Freshwater Plant Diversity in Punakha District, Bhutan

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Abstract

Freshwater plant diversity in different waterbodies of Punakha District was studied to determine and compare the diversity among different waterbodies, study the correlation of freshwater plant diversity with environmental variables, and to record and assess the ideas and knowledge of the locals about freshwater plants. Area based survey was used to record the plant species from a total of 80 plots, 100m^2 area consisting of 4 plots of 5m × 5m from each water body. The area-based survey method directly compares the plant diversity of different waterbodies with different dimensionality and characteristics. Simpson's diversity index, Pearson correlation, Canonical correspondence analysis and Chi Square analysis were used to analyze the diversity, correlation of the freshwater plant species with environmental variables and social data. A total of 72 freshwater plant species distributed among 57 genera and 37 families were recorded. The diversity index for the freshwater plant was found to be the highest in the ponds (0.951), followed by ditches (0.939), streams (0.928), and lakes (0.913). Correlation and Canonical correspondence analysis showed decreasing diversity with increase in water velocity, depth, and altitude. Chi Square statistics analysis showed the association of gender, age, and education level of the respondents with awareness on freshwater plants. Although the locals are aware of the benefits of freshwater plants, their awareness and knowledge did not include the vital ecological roles of the freshwater plants.

Keywords: Awareness**,** Diversity, Correlation, Freshwater plant, Waterbodies

Introduction

Freshwater plant is a common yet important component of the freshwater systems (Netherland & Schardt, 2009). It exhibits strong physiological resemblance to its terrestrial ancestors with adaptation to waterlogged and submerged conditions (Sculthorpe, 1967). Freshwater plants hold an important role in the ecological functions and stability of the freshwater ecosystem (Silva, Costa, Melack & Novo, 2008). Freshwater plant diversity and richness is an essential parameter that is entirely reliant on the water quality and nutritional content of a water body (Ansari et al., 2017). Also, several studies have concluded altitude, water velocity, and depth as prime factors that influence the diversity and the distribution pattern of freshwater plants and better predict their species richness (Chambers et al., 1991, Jones; Li & Maberly, 2003; Bao et al., 2018).

Bhutan is blessed with rich supplies of fresh water, with an average flow of 2,238 m^3/s accounting up to 70,572 million m^3 per annum (Rizal, 2020). Freshwater encompasses the freshwater ecosystem which includes rivers, streams, ponds, wetlands, and lakes.

These freshwater ecosystem forms a vital aquatic ecosystem connecting the aquatic biodiversity (Wangchuck Centennial Park, 2012) which includes micro and macroinvertebrates, birds, mammals, fishes, and plants. Mostly studies have been conducted on fishes, birds, invertebrates, and mammals (NBC, 2019), however, a complete description and report on aquatic floras in Bhutan still not available. A major focus has been placed on iconic species and very little on other, especially plant, species has been studied, resulting in a significant literature gap and low social awareness of the diversity of smaller plants, including freshwater plants.

Studies on the diversity and distribution pattern of freshwater plants in different water bodies of Punakha District has not been conducted previously. Information regarding the biodiversity value of different water bodies is an important requirement for planning strategic conservation goals (Williams et al., 2004). This study, therefore, aims to collect data for an inter-water body comparison for Punakha District.

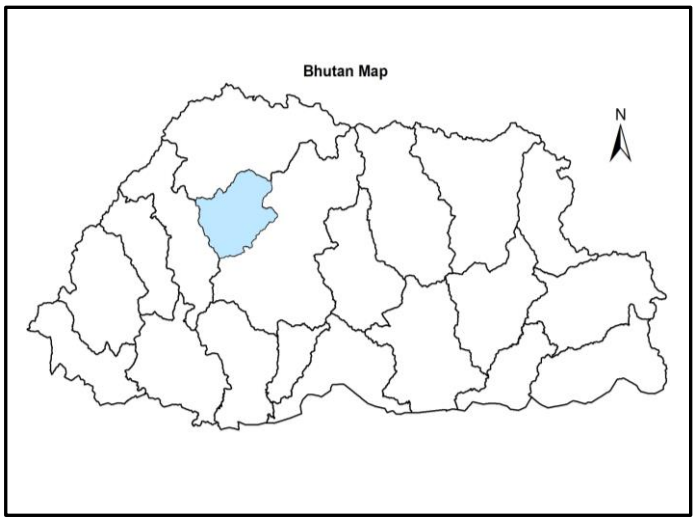
Objectives

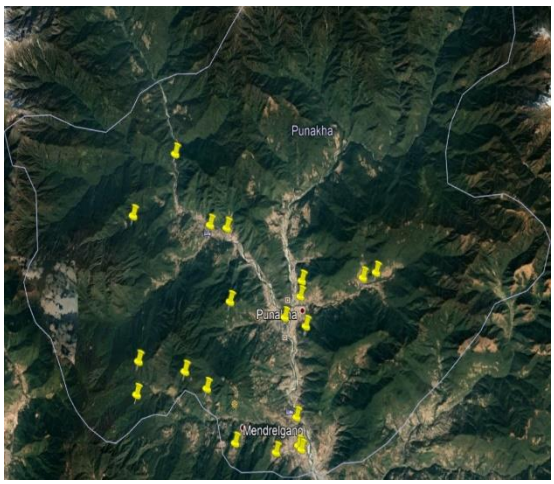
i. To determine freshwater plant diversity in different waterbodies.
ii. To study the species diversity with environmental variables.
iii. To assess local people's ideas and knowledge about the plant species prevalent in the freshwater bodies.

Materials and methodology

Study area

Punakha is situated in west-central part of Bhutan and lies at an altitude of 1,242masl (Dorji, 2014). The District is located within geographical coordinates 27.5921^0 N and 89.8797^0 E and is bordered by Thimphu, Gasa, and Wangdue Phodrang districts. The area receives an annual rainfall of 500-1500mm and has a temperature ranging from 5 degrees Celsius to 30 degrees Celsius. The District is located in the temperate region and experiences hot and humid summers with heavy rainfall during the monsoon months of June, July, and August and has moderate winters. The elevation, climatic condition, and temperature range make the District home to diverse floral species (Tshering, 2019). The District is home to Punatshagchhu, one of the largest rivers in the country. The river is fed by snow and melting glaciers from the Himalayas, its two main tributaries, Pho chhu and Mho chhu, and numerous streams and ditches. Punakha has abundant freshwater.





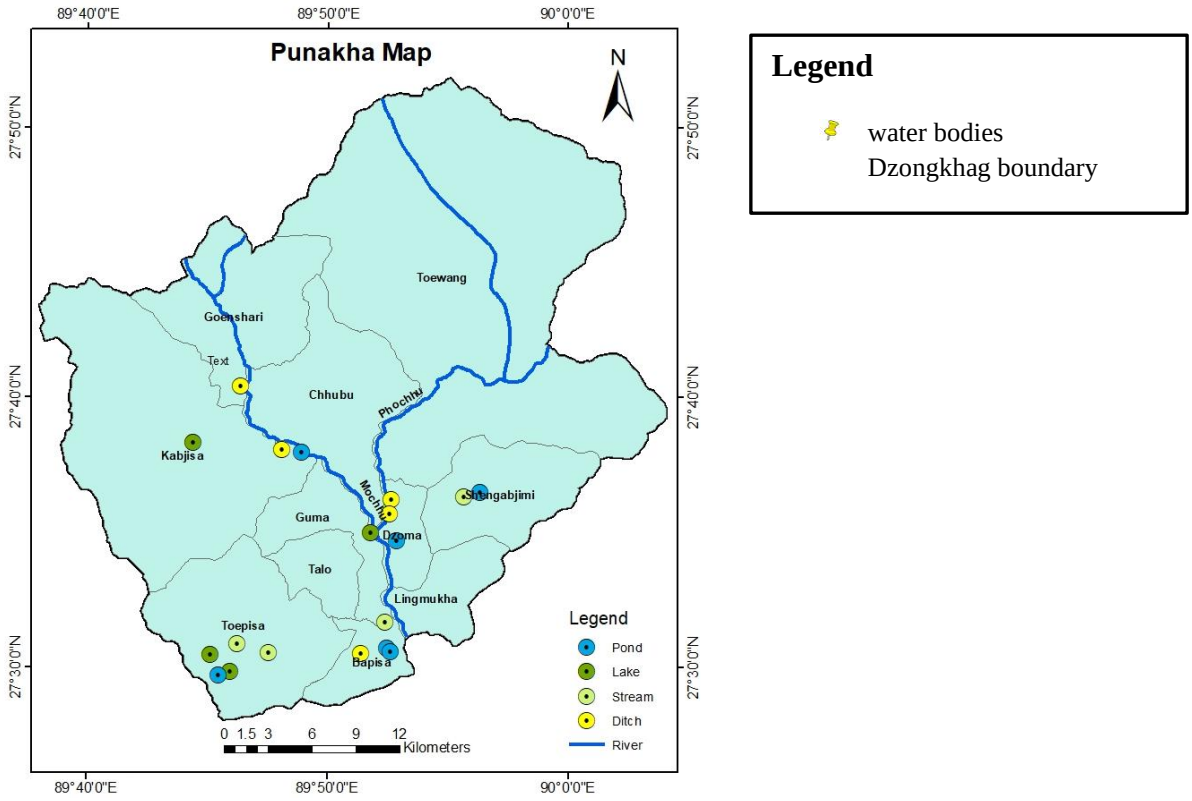


Figure 1. Study area

Sampling design

Selection of water bodies

A preliminary survey was conducted to stratify the waterbodies in Punakha District into two categories, namely, lotic and lentic water. Stratification was done as the diversity of freshwater plant species prevalent in lotic and lentic waters differ (Davies et al., 2008; Aslan, Biggs & Willaims, 2018). A total of 20 water bodies (10 for each stratum) were selected randomly using excel random generator.

Selection of the interviewee

In order to assess people's ideas and knowledge about freshwater plants available in the water bodies of Punakha, people residing near the water bodies were interviewed using a semi structured questionnaire. The interviewees were selected based on the random sampling method. A preliminary survey was conducted to obtain the number of households near water bodies where the freshwater plant species were recorded. The preliminary survey recorded a total of 78 households. A sample size of 65 respondents (one from each household) was determined using Taro Yamane's statistical formula:

$$n = N/1 + N(e)2 \text{ (Yamane, 1973).}$$

Where n = sample size, N = the whole population, and e = sampling error. A sample size of 65 respondents was determined out of the total population in order to maintain 95% confidence level.

Assessment of freshwater plant diversity

To ensure that the ecological data of the freshwater plant diversity gathered from different waterbodies with widely differing dimensions and characteristics can be directly compared, the sampling was area limited (Williams et al., 2004). Following the area-based survey, data was collected from 80 plots of 100m^2 area for

each water body as the size of the smallest lentic water including its surrounding was 100m^2. The 100m^2 area comprised of 4 sampling sites of 5m x 5m quadrats. The quadrats were laid randomly on the potential sites of each water body. As the riparian vegetation around the water bodies consisting of freshwater plants vary tremendously from 3 to 25m (Diop, 2010), a quadrat size of 5m^2 was employed for recording the freshwater plants.

Analysis

Freshwater plant diversity analysis

The diversity of the aquatic hydrophyte species was determined by the Simpson's diversity index using the following formula:

$$D = 1 - \sum_{i=1}^{s} \frac{ni\ (ni-1)}{N\ (N-1)}$$ (Simpson, 1949)

Where, N = the total cover percent of all species and n = cover percent of a particular species.

Canonical correspondence analysis

Canonical correspondence analysis was conducted using PAST in order to determine the correlation of freshwater plant diversity and occurrence of different waterbody habitats with the environmental variables.

Species rarefaction analysis

The species rarefaction curve analysis was conducted using PAST to determine the expected number of species observed in different water body habitats and to identify if the sampling effort was adequate or not.

Pearson correlation coefficient

The correlation of freshwater plant species with the environmental variables and the correlation between the environmental variables were analysed using PAST. Pearson correlation coefficient test was run to analyze the correlation among the species number and environmental variables.

Social data analysis

The social data was compiled and coded in Microsoft Excel and analysed in SPSS. People's awareness and use of freshwater plant were reported using descriptive statistics. Chi Square test was used to test the relationship of gender, age, and education level of the interviewees with awareness on freshwater plants.

Results

Floristic composition of freshwater plants

A total of 72 freshwater plant species distributed among 57 genera and 37 families were recorded from the study sites. The maximum number of plant species was found under Polygonaceae family (9 species) and the least was recorded under the following families: Acoraceae; Apiaceae; Araceae; Araliaceae; Boraginaceae; Campanulaceae; Cannaceae; Cyperaceae; Gentianaceae; Hydrocharitaceae; Hypoxidaceae; Juncaceae; Lamiaceae; Mniaceae; Onagraceae; Saururaceae; Scrophulariaceae; Selaginellaceae; Tropaeolaceae; Violaceae; and Zingiberaceae with one species each. Fourteen species from the total recorded were not listed in the Flora of Bhutan (Table 1).

Table 1. List of species not recorded in Flora of Bhutan

Species	Family
Campanula erinus Linnaeus	Campanulaceae
Canna indica Linnaeus	Cannaceae
Conyza floribunda Kunth	Asteraceae
Equisetum arvens Linnaeus	Equisetaceae
Equisetum diffusum D.Don	Equisetaceae
Laphangium luteoalbum (L.) Tzvelev	Asteraceae
Nasturtium officinale W.T. Aiton	Brassicaceae
Oxalis accetosella Linnaeus	Oxalidaceae
Pilea pumila (L.) A.Gray	Urticaceae
Plantago major Linnaeus	Plantaginaceae
Ranunculus repens Linnaeus	Ranunculaceae
Rhizomnium punctatum (Hedw.) T.J. Kop.	Mniaceae
Saxifraga hirsuta Linnaeus	Saxifragaceae
Spermacoce latifolia Aublet	Rubiaceae

plant species recorded in and around water bodies

Diversity of freshwater plant species

The highest number of freshwater plant species was found in ponds (n = 46), followed by ditch (n = 44), stream (n = 39), and lake habitat (n = 34). With the evenness value close to 0.5 for all the habitats, the analysis showed that the freshwater plant species were moderately uniformly distributed in all the habitats (Table 2).

Table 2. Diversity indices of freshwater plants of different habitats

	Stream Habitat	Ditch Habitat	Pond Habitat	Lake Habitat
Species number	39	44	46	34
Individual	45	59	44	53
Dominance	0.07195	0.06042	0.04851	0.08683
Simpson diversity	0.928	0.9396	0.9515	0.9132
Shannon diversity	3.073	3.162	3.3	2.836
Evenness	0.5543	0.537	0.5892	0.5015

Margalef	9.982	10.55	11.89	8.312

Beta Diversity

Beta diversity shows the change in species composition among groups or areas (Anderson, Ellingsen & McArdle, 2006). Table 3 shows the Whittaker beta diversity pairwise comparison for different waterbody habitats. The beta values ranged from zero to one with higher values indicating dissimilarity between two habitats (Legendre, 2008). The analysis showed that ponds and lakes were more similar in terms of freshwater plant species richness and abundance. Ditch habitat showed dissimilarity to lake habitat as the Whittaker comparison value was high. Table 5 further shows that the similarity between stream and ditch and ditch and pond were up to the same extent with same beta diversity values.

Table 3. Whittaker beta diversity pairwise comparison between habitats

Habitat	**Stream**	**Ditch**	**Pond**	**Lake**
Stream	0	0.422	0.365	0.452
Ditch		0	0.422	0.513
Pond			0	0.300
Lake				0

Alpha diversity

Ditch and stream habitats were most similar to each other in freshwater plant richeness and abundance with 0.527 value. Ditch and pond habitat were also found to be similar. Stream habitat was found to be highly dissimilar to lake habitat with 0.239 value.

Table 4. Bray-Curtis similarity matrices of habitats based on freshwater plant species distribution and abundance

Habitat	**Stream**	**Ditch**	**Pond**	**Lake**
Stream	1	0.527	0.358	0.239
Ditch		1	0.459	0.251
Pond			1	0.375
Lake				1

Diversity index between different waterbody habitats

One-way ANOVA test revealed a significant difference in the freshwater plant diversity between different habitats (p = .000) while the Bonferroni Post Hoc test determined insignificant difference in freshwater plant diversity within habitats.

Correlation among Freshwater habitats, species, and environmental variables (CCA)

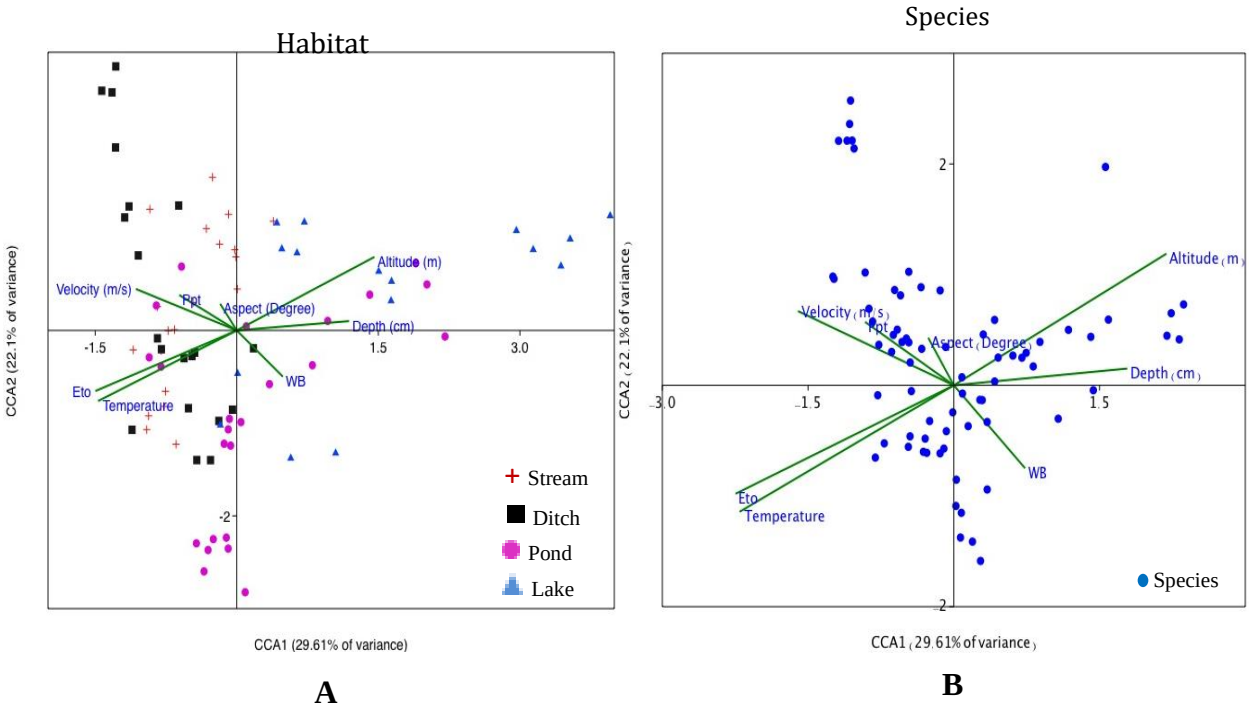


Figure 2: A-Canonical correspondence analysis showing the correlation of habitat with environmental variables, B-Canonical correspondence analysis showing the correlation of freshwater plant with environmental variables

Occurrence of lake habitat showed positive correlation with altitude and depth and showed a significant negative correlation with temperature and Eto (evapotranspiration). The majority of the stream habitats were associated with high velocity and Ppt (precipitation), whereas a majority of the ditch and pond habitats showed negative correlation with velocity and Ppt. Ditches and pond habitats showed significant positive correlation with temperature and Eto (Figure 2A). The closer grouping of ditches and pond habitats indicated that the two habitats are similar and have similar correlation with the environmental variables. The test also concluded that a majority of the freshwater plant species showed negative correlation with water velocity, depth, and altitude (Figure 2B).

Pearson correlation coefficient between species count and environmental variable

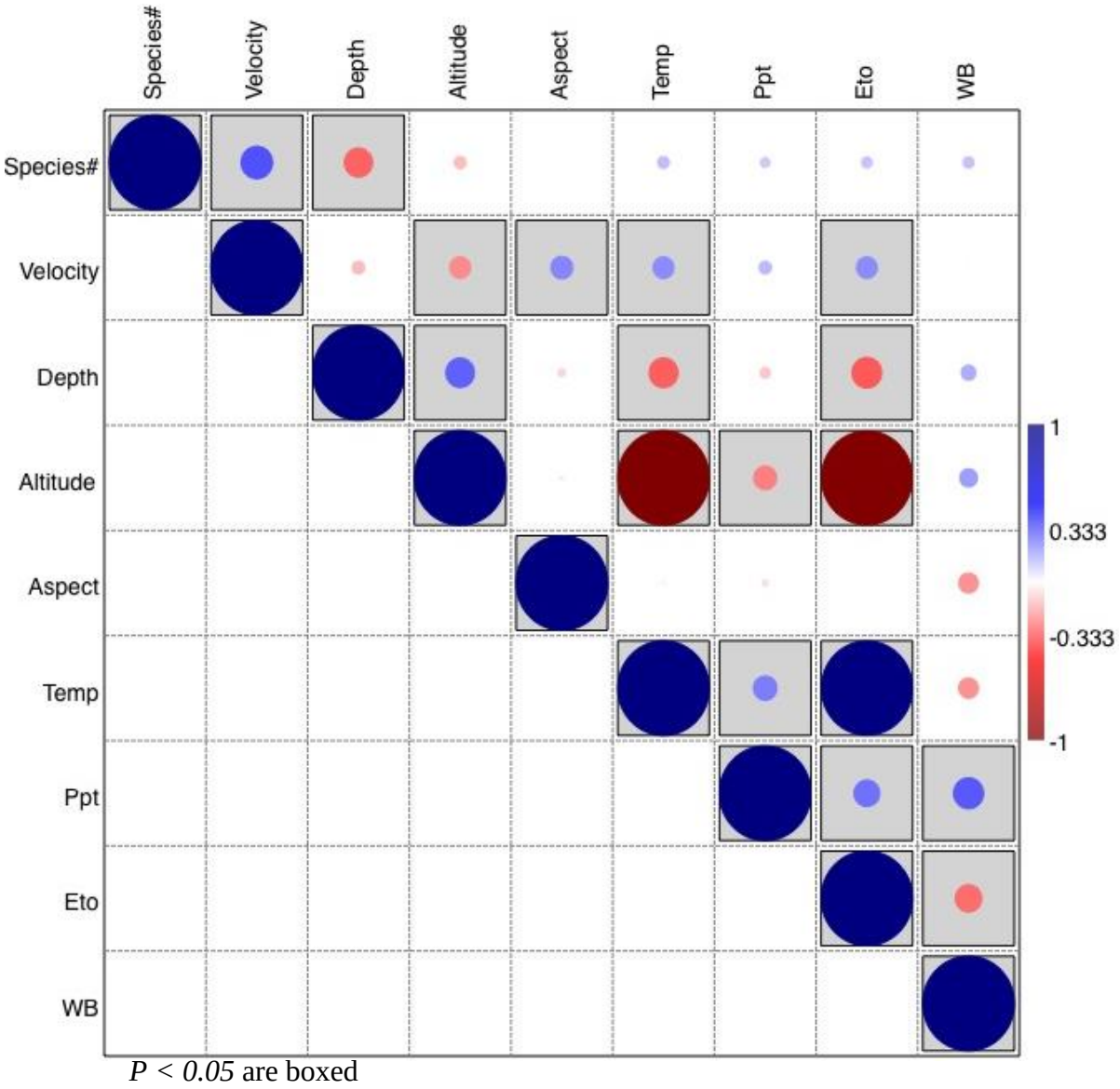


Figure 3. Pearson correlation coefficient

The environmental variables that had major influence on the freshwater plant species count during the present study were water velocity (p = 0.001) and depth (p = 0.005) where by, the species number showed significant negative correlation towards these variables. The species count had a slight negative correlation with altitude (p = 0.260) and a slight positive correlation with temperature (p = 0.273) and Ppt (p = 0.373).

Species rarefaction curve

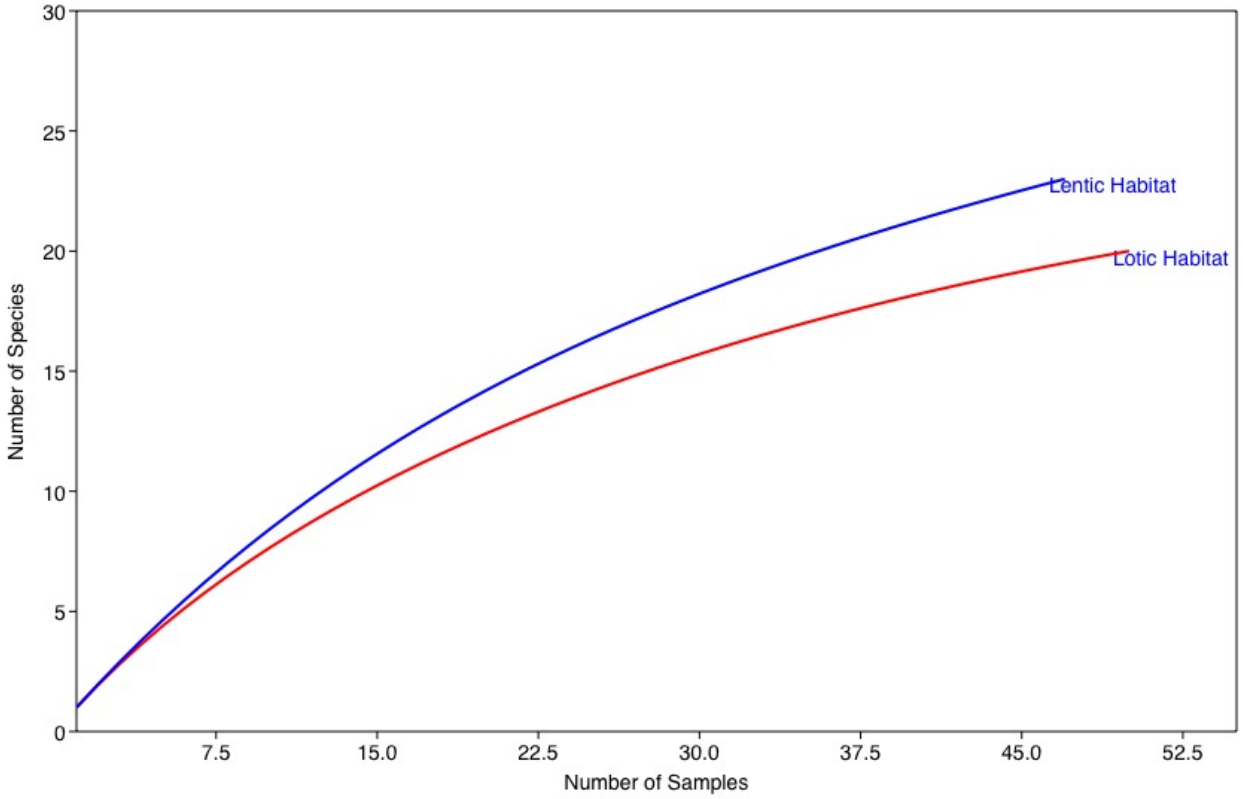


Figure 4. Species rarefaction curve

Higher number of freshwater plant species was recorded from the lentic habitats as compared to the lotic habitats with 23 species in lentic habitats and 20 species in lotic habitats. Owing to the species richness in the lentic habitats, a sampling of 47 plots was adequate to record all the species whereas in case of lotic habitats a sampling of 50 plot was required for recording all the species which is slightly more compared to lentic habitats.

Indicator species

A total of 72 plant species were recorded from the lotic and lentic habitats comprising of streams, ditches, ponds, and lakes. Total freshwater plant species estimated in the lotic and lentic habitats were 20 and 23 respectively. The SIMPER analysis was used to identify freshwater species that contributed to the maximum dissimilarity between lotic and lentic habitats. More than 2% dissimilarity was contributed by 10 species. Results from the analysis concluded that freshwater species contributing to the maximum dissimilarity was Pilea glaberrima in lotic habitat and Juncus inflexus in lentic habitat (Table 5).

Table 5. SIMPER analysis on percent cover of freshwater plants according to Bay-Curtis Groups: Mean 1-Lotic habitat and Mean 2-Lentic habitat Average dissimilarity: 67.28

Species	Av. dissim	Contrib.%	Cumulative%	Mean 1	Mean 2
Pilea glaberrima (Bl.) Bl.	7.558	11.23	11.23	10.4	0.167
Juncus inflexus L.	4.398	6.537	17.77	0	5.99
Nasturtium officinale W.T. Aiton	3.587	5.331	23.1	6.25	2.56

Rumex nepalensis Spreng.	3.154	4.688	27.79	2.35	5.7
Fragaria nubicola Lindl. ex Lacaita	3.1	4.608	32.4	1.6	5.8
Drymaria cordata (L.) Willd. ex Roem. & Schult.	2.519	3.744	36.14	4.15	1.43
Oplismenus compositus (L.) P. Beauv.	2.335	3.471	39.61	3.23	0
Chryososplenium tenellum Hook. F. & Thoms.	2.239	3.328	42.94	0	3.03
Persicaria lapathifolia (L.) Delarbre	2.181	3.243	46.18	1.52	2.94
Selaginella monospora Spring	2.124	3.157	49.34	2.83	0

only floral cover that accounted for at least 2% of the dissimilarity are included.

Demographic information

Demographic information determined that 47.7% of the respondents were females (Table 6). The literary rate of the respondents was encouraging with 75.4% of respondents educated and only 24.6% without education. The education level was categorized into five types and the percent of educated respondents for each category is as follows: primary (15.4%), secondary (13.9%higher (18.4%), tertiary (7.7%), and non-formal education (20%). The respondents were categorized into two age groups of below 30 and greater than 31, with 63% below the age 30.

Table 6. Percentage and frequency of the respondents as per demographic information

Demographic parameters	Respondents	Frequency	Percentage
Gender	Male	34	48
	Female	31	52
	Total	65	100
Age	Less than 30	41	63
	Greater than 31	24	37
	Total	65	100
Education	Non educated	16	24.6
	Primary	10	15.4
	Secondary	9	13.9
	Higher	12	18.4
	Tertiary	5	7.7
	Non formal	13	20
	Total	65	100

Table 7. Chi-Square table: Gender, age, and education level in relation awareness on freshwater plant

	Awareness on freshwater plants			
	Yes n(%)	**No n(%)**	**x^2**	**p-value**
Gender				
Female	16(51.6)	15(44.1)		
Male	15(48.4)	19(55.)	9.581	.002
Age				
Less than 30	9(29.0)	32(94.1)		
Greater than 31	22(71.0)	2(5.9)	29.493	.000
Education level				
Not educated	13(41.9)	3(8.8)		
Educated	18(58.1)	31(91.2)	9.581	.002

Association between gender, age, education level, and awareness on freshwater plants

A Chi Square test of independence showed there is a strong association between awareness on freshwater plants with gender (p = .002), age (p = .000), and education level (p = .002) (Table 7). Females, non-educated respondents, and respondents above age 31 knew significantly more about freshwater plants.

Perception on benefits and number of known freshwater plant species in relation with gender, age, and education

The Chi Square test of independence showed a strong association between respondent's perception on benefits of freshwater plants with gender (p = .014), age (p = .000) and education level (p = .003) (Table 8). The test concluded that females and non-educated respondents with age 31 and above knew significantly more about the benefits of freshwater plant as compared to males and educated respondents aged 30 or below. However, there was no significant association between gender and the number of species known (p = 0.115).

Table 8. Chi-Square table showing association of benefits and number of known freshwater species with gender, age, and education

	Perception on benefits of freshwater plants			
	Yes n(%)	**Not sure n(%)**	**x^2**	**p-value**
Gender				
Female	23(60.5)	8(29.6)		
Male	15(39.5)	19(70.4)	6.040	.014
Age				

Less than 30	17(44.7)	24(88.9)		
Greater than 31	21(55.3)	3(11.1)	13.212	.000
Education level				
Not educated	13(34.2)	3(11.1)		
Educated	25(65.8)	24(88.9)	4.539	.033

Number of known freshwater plant species

	Less than 20 n(%)	Greater than 20 n(%)	x^2	p-value
Gender				
Female	17(40.5)	14(60.9)		
Male	25(59.5)	9(39.1)	2.478	.115
Age				
Less than 30	38(90.5)	3(13.0)		
Greater than 31	4(9.5)	20(87.0)	38.260	.000
Education level				
Not educated	5(11.9)	11(47.8)		
Educated	37(88.1)	12(52.2)	10.334	.001

Benefits of freshwater plants

Fifth five percent of the respondents (n = 39) were aware about the uses and benefits of freshwater plant species prevalent in the waterbodies of the District. The number of respondents aware about each benefits of freshwater plants was as follows: Food (n = 26), Medicine (n = 30), fodder (n = 6), religious purpose (n = 8), and commercial (n = 1) (Figure 4.4). Although aware, their awareness did not include the vital ecological role of freshwater plants in maintaining the health of the freshwater ecosystem

Discussion

The Simpson's diversity index, Shannon diversity index, and Margalef's index of species richness were highest for pond habitat, lowest for lake habitat, and had almost the same diversity value for stream and ditch habitat. This was similar to the findings of the study conducted by Willaims et al. (2004) and Vannote et al. (1980), which concluded that pond habitat supported greater diversity of plant species and that the comparison of species-richness between the streams and the ditches were moderately common.

One-Way ANOVA test concluded a significant difference in the freshwater plant diversity between habitats, however, the Bonferonni Post Hoc test revealed insignificant difference among the habitats. The insignificant difference could be because of the locations of the habitat as they were almost at the same elevation and had similar environmental variables. It could, therefore, have resulted in similar species composition and insignificant difference in the diversity index.

CCA analysis showed a closer grouping of ditches and pond habitats indicating that the two habitats have similar environmental variables. This was contradictory to the findings of Vannote et al. (1980), which concluded that stream and ditch habitats are similar and have similar composition. The test also revealed that majority of the freshwater plant species showed negative correlation with water velocity, depth, and altitude which is similar to the finding of Chamber (1991), Zhou et al. (2017), Weisner (2001), Bucak et al. (2012), Gong et al. (2019) and Lacoul and Freedman (2006) which reported that the diversity of freshwater plant species decreased with the increase in the water velocity, depth, and altitude and vice versa.

Pearson correlation coefficient showed that species count had a slight negative correlation with altitude (p = 0.260) and a slight positive correlation with temperature (p = 0.273) and Ppt (p = 0.373)

which were similar to the findings of Classen et al. (2015) which also concluded that floral species richness increased with increase in the temperature and precipitation due to resource availability.

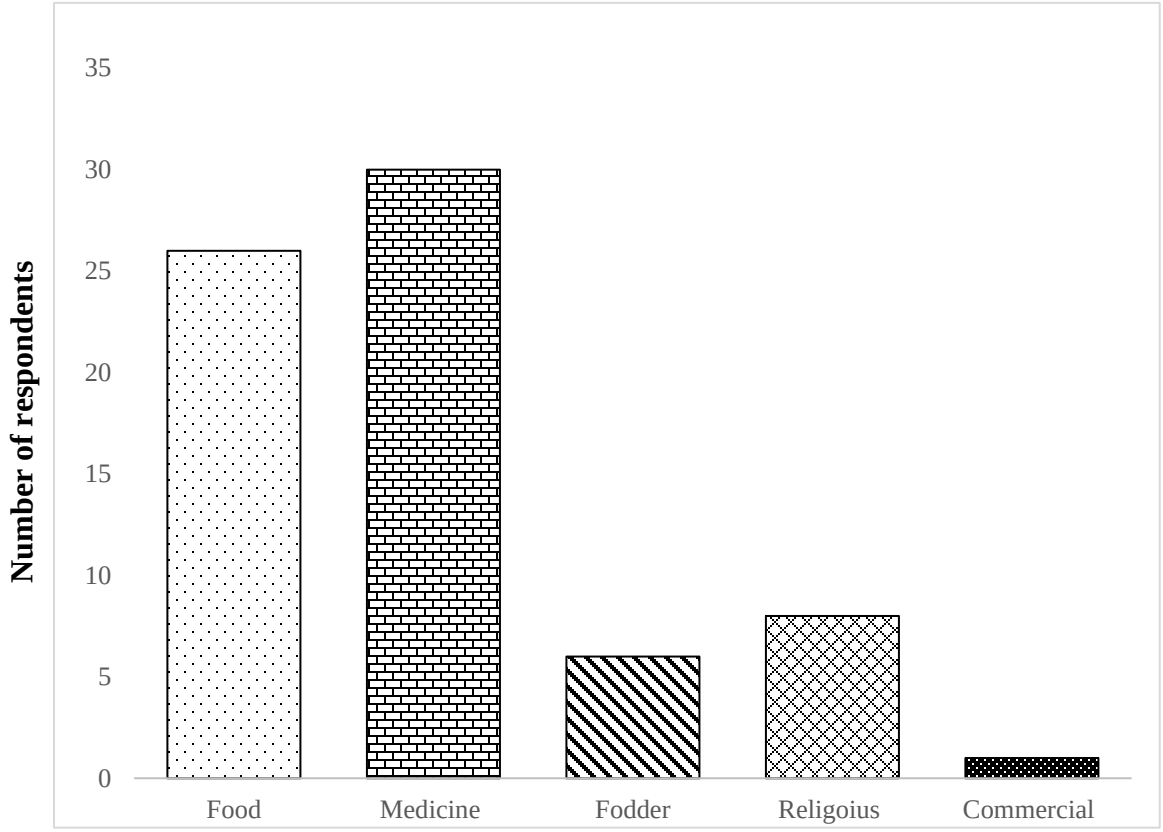


Figure 5. Knowledge of freshwater plants' benefits by respondents

Species rarefaction curve determined that a higher species of freshwater plant was found in lentic waterbodies as compared to lotic water and that a less sampling effort was required for lentic water bodies. This was in line with the findings of Williams et al. (2004) that lentic habitats (ponds and lakes) generally have more variable in their richness than the lotic habitats, particularly in terms of macrophytes and invertebrate communities.

The Chi Square test of association was conducted for analyzing the social data. The test determined that females, respondents with age greater than 31, and farmers knew significantly more about freshwater plants. The findings were similar to those by Geissler et al. (2002) and Avilez et al. (2016) which also reported that women knew significantly more about the plants and used the resource as food and herbal medicine for the care of their children and family. The findings were quite consistent with the study conducted by Shaheen et al. (2017) and Cole (2019) which reported that the older generations had comparatively more awareness and knowledge on aquatic plants than the younger generations and Bruyere et al. (2016) and Novoa et al. (2017) who concluded that educated people often remain unaware of plants and their ecological impacts and that although education benefits students' learning, the local herders knew and identified plants significantly more than the students.

Conclusion and Recommendation

The current study analyses the diversity of freshwater plant in different freshwater bodies of Punakha District. The study recorded a total of 72 freshwater plant species distributed among 57 genera and 37 families. The diversity of the freshwater plant was found to be the highest in ponds, followed by ditches, streams, and lakes.

The study also analyses the diversity of freshwater plants' correlation to various environmental variables. Pearson correlation and Canonical correspondence analysis concluded that the diversity of the freshwater plants decreases with an increase in the water velocity, depth, and altitude. The freshwater plant species were prominent in waterbodies with low depth and velocity. Chi Square statistics showed that the knowledge of the local people about freshwater plants prevalent in the freshwater bodies in the District was stronger in females, non-educated farmers, and people above age 31. However, apart from the domestic uses of freshwater plant, people were not aware of the vital ecological role that freshwater plants holds in an ecosystem.

The current study only focuses on the diversity of freshwater plant in different waterbody habitats and distribution pattern of freshwater plants in relation to environmental variables. It does not include information with respect to physio-chemical parameters and macroinvertebrate communities of the water bodies. Also, the data collection for the study was carried out for only one month which reduced the probability of recording a greater number of plant species. A comparative study of the diversity of freshwater plants in different seasons along with physio-chemical parameters and macroinvertebrate communities of the water bodies could be conducted to generate a more detailed dataset for future reference.

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