829	19	2.861
		Cybister lateralimarginalis De Geer, 1774	9	1.355
		Hydaticus conspersus Regimbart, 1899	43	6.476
4	Hydroporinae	*Nebrioporus melanogrammus* Regimbart, 1899	20	3.012
		Methles cribatellus Fairmarie, 1880	20	3.012
		Liodessus sp. Sharp, 1882	96	14.458
		Hyphydrus ovatus Linnaeus, 1761	12	1.807
5	Laccophilinae	*Laccophilus undatus* Aube, 1838	63	9.488
		Laccophilus minutus Linnaeus, 1758	21	3.163
		Laccophilus poecilus Klug, 1834	61	9.187
		Laccophilus flexuosus Aube, 1838	23	3.464
	5	**17**	**664**	**100**

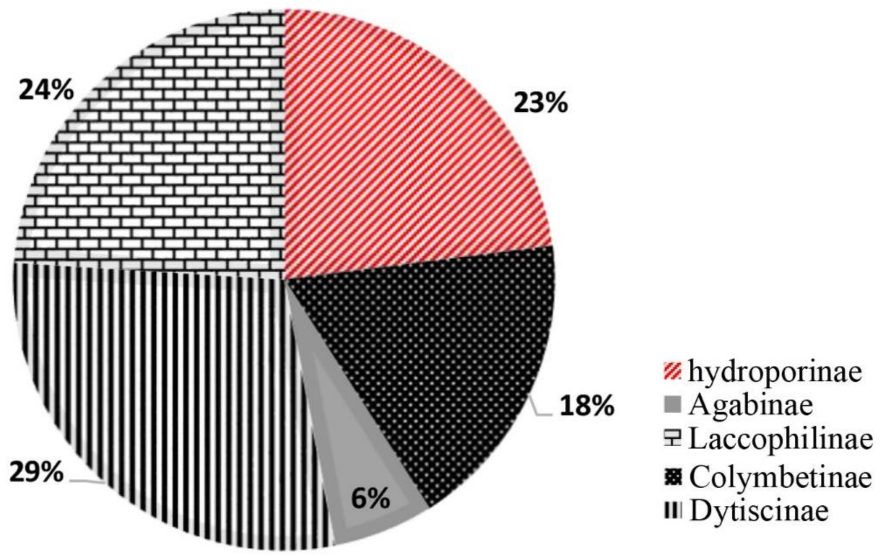


Figure 2: Species composition in whole study area

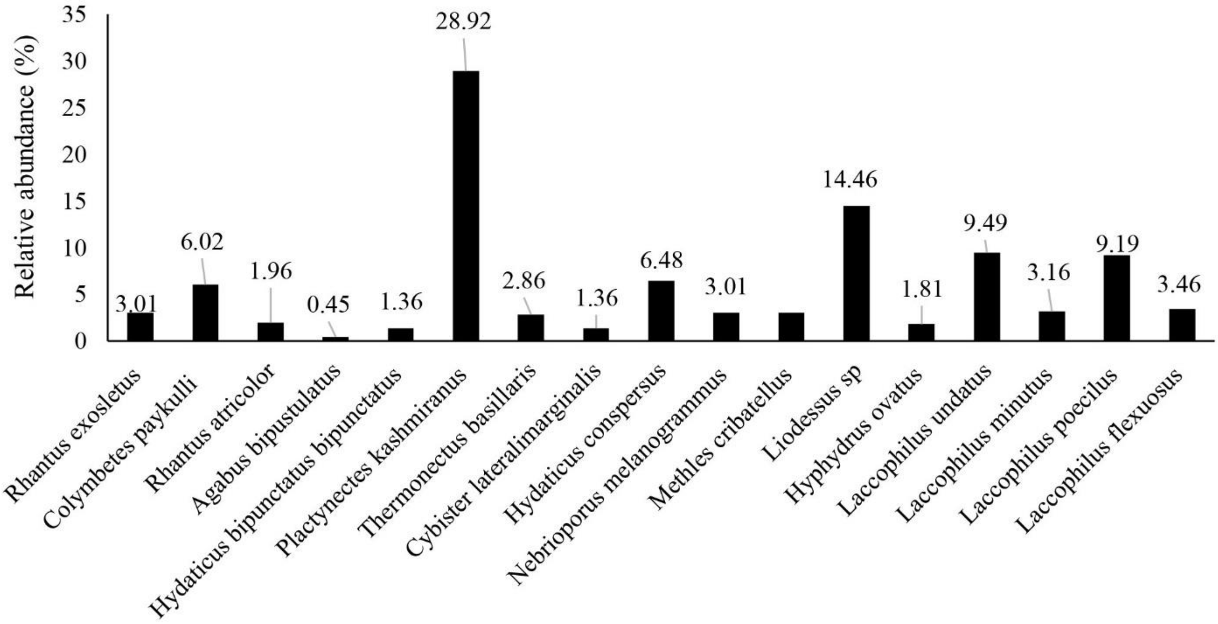


Figure 3: Relative abundance of species in whole study area

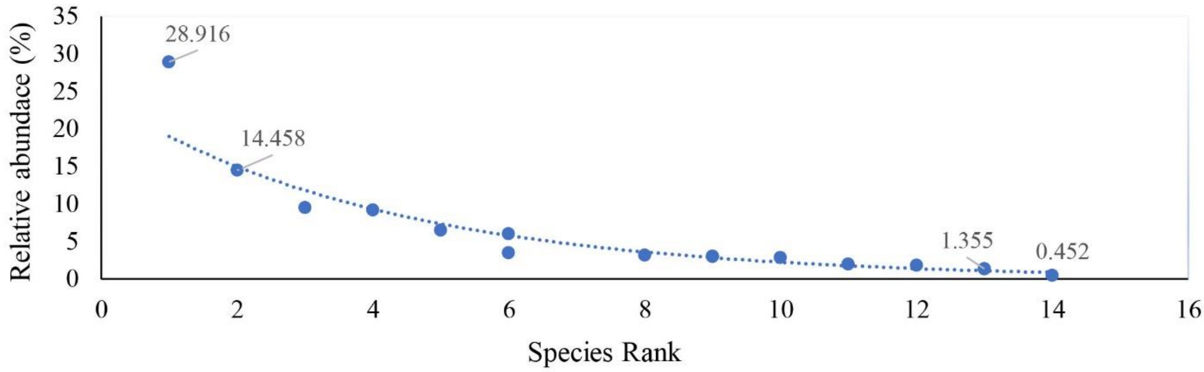


Figure 4: Species ranking of Dytiscidae in whole study area

3.2. Diversity along the altitudinal gradient

The comparison of indices comparison among the altitudinal gradient showed the stratum two with the altitude ranging from 800 masl – 1200 masl had the highest Shannon's diversity index *H'* = 2.07, evenness E_H = 0.87, taxon richness S_R = 2.19 and dominance P_{imax} = 0.21 followed by stratum one (400 masl – 800 masl) with diversity *H'* = 1.90, evenness E_H = 0.88, taxon richness S_R = 1.17 (Figure 5) and dominance P_{imax} = 0.212. The difference in these results among different altitude gradients is because of the diversity of macroinvertebrates which are indirectly influenced by the nearby land use types which influence the chemical parameters of the water (Dhakal, 2006).

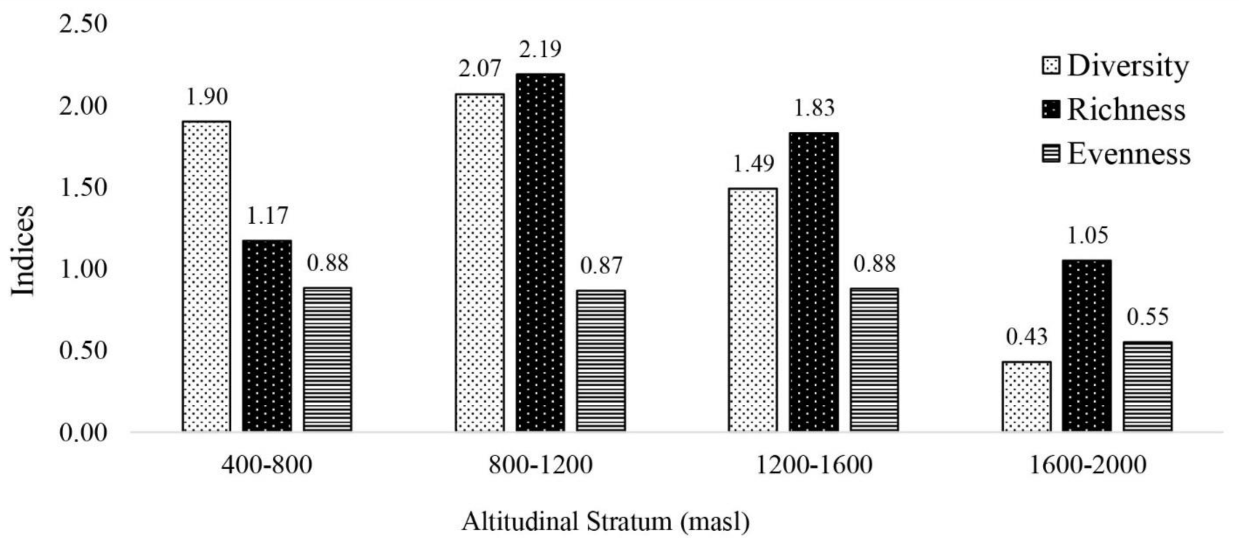


Figure 5: Shannon Wiener diversity, evenness and evenness in four altitudinal strata

The Kruskal Wallis test among four different altitudinal strata also showed significant difference in Dytiscid diversity $X^2(3) = 8.502$, $P = 0.037$ and taxon richness $X^2(3) = 9.893$, $P = 0.037$ (Table 2). According to Li et al. (2012), it is mentioned that it is difficult to pinpoint the driving forces of deterioration of macroinvertebrate biodiversity; the macroinvertebrate community varies from habitat to habitat depending on various environmental parameters. Che Salmah et al. (2013) also supported that the hydrological and physico-chemical variables affect the aquatic macroinvertebrates.

Table 2: Kruskal Wallis test for Shannon diversity, richness, evenness and abundance among four different altitudinal strata

SN.		*Diversity*	*Richness*	*Evenness*
1	*Chi Square*	8.502	9.893	7.403
2	*Df*	3	3	3
3	*p*	0.037	0.019	0.06

The diversity and richness have been found to decrease with increase of temperature of water (Jacobsen, 2004). Several studies have revealed that the temperature influences species diversity, richness, composition and distribution of aquatic organisms, among aquatic habitats (Jacobsen et al., 1997; Rivers-Moore et al., 2013; Dallas et al., 2015). Temperature is an abiotic variable which changes with the latitude and the altitude impacting the aquatic community structure and species diversity (Jacobsen, 2004; Dallas et al., 2015).

Table 3: One way ANOVA test for temperature among four altitude strata

SN.		*SS*	*df*	*MS*	*F*	*p*
1	Between Groups	1881.45	3	627.15	4.588	0.006
2	Within Groups	7928.06	58	136.691		
	Total	9809.51	61			

Table 4: One way ANOVA test for TDS among for altitude strata

SN.		*SS*	*df*	*MS*	*F*	*p*
1	Between Groups	89.533	3	29.844	4.866	0.004
2	Within Groups	355.694	58	6.133		
	Total	445.227	61			

In agreement with that, in the current study species diversity and richness, it was found to decrease as the altitude increases (Figure 5) because of different habitats and altitudes with different physicochemical properties. On the other hand, it's because there were more prey of Dytiscidae such as mosquitoes, fingerlings of fish and tadpoles of frogs towards the lower altitude in warmer temperature. One way ANOVA among three habitats was performed to compare the values of physico-chemical parameters among four different altitude strata. The result indicated significant difference in temperature $F(3, 58) = 4.866$, $p = 0.004$ (Table 3), TDS $F(3, 58) = 4.588$, $p = 0.006$ (Table 4), EC $F(3, 58) = 3.708$, $p = 0.016$ (Table 5) and salinity $F(3, 58) = 4.992$, $p = 0.004$ (Table 6). Thus, these physico-chemical parameters have influenced them and responded to these differences and showed significant differences in dytiscid diversity.

Table 5: One way ANOVA test for electrical conductivity (EC) among four altitude strata

SN.		***SS***	***df***	***MS***	***F***	***p***
1	Between Groups	6972.48	3	2324.2	3.708	0.016
2	Within Groups	36353.8	58	626.79		
	Total	43326.3	61			

Table 6: One way ANOVA test for salinity among four altitude strata

SN.		***SS***	***df***	***MS***	***F***	***p***
1	Between Groups	196394	3	65464.6	4.992	0.004
2	Within Groups	760542	58	13112.8		
	Total	956935	61			

3.3. Relationship between dytiscids and physico-chemical parameters among different altitude strata

Maximum mean for pH (7.58), temperature (16.86ºC) and DO (10.19 mg/L) was recorded in fourth stratum 1,600 - 20000 masl, first stratum 400-800 masl and second stratum 800-1,200 masl, respectively. The minimum mean for pH (6.98), temperature (14.01ºC) and DO (10.01 mg/l) was recorded in third stratum 1,200 -1,600 masl, fourth stratum 1,600-2,000 masl and first stratum 400-800 masl, respectively. The maximum mean for salinity (306.67 ppm), TDS (31.03 mg/L) and electrical conductivity (16.86 µ.s/cm) was recorded in first stratum 400-800 masl while for resistivity (32.28 Kω.cm) was recorded in the fourth stratum 1,600-2,000 masl (Table 7). Pearson's correlation had shown moderately negative correlation between diversity of Dytiscidae and altitude ($r_s = -4.06$, $p = 0.013$), species richness and pH ($r_s = -0.055$, $p = 0.00$). The species richness showed negatively moderate correlation with resistivity ($r_s = -0.557$, $p = 0.00$) (Table 8).

Table 7: Mean, standard error, minimum and maximum values of physico-chemical parameters in different altitude strata

SN.	***Altitude Strata***	***400-800 (masl)***	***800-1200 (masl)***	***1200-1600 (masl)***	***1600-2000 (masl)***
1	pH	7.25 ±0.36 (6.68-7.92)	7.31 ±0.48 (6.45-8.51)	6.98±0.70 (5.58-8.00)	7.58±2.71 (5.65-17.10)
2	EC	63.76 ±28.90 (3.16-97.20)	47.43 ±31.31 (13.25-133.70)	36.79 ±15.16 (13.84-73.00)	38.42 ±21.57 (14.23-96.60)
3	Temp	16.86 ±1.83 (14.30-21.00)	16.50 ±2.97 (11.00-22.70)	14.62 ±2.37 (10.70-19.40)	14.01 ±2.58 (10.20-20.70)
4	TDS	31.03 ±13.41 (4.50-47.00)	24.11 ±14.53 (11.84-63.60)	17.24 ±7.14 (6.42-34.50)	17.99 ±10.28 (6.63-45.50)
5	DO	10.01 ±0.76 (8.88-11.21)	10.19 ±0.61 (9.55-11.55)	10.13 ±0.60 (9.08-11.222)	10.18 ±0.95 (8.99-11.8)
6	Salinity	306.67±138.7 (100-500)	218.75±132.76 (100-600)	162.50 ±71.88 (100-300)	173.33±103.28 (100-400)
7	Resistivity	21.35 ±17.26 (10.14-71.80)	22.88 ±11.52 (0.01-39.60)	31.95 ±13.63 (13.62-71.10)	32.28 ±13.71 (10.36-70.40)

Table 8: Pearson correlation between diversity indices, richness, evenness, altitude and physiochemical parameters

	Diversity	***Richness***	***Evenness***	***Altitude***	***pH***	***Tempt***	***EC***	***DO***	***TDS***	***Salinity***	***Resistivity***
Diversity	1	-0.159	0.967*	-0.406*	0.104	0.535	0.06	0.066	0.037	-0.074	0.252
Richness		1	-0.382*	-0.107	0.563*	-0.005	-0.039	0.294	0.022	0.207	-0.557*
Evenness			1	-0.414*	-0.001	0.244	0.101	0.006	-0.045	-0.08	0.371
Altitude				1	-0.399*	-0.462*	-0.293	-0.001	-0.14	-0.345*	-0.04
pH					1	0.17	0.105	-0.058	-0.172	-0.045	-0.07
Temp						1	0.380*	0.04	0.099	0.337*	-0.262
EC							1	0.036	0.033	0.016	-0.083
DO								1	0.164	0.349*	-0.277
TDS									1	-0.058	-0.124
Salinity										1	-0.234
Resistivity											1

The pH range of 6.5 to 9.0 is optimal for the freshwater macroinvertebrates (United States Environmental Protection Agency, 1986). Complying this fact, the current study too found a mean of pH within the range recommended by USEPA. The positive correlation between diversity and richness with water temperature explains that water beetles tolerate warmer water. The temperature increases due to the absorption of heat by the water molecules as a result of accumulation of suspended particles in the water (Paaijmans et al., 2008). However, the study of Flores and Zafaralla (2012) on macroinvertebrate composition, diversity and richness in relation to water quality status of Mananga river in Philippines discovered that taxa richness, diversity, abundance and evenness were negatively correlated with water temperature. This may be because the study was conducted in a single habitat. The species richness of Dytiscidae subfamilies has been reported to increase with increasing variety of water bodies (Lawrence and Slipinski, 2013; Yee, 2014). The Canonical Correspondence Analysis had shown that temperature was the most effective factor influencing the distribution of Dytiscidae.

Canonical correspondence analysis between dytiscid species, physicochemical parameters and the altitude strata

Canonical correspondence analysis (CCA) was performed to determine the distribution of dytiscids according to the physico-chemical parameters (Figure 4). The eigenvalues for CCA axis 1 and axis 2 were 0.385 and 0.297, respectively. The eigenvalue for axis 3 was 0.284.

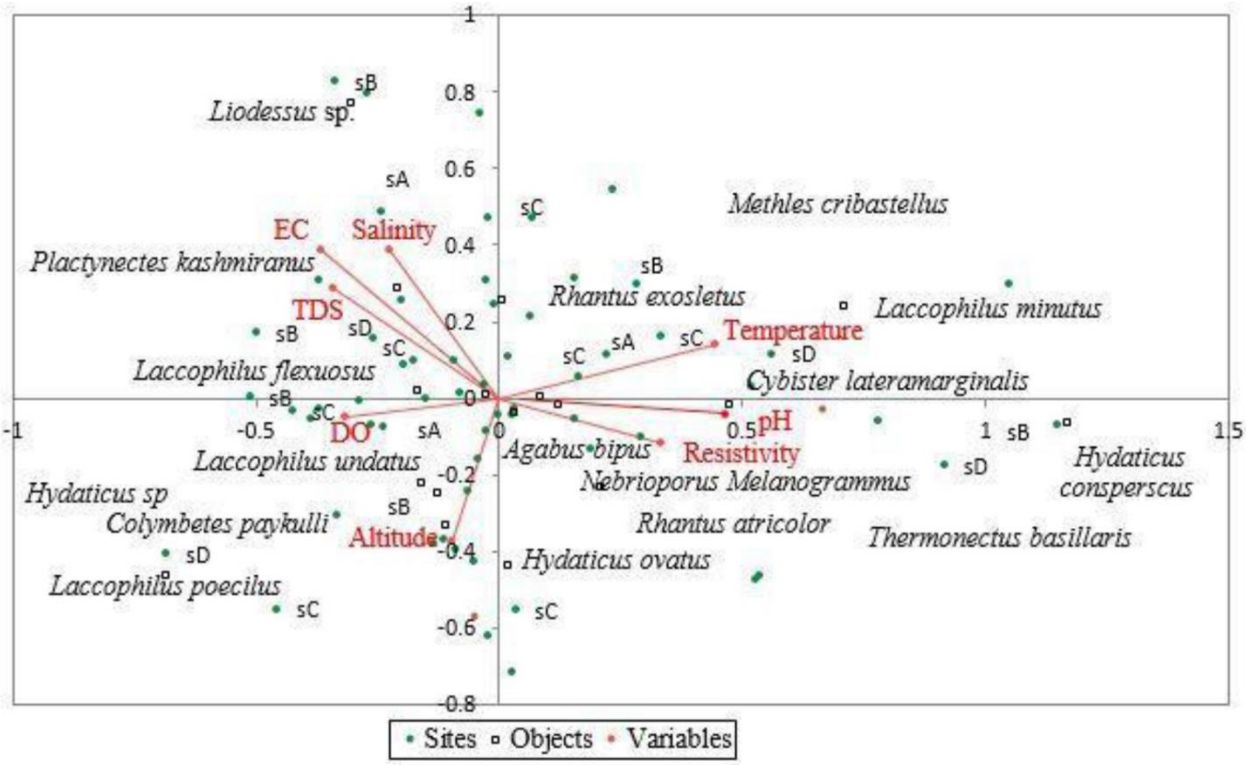


Figure 5: Canonical correspondence analysis showing species, altitude stratum and water parameters

Eigen values measures niche separation in which the competing species use the space differently in a way that lets them coexist (Ter Braak and Verdonschot, 1995). The highest eigen value was found in axis 1 and the variable is temperature (0.667). The pH (r = -0.446) was negatively correlated with axis 1, EC (r = - 1.065) positively correlated with axis 2, TDS negatively correlated with axis 3, DO (r = -0.373) negatively correlated with axis 3, salinity (r = -0.226) negatively correlated with axis 1 and resistivity (r = 0.543) positively correlated with axis 3. Temperature was positively correlated with axis 1 with highest eigen value proving it as the main determining factor of the Dytiscidae family. Similar finding was stated by Chuche and Thiery (2012), where the water temperature was found to be the most effective variable in development rate in terrestrial and aquatic insects.

Distribution of *Rhantus exolstelus*, L*accophilus minutus*, *Methles cribatellus* and *Cybister lateralimarginalis* were positively correlated with temperature (Figure 5). For the distribution of species like *Agabus bipustulatus, Nebrioporus melanogrammus, Rhantus articolor, Hydaticus conspersus, Hydaticus ovatus, Thermonectus basillaris* and *Colymbetes paykulli*, the parameters like pH and resistivity are the most effective factor. Similarly, species like *Laccophilus poecilus, laccophilus undatus* and *Hydaticus bipunctatus* are associated with DO and altitude. Other factors in which the species associated were electrical conductivity, salinity and total dissolved solids (TDS) for *Platynectes kashmiranus, Liodessus* sp. and *Laccophilus flexuosus*. The most effective water parameter found was the temperature as it was positively correlated with the highest Eigen value with the axis 1. However, the study was carried out in a limited time; in one month with small study area, and it do not display the data at large to represent the study area.

Conclusions and recommendations

So far, the Dytiscidae family has more than 4,000 described species in 12 numerous genera around the globe. Bhutan has recorded 24 species of Dytiscidae till now. The current study recorded 17 species of dytiscidae belonging to five subfamilies and 12 genera out of which eight species were new to Bhutan. The subfamily Dytiscinae was the most common, and the subfamily Agabinae was the least in the whole study area. The marshy area and the second altitude stratum (800 – 1,200 masl) were found to be the most suitable habitats for the dytiscids, because these habitats had shown the highest Shannon diversity indices, i.e., H = 2.44 and H = 2.19, respectively. The altitudinal gradient had shown a significant effect on the diversity of Dytiscidae. It was found to decrease as the altitude increases which is in line with the finding of the past studies. It is because the sampling was undertaken in different habitats and altitude where the physico-chemical parameters were also found to differ significantly.

The significant positive correlation between diversity and richness with water temperature was determined. The temperature was found warmer towards the lower altitude. This explained that water beetles can tolerate warmer water. The tolerance of dytiscids in warmer temperatures were because of their ability to carry a bubble of air (Oxygen) at the end of their body. They had the ability to recycle air bubbles from the surface of the water. Moreover, warmer the temperature, more available will be the prey for predaceous diving beetles. In agreement to this observed behavior, the Canonical correspondence analysis also found temperature, pH, EC and altitude as an effective factor in the distribution of Dytiscidae.

However, this study was done based on the data collected in a single month. Hence, similar studies on water beetles in different regions of the country focusing on foraging behavior is suggested. A robust study covering large areas in different seasons should be done because their habitats are more available during summer due to availability of water. Therefore, better results may be found if it is taken up in all four seasons. Moreover, Bhutan falls in the junction of Palearctic and Oriental zoogeographic regions which are assumed to have rich diversity of water beetles.

Authorship contribution statement

Wangchuk Blon devised the general idea, collected data, analyzed data, wrote and edited the content. Tashi Dendup, Cheten Dorji and Wangdi contributed ideas, provided insights and suggestions for better results and edited the contents.

Conflict of interest

None.

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