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ASSESSMENT OF FRESHWATER PLANT DIVERSITY AND LOCAL COMMUNITY DIFFERENCE IN FRESHWATER PLANT USE KNOWLEDGE IN PUNAKHA DISTRICT, BHUTAN

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Abstract

Freshwater plants play a paramount role in an aquatic ecosystem. However, only limited studies have been carried out on freshwater plants. Therefore, the study aimed to assess the diversity of freshwater plants in different freshwater bodies of Punakha District, the correlation of freshwater plant species with the environmental variables, and the local community difference in freshwater plant use knowledge in Punakha district. A total of 20 water bodies were selected using a stratified sampling method. Area-based surveys were employed to assess the plant species from a total of 80 plots, each with the quadrat of 5 m x 5 m. Environmental variables such as altitude, water depth, water velocity, temperature, precipitation and evapotranspiration were measured. The study recorded a total of 72 freshwater plant species distributed among 57 genera and 37 families. Shannon-Weiner diversity index revealed high diversity of freshwater plants from ponds (H' = 3.3) followed by ditches (H' = 3.16), streams (H' = 3.07) and lakes (H' = 2.83). The study revealed the decrease of freshwater plant species with an increase in the water velocity, depth and altitude. There was a significant association between respondents' freshwater plant use knowledge with gender ($x^2(1) = 6.04$, p = .014), age ($x^2(1) = 13.21$, p = .000) and education level ($x^2(1) = 4.53$, p = .003). Females and illiterate respondents with aged 31 years old and above had more knowledge on freshwater plant use compared to males and educated respondents with aged 30 years old and below.

Keywords: Ditches, Freshwater plants, Lake, Pond, Stream

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Introduction

Freshwater plants play a crucial role in the ecological functions and stability of freshwater biological systems (Silva *et al*., 2008; O'Hare *et al*., 2018) to provide aquatic ecosystem amenities (Nunez *et al.*, 2019). The higher diversity of freshwater plants supports greater numbers of fish, macroinvertebrates and other benthic species (Kurbatova and Yershov, 2020) by rendering them food and shelter (O'Hare *et al*., 2018; Kurniawan and Paramita, 2020).

Diverse freshwater plant species inhabit different ecosystems due to the influence exerted by different environmental variables such as temperature, precipitation and altitude. The influence of these potential drivers are known to shape the diversity and richness of freshwater plant species (Bolpagni *et al*., 2018). Therefore, the presence or the absence of certain diversity of freshwater plant species not only serves as an important biological tool for monitoring the ecological condition of the waterbodies but also reflects changes in the environmental variables (Ansari *et al*., 2017). Freshwater plants also serve as a significant source of food and medicine and hold a vital role in agriculture. Owing to the ability of the freshwater plants to recycle nutrient, increasing studies have reported an improvement in the soil structure and crop productivity (Owamah *et al*., 2014). The multitude benefits of freshwater plant species have a potential to support livelihood of the local community. However, the multiple use of freshwater plants as human food and in agriculture is known only to a specific community through traditional knowledge (Aasim *et al*., 2018).

Punakha district, Bhutan, harbors the largest river basin covering 25 % of the country's land and stretches over 320 km long (National Environment Commission [NEC], 2016). Punakha also anchors the largest glacier of the country with an area of 36 km^2 (Ugyen Wangchuck Institute for Conservation and Environment Research [UWICER], 2018). Numerous studies have been conducted on the freshwater of Punakha district such as changes in the water availability (Kusters and Wangdi, 2013) and on various aquatic organisms including Himalayan newt (Wangyal and Gurung, 2012a), amphibians (Wangyal and Gurung, 2012b), dragonflies (Dorji, 2014), macroinvertebrates (Gurung and Dorji, 2014), otters (Chettri and Savage, 2014) and fishes (Gurung *et al*., 2013; Tashi *et al*., 2022). However, the data and scientific evidence on one of the important components of the freshwater ecosystem, plants, remain vague.

Regardless of freshwater plants' potential of indicating and maintaining the quality of the water body, supporting a diverse species of aquatic organisms and operating many other critical functions, freshwater scientific research and conservation remains disproportionately focused on iconic species. Only limited research has been carried out regarding the plant species thus resulting in a significant literature gap and less social awareness on the diversity of freshwater plants that plays an important role in the aquatic ecosystem as a food source and nutrient recycler (Wangdi and Gyeltshen, 2020).

Likewise, biodiversity value of different water bodies is a prerequisite for planning most of the strategic conservation goals (Williams *et al*., 2004), however, no inter-water body comparisons have been made in the district. Therefore, this study aimed to assess the freshwater plant diversity in different freshwater bodies, the correlation of freshwater plant species with environmental variables and local community knowledge about the freshwater plant use prevalent in freshwater bodies.

Materials and Methods

Study Site

Punakha district lies in the west-central part of Bhutan at an altitude of 1,242 masl (Dorji, 2014) and the geographical coordinate of 27° 39' 59.99'' N and 89° 49' 59.99'' E (Tshering and Rinzin, 2022).

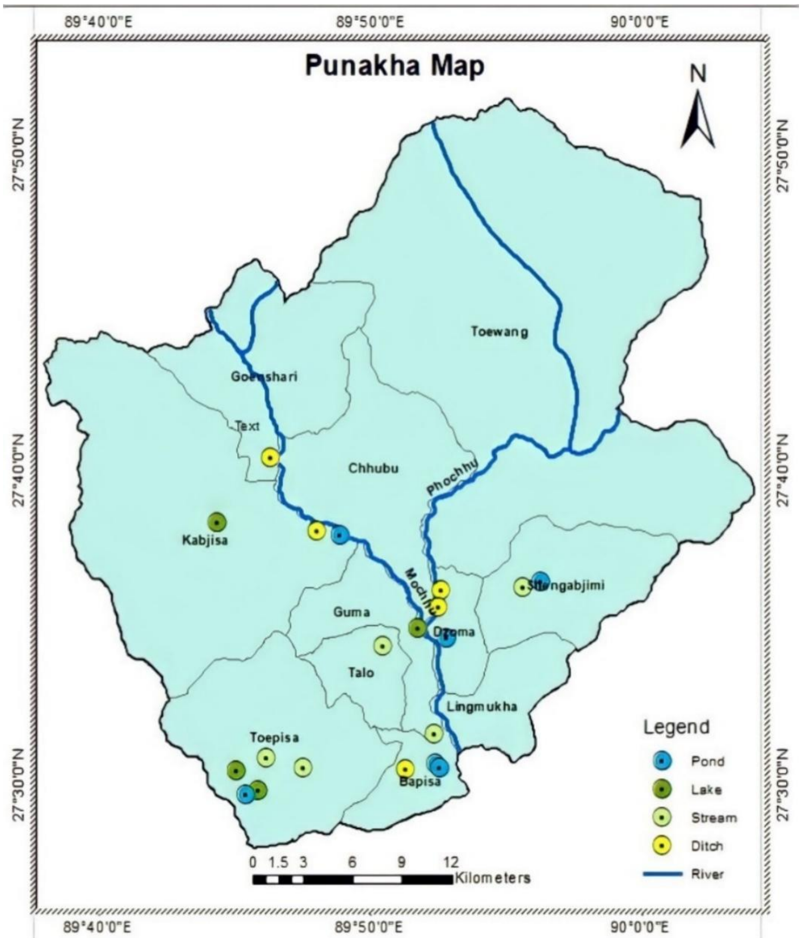


Figure 1. Location map showing water bodies of Punakha District

The district receives an annual rainfall ranging from 500 to 1,500 mm and an average temperature ranging from 5 ˚C to 30 ˚C. Punakha district experiences warm and temperate climatic conditions. The elevation, climatic condition and temperature range make the district home to a diverse floral species (Tshering, 2019).

The river system of the district, Punatsangchhu is fed by snow and melting glaciers from the Himalayan mountains, two main tributaries Pho chhu and Mo along with numerous streams and ditches (NEC, 2016).

Sampling Methods

The study was conducted in early spring in 2021. Prior to sampling of freshwater plants, preliminary surveys of different water bodies were carried out in Punakha District. Freshwater bodies were stratified into lotic and lentic water. Stratified sampling was employed owing to the difference of the diversity of freshwater plant species prevalent in lotic and lentic waters (Davis *et al*., 2008; Madsen and Wersal, 2017; Aslan *et al*., 2018). For each stratum, ten water bodies from the total water bodies were randomly selected using excel random generator. A total of 20 water bodies consisting of four numbers of ponds, lakes, ditches and streams were selected for studying the freshwater plants diversity and its correlation with the environmental variables. Freshwater plant species, altitude, water depth, water velocity, temperature, precipitation and evapotranspiration of each water body was assessed quantitatively whereas social data of local people's knowledge on freshwater plant use were obtained qualitatively through semi-structured interview.

Sampling of freshwater plants

In order to ensure that the ecological data of the freshwater plant diversity gathered from different water bodies with widely differing dimensions and characteristics can be directly compared, the sampling was conducted in a limited area (Williams *et al.*, 2003; Fenu *et al*., 2022). Following the area-based survey, a total of 80 plots from 20 water bodies, each with the quadrat of 5 m x 5 m was placed on a 100 m^2 area of each of the water body (Müllerová *et al*., 2020). The first quadrat was randomly selected (Bubíková and Hrivnák, 2018a) and the following quadrats were systematically laid at the periphery of each water body (Pem *et al*., 2022). The maximum and the minimum area of the sampled water bodies were of 0.48 km^2 to 100 m^2 respectively. The data were collected from 100 m^2 area for each water body taking into consideration the size of the smallest lentic water body. Although systematic sampling can lead to either over or under-representation of the freshwater plant communities, systematic sampling provides more coverage of the study area and can be employed in a limited time. Plant species were identified using Flora of Bhutan (Grierson and Long, 1984), Weeds of Bhutan (Parker, 1992) and available literature on the internet.

Recording of altitude, slope, aspect, water depth and velocity

The geographical coordinates and the altitude of each water body were recorded using GPS Garmin. The water depth of both lotic and lentic water bodies was measured by inserting a meter ruler in the water until it came in contact with the bed. The velocity of the water was determined using floats (Hundt and Blasch, 2019) whereby the float was released upstream to cover a distance of 10 m. The time taken by the float to cover the

10 m distance was recorded. With the recorded time and distance, the velocity of the water was calculated as per the formula adopted by Hundt and Blasch (2019).

$$\text{Velocity} = \frac{\text{Distance}}{\text{Time}} \;\ldots\ldots\ldots\ldots \text{Eq. (1)}$$

Selection of the interviewee

The interviewees were selected using a random sampling method. To assess people's knowledge on freshwater plant, people residing near the water bodies were assessed using a semi structured interview. A preliminary survey was employed to obtain the number of households near the water bodies where the freshwater plant species were recorded. The preliminary survey recorded a total of 78 households. Yamane (1967) statistical formula was employed for selecting respondents. A total of 65 respondents (one from each household) were selected for semi-structured interview.

$$n = \frac{N}{1+N\,(e)^2} \ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. (2)}$$

Where n is the sample size, N is the whole population and e is a sampling error.

Data and statistical analysis

Freshwater plant diversity

The diversity and evenness of the freshwater plants was determined as per the formula modelled by Shannon and Weaver (1949) and Pielou (1996).

$$\text{Shannon diversity index } (H') = \textstyle\sum_{i=1}^{S} Pi \times Ln(Pi) \ldots\ldots\ldots\ldots \text{Eq. (3)}$$

Where *Pi* is the proportion of the i^{th} taxon ($Pi = n_i/N$, *ni* is the individuals of one particular species found [*n]* divided by the total number of individuals found *N*). *Ln* is the natural log and Σ is the sum of the calculations. Shannon Weiner Diversity Index takes into account the relative abundance of large *H'* values which indicates greater diversity (Ghosh and Biswas, 2015).

$$\text{Pielou's evenness index (e)} = \frac{H}{In\,S} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. (4)}$$

Where H is a Shannon-Weiner diversity index and S is the total number of species.

Canonical correspondence analysis

Canonical correspondence analysis was conducted using PAST to determine the correlation of freshwater plant diversity in different water bodies as well as its habitat with environmental variables such as altitude, water depth, water velocity, temperature, precipitation and evapotranspiration.

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 such as altitude, water depth, water velocity, temperature, precipitation and evapotranspiration and the correlation between the environmental variables was analyzed using PAST. Pearson correlation coefficient test was run to analyze the correlation between the species number and the mentioned environmental variables.

Social data analysis

The social data was compiled and coded in Microsoft Excel and analyzed in SPSS. People's knowledge on use of freshwater plants was reported using descriptive statistics. Chi Square test was used to test the relationship of gender, age, and education level of the interviewees to either having knowledge on the freshwater plant use or not.

Results and discussion

Floristic composition of freshwater plants

A total of 72 freshwater plant species belonging to 57 genera and 37 families were recorded from the study sites. The maximum number of plant species was found under Polygonaceae family (nine species), and the least number of species was recorded under the following family: 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. The study was carried out only for a month in early spring. Since the study was carried out before the flowering season, this may have affected the probability of recording a greater number of plant species.

Freshwater plants' diversity

The highest number of freshwater plant species was found in ponds (n = 46) followed by ditches (n = 44), streams (n = 39) and lake habitats (n = 34). The Shannon-Weiner diversity index revealed the highest and lowest diversity in the pond (*H'* = 3.3) and lake (*H'* = 2.83) habitats respectively. The plant diversity value did not differ much between stream and ditch habitats. However, the freshwater plant diversity was statistically significant ($p > .005$) between different freshwater bodies. The findings are in accordance with Williams *et al*. (2020) and Williams *et al*. (2003) who concluded that pond habitat supports greater diversity of plant species. Cèrèghino *et al.* (2008) stated the presence of richer biodiversity in small-sized ponds compared to large lakes. Davies *et al*. (2008) also argued that the small waterbodies have high species richness and more rare species compared to large water bodies.

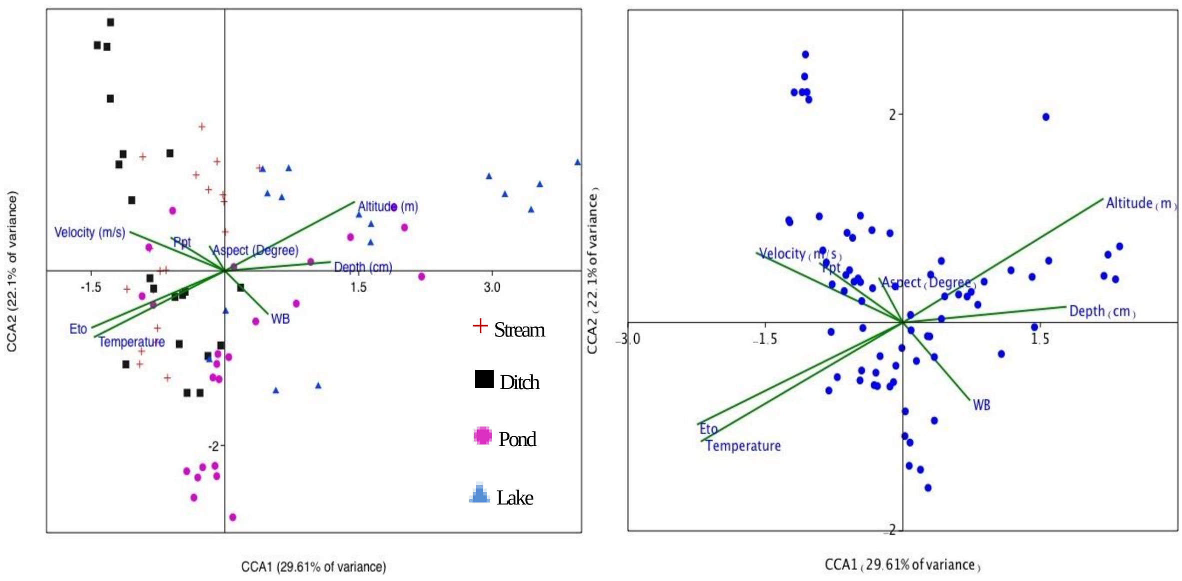
Table 1. Richness, evenness and diversity of freshwater plants of different habitats

	Stream Habitat	**Ditch Habitat**	**Pond Habitat**	**Lake Habitat**
Individuals	45	59	44	53
Richness	39	44	46	34
Shannon diversity	3.07	3.16	3.3	2.83
Pielou's evenness	0.55	0.53	0.58	0.50

The high diversity observed in ponds may be due to its shallow depth and lengthy banks which renders rooted aquatic plants to receive sunlight and accumulate nutrients from plants and animals in the riparian zone (Vispo and Knab-Vispo, 2007). Hill *et al.* (2021) stated that the pond also sustains many uncommon and threatened aquatic organisms. The diverse freshwater plants aid as an important resource for many herbivores as well as provide refuge from landscape alteration and predators (Hoverman and Ohnson, 2012). Lopez *et al.* (2020) stated that the extended shoots of freshwater emergent plants serve as forage for terrestrial herbivores. 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. Bubíková and Hrivnák (2018b) stated that all the water bodies have similar freshwater plant species richness. The authors argued that it is the environmental factors that influences the freshwater plants' diversity and richness and not the water body type.

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

Canonical correspondence analysis (CCA) revealed positive correlation of lake habitats with altitude and depth and a negative correlation with temperature and evapotranspiration. The positive correlation may be attributed to the water melts from high altitude mountain glaciers and snow that feeds large amount of water into the lake (Srivastava *et al*., 2013). Bhateria and Jain (2016) and Caballero *et al*. (2020) stated that the low temperature and nutrient content at high altitude reduces the water loss by evaporation and prevents eutrophication by limiting algal growth. Similarly, the water depth showed a positive effect on the lake habitat. This could be due to the influence of water depth on light intensity, temperature and nutrient content (Zhou *et* *al*., 2017). Qin *et al.* (2020) reported a lower probability of eutrophication in deep lakes compared to shallow lakes.



A B

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

CCA illustrated a positive association for most of the stream habitats with high velocity and precipitation (Figure 2A). According to the United States of Environmental Protection Agency (EPA) (2012), streams with high velocity contain high concentrations of dissolved oxygen and are less affected by pollution compared to streams with low velocity. EPA (2013) also stated that the precipitation enhances the flow of the seasonal streams as well as serves as primary source of water for some of the streams.

In contrast, CCA showed negative association for most of the ditch and pond habitats with velocity and precipitation (Figure 2A). The negative association with precipitation could be due to the ingress of contaminants and total dissolved solids (TSS) from runoff. Sharma *et al.* (2016) reported an increased concentration of TSS and trace elements existing in the pond during precipitation.

Ditches and pond habitats showed a significant positive correlation with temperature and evapotranspiration. Shen *et al.* (2021) reported that ditches and ponds serve as a sink for pollutants and thrive better in warmer temperatures as they enhance water treatment and waste removal. Similarly, the loss of pond water by evaporation and transpiration through dense foliage lowers the pond water (Fortuna, 2020), thus rendering the pond with concentrated residuals for effective treatment of waste or residuals (Gulliver *et al.*, 2010). The closer grouping of ditch and pond habitats indicates that the two habitats are similar and have a similar correlation with the environmental variables.

Most of the freshwater plant species showed a negative correlation with water velocity, depth and altitude (Figure 2B). This is in line with the findings reported by Bucak (2012), Zhou *et al*. (2017) and Gong *et al.* (2019) who concluded the decreasing diversity of freshwater plant species with increase in the water velocity, depth and altitude. The diversity of freshwater plants decreases due to the restrictions imposed by increasing altitude on temperature and oxygen (Labat *et al*., 2021). Cornacchia *et al.* (2020) stated that the water velocity alters the occurrence, biomass and species composition of aquatic plants. In addition, Janauer *et al*. (2013) reported a significant reduction of morphological characteristics and further argued that the water velocity and water depth more than 1.5 m limit the growth of aquatic plants.

Pearson correlation coefficient between species count and environmental variables

Pearson correlation revealed a major influence of water velocity and depth on the freshwater plant species whereby the species count was positively correlated with water velocity (r = 0.34, p = 0.001) and negatively correlated with depth (r = -0.30, p = 0.005). The species count had slight negative correlation with altitude (r = -0.12, p = 0.26) and slight positive correlation with temperature (r = 0.01, p = 0.273) and precipitation (r = 0.011, p = 0.373). The results are in accordance with the findings reported by Kochjarová *et al.* (2017) that concluded that floral species richness increased with an increase in temperature and precipitation and decreased with increasing altitude. There was a significant negative correlation of altitude with temperature (r = -0.99, p = 0.001) and evapotranspiration (r = -0.99, p = 0.0001). Temperature was significantly correlated with evapotranspiration (r = 0.99, p = 0.0014).

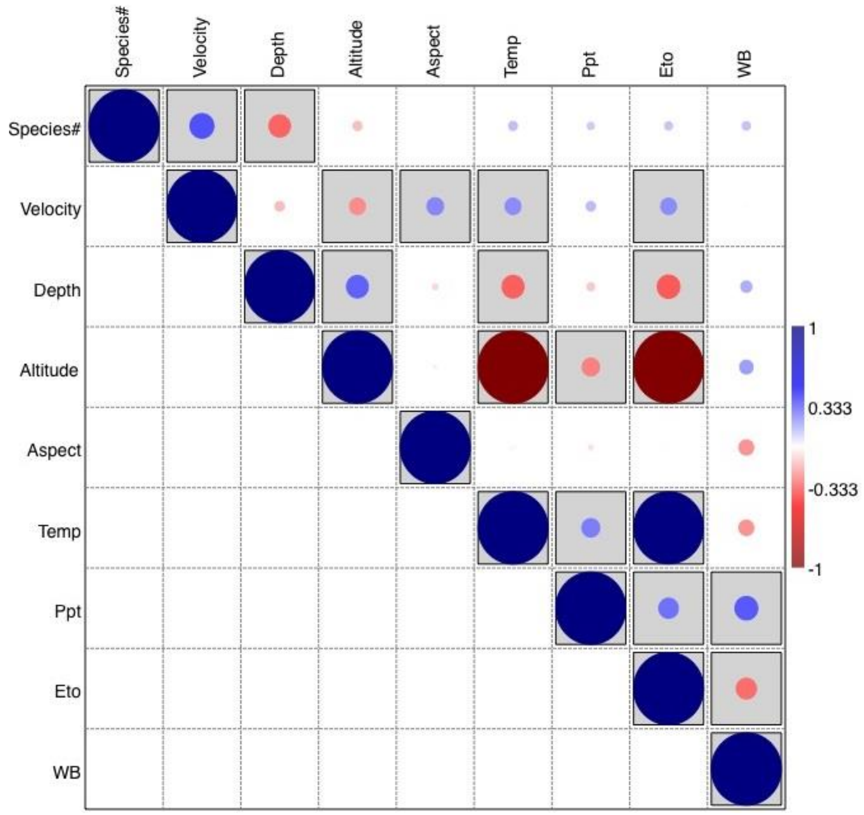


Figure 3. Pearson correlation coefficient ($p < 0.005$ are boxed)

Species rarefaction curve

A 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. *Fragaria nubicola* and *Pilea glaberrima* was the dominant species in lentic and lotic habitats respectively. Lentic habitat supports a high diversity of freshwater plant species owing to the multiformity of landscape and habitat provided by lentic habitat (Pérez-Bilbao *et al*., 2015; Deacon *et al*., 2018). Bubíková and Hrivnák (2018) enunciated that the lentic habitats have comparatively smaller catchment areas than lotic habitats which renders them to limited exposure to detrimental anthropogenic activities. In supplementary to smaller catchment areas, Bubíková and Hrivnák (2018) further stated that the isolation of lentic bodies supports heterogeneous landscape and species composition.

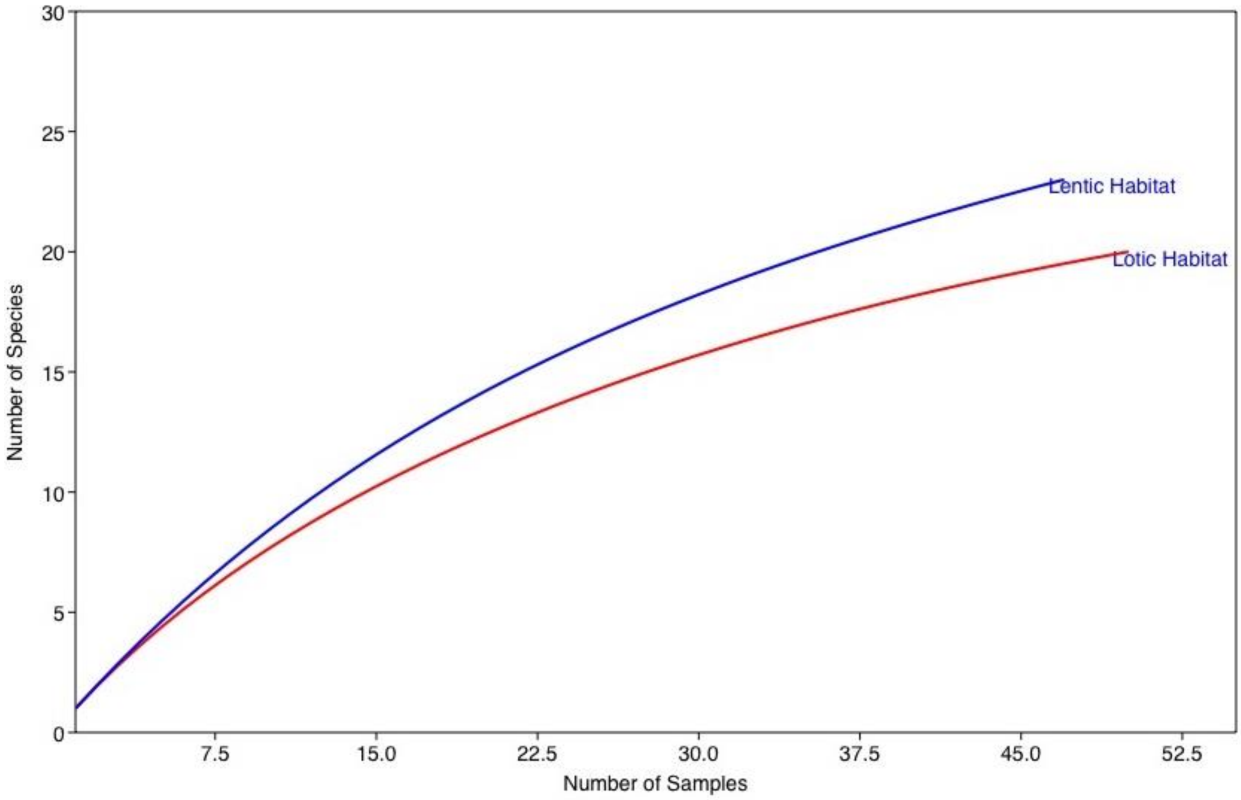


Figure 4. Species rarefaction curve

Demographic Information

A total of 65 respondents were classified into two groups according to their age. One group consisted of individuals aged 30 years old and below whereas the other group consisted of individuals aged more than 31 years old. The majority of the respondents (*n* = 41, 63 %) was represented by those aged 30 years old and below. Demographic data revealed that the respondents were slightly more represented by males (*n* = 34, 52.3 %) than females (*n* = 31, 47.7 %). The literacy rate of the respondents was encouraging with 75.4 % (*n* = 49) of respondents being literate and only 24.6 % (*n* = 16) of respondents being illiterate. The literacy of the respondents was classified into five education levels. The highest percent of literate respondents were represented by individuals who pursued non-formal education (*n* = 13, 20 %), followed by higher (*n* = 12, 18.4 %), primary (*n* = 10, 15.4 %), secondary (*n* = 9, 13.9 %) and tertiary (*n* = 5, 7.7 %).

Table 2. Percentage and frequency of the respondents as per demographic information

Demographic parameters	Respondents	Frequency (n)	Percentage (%)
Gender	Male	34	47.7
	Female	31	52.3
	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

Local community difference of freshwater plant use knowledge in relation with gender, age and education

Chi Square test of independence showed a strong association between respondents' knowledge on freshwater plant use with gender (x^2(1) = 6.04, p = 0.014), age (x^2(1) = 13.21, p = 0.000) and education level (x^2(1) = 4.53, p = 0.003). No study has been carried out on the gender difference on knowledge of freshwater plant use till date. However, some studies have reported that females possess greater knowledge on plants use, while other studies have argued that male possesses more knowledge on plant use, especially on the planta used for construction purposes (da Costa et al., 2021). The test concluded that females and non-educated respondents with aged above 31 years old had significantly more knowledge on the use of freshwater plants compared to males and educated respondents with aged 30 years old and below.

The gender difference in regards to knowledge on freshwater plant use may be attributed to the social role of the female as a housekeeper responsible for the care of the family (da Costa *et al*., 2021; Tng *et al*., 2021). da Costa *et al*. (2021) also stated that females share more information with each other compared to men. Although no comparative study of knowledge, in particular freshwater plants between younger and older generations has been carried out, Bruschi *et al*. (2019) reported that the older generation are comparatively more aware of the plants than the younger generations. Shaheen *et al*. (2017) stated that the older people living in the rural communities have extensive ideas and knowledge about various ethno-medicinal plants.

Conclusion

A total of 72 freshwater plant species belonging to 37 families from 20 different waterbodies of Punakha district were recorded. The freshwater plant diversity was highest in ponds despite its small size. The study revealed that the species richness depends on the environmental factor and not on the type of the waterbodies. Pearson correlation and Canonical correspondence analysis concluded that the freshwater plant diversity decreases with an increase in the water velocity, depth and altitude, and increases with temperature and precipitation. Chi Square test concluded that the females, non-educated farmers, and people aged 31 years old and above have more knowledge on the freshwater plant use. The study also concluded that knowledge on freshwater plant use is structured by gender, age and education level. This study does not include an assessment of freshwater plant diversity with respect to the physico-chemical variables and macroinvertebrate communities of the water bodies. Therefore, a comparative study of the freshwater plant diversity in different seasons along with the physico-chemical variables and the macroinvertebrate communities is recommended for an extensive study and to generate a comprehensive data for reference in the future.

Authors contribution statement

The research design, planning and data collection were done by Tenzin Dema while the write up and editing was done by Tshering Pem. Sangay Tshomo and Sonam Tshering assisted in data collection while Jambay supervised the research.

Conflict of interest

The authors declare no conflicts of interest.

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