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Cover: Himalayan Gray Langur *Semnopithecus ajax* (adult female) © Rupali Thakur.



Environmental factors affecting water mites (Acari: Hydrachnidia) assemblage in streams, Mangde Chhu basin, central Bhutan

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Abstract: Water mites were sampled from 15 tributary streams of Mangde Chhu river in Zhemgang and Trongsa districts, Central Bhutan in pre-monsoon (April–May) and post-monsoon (October–November) of 2021. A total of 802 individuals were collected belonging to seven families and 15 genera. The accumulation curve suggests that the sampling efforts were adequate to give a proper overview of genera composition for elevations 500–2,700 m. Eleven genera—*Aturus*, *Kongsbergia*, *Woolastookia*, *Atractides*, *Hygrobatas*, *Lebertia*, *Piona*, *Sperchonopsis*, *Monatractides*, *Pseudotorrennicola* and *Testudacarus*—and five families—Aturidae, Hygrobatidae, Lebertiidae, Pionidae, and Protziinae—are new records for Bhutan. Independent sample *t*-tests of genera richness (t , (26) = 0.244, p = 0.809); genera evenness (t , (26) = 0.735, p = 0.469); Shannon diversity index (t , (26) = 0.315, p = 0.755) and dominance (t , (26) = -0.335, p = 0.741) showed no significant differences between pre- and post-monsoon assemblages. Species abundance was also not significantly different (t , (28) = -0.976, p = 0.330). Principal component analysis indicated that the diversity of water mites is negatively associated with several environmental variables including chloride (r = -0.617), ammonia (r = -0.603), magnesium hardness (r = -0.649), total hardness (r = -0.509), temperature (r = -0.556), salinity (r = -0.553), total dissolved solids (r = -0.509) and electrical conductivity (r = -0.464). Diversity was positively correlated with altitude, mainly caused by the higher Palaearctic genera diversity. Similarly, Pearson's correlation test showed that there was significant negative correlation between mite abundance and the water physio-chemical parameters salinity (r = -0.574, p = 0.032), electrical conductivity (r = -0.536, p = 0.048), total dissolved solids (r = -0.534, p = 0.049), total hardness (r = -0.621, p = 0.018), and chloride concentration (r = -0.545, p = 0.036), indicating sensitivity of water mites to pollution.

Keywords: Biotic assessment, climate change, fresh water, macroinvertebrates.

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INTRODUCTION

Most lotic freshwater habitats in Bhutan, such as streams, springs, and rivers, harbor a rich aquatic diversity of macroinvertebrates and fishes (Gurung et al. 2013; Gurung & Dorji 2014; Wangchuk & Dorji 2018; Dorji et al. 2020; Rai et al. 2020; Norbu et al. 2021). These habitats are also the major sources of drinking water in Bhutan, thus their preservation is essential for both the conservation of biodiversity as well as the economic well-being of Bhutanese (Dorji 2016b). Lotic freshwater systems are, however, under pressure due to anthropogenic pollution and climate change (Xu et al. 2009; Tsering et al. 2010). Thus biotic assessment of these habitats is essential to understand the health of the water.

Water mites are greatly influenced by water parameters and environmental factors (Stryjecki et al. 2016; Savić et al. 2022). Abundance of water mites is negatively influenced by water temperature and velocity (Stryjecki et al. 2018), with temperature being the major factor that influences distribution of Hydrachnidia along altitudinal gradients. Mite abundance is positively impacted by pH (range 7.3–8), dissolved oxygen, and total hardness (Stryjecki et al. 2016; Negi et al. 2021). Seasonality also impacts mites in freshwater habitats. According to Negi et al. (2021), Hydrachnidia abundance was maximum during pre-monsoon (January) and minimum in monsoon (July).

Water quality monitoring is commonly done in two ways: a) determination of physico-chemical properties of the water and b) biotic assessment of aquatic organisms. In most cases, physico-chemical assessment of water only gives an indication of the water quality at the moment when the sample was taken, and poor water quality or pollution during other parts of the year might go unnoticed. Results based on biotic assessment, in contrast, give a direct indication of water quality throughout the year, as poor water quality or pollution will be reflected in the faunal composition (Ofenböck et al. 2010; Wangchuk & Dorji 2018; Rai et al. 2020). For this reason, biological water monitoring is at least as valuable for freshwater management as physico-chemical monitoring.

Aquatic meiofauna such as water mites are excellent bioindicators of water quality (Smit & Van Der Hammen 1992; Miccoli et al. 2013; Wieçek et al. 2013; Goldschmidt 2016). They are present in both lotic and lentic habitats (Smith et al. 2001) at a large range of elevations (Mani 2013). Thus far, water mites have been ignored during freshwater habitat assessments in

Bhutan and other regions, which have focused on other macroinvertebrates (Ofenböck et al. 2010; Giri & Singh 2013; Patang et al. 2018; Wangchuk & Dorji 2018; Rai et al. 2020) and fishes (Gurung et al. 2013; Wangchuk et al. 2018; Dorji et al. 2020; Norbu et al. 2021). Neglect of water mites as bioindicators can partially be explained by their diminutive size and complex life cycle (Goldschmidt 2016).

A few aquatic diversity assessments in Bhutan recorded water mites and in all cases they were lumped together as Hydrachnidia or Acari (Ryder et al. 2015; Currinder 2017; Wangchuk & Dorji 2018). However, recent faunistic studies have described and recorded several new species of water mites from Bhutan (Pešić et al. 2022a; Smit & Gurung 2022; Smit et al. 2022; Pešić et al. 2022b) and currently there are 30 species documented from Bhutan (Gurung et al. 2022). Several major biotic assessment studies in Bhutan did not record mites (Dorji 2016a; Rai et al. 2020) despite fulfilling all the criteria (Resh 2008) for use as bioindicators, including: (1) wide geographical and habitat distribution, (2) high species richness, (3) relatively sedentary to a localized microhabitat (ideal for examination of contamination spatially), (4) long life cycle for long-term integration during the biotic assessment, (5) easy and cost effective sampling, (6) clear and well known taxonomy, (7) sensitive to contamination, (8) availability of experimental data on effects of contamination for different species (Di Sabatino et al. 2002; Smith et al. 2010; Miccoli et al. 2013; Goldschmidt et al. 2016). Therefore, during biotic assessment of freshwater habitats in Bhutan, water mites should be regarded as important for monitoring.

One of the reasons that water mites have not been used as bioindicators in Bhutan is that basic data on identification and distribution is largely absent for the Himalayan region (Pesić & Smit 2007; Gerecke & Smit 2022). Identification to species level is problematic, and even identification to genus level takes some experience (Smit 2020). The key to the genera of the world recently published by Smit (2020) makes identification to genus level easier, but papers like Gerecke & Smit (2022) describing species new to science are needed to enable people to identify material to species level. Taxonomic progress is partially hampered by the complex life cycle of water mites that includes pre-larval stage, parasitic larva, protonymph (i.e., initial resting stage), deutonymph (free living form), tritonymph (second resting stage), and the final adult stage (Di Sabatino et al. 2000).

Besides problems with identification, another reason

for the lack of the use of water mites as bioindicators is the lack of ecological studies for the Himalayan region. Although the link between water mite assemblages and water quality has been studied at least some times in other parts of the world basic studies on this subject from Bhutan and surrounding countries are lacking. A study on this subject would be of interest, especially as Bhutan is part of the border between the Oriental and Palaeartic region, with the species composition above 1,000 m elevation in general being of Palaeartic origin while that below 1,000 m elevation has a more Oriental affinity (Rasaily et al. 2021).

In 2021 and 2022, the first author studied water mites in Bhutan with the aim of producing new faunistic and taxonomical data providing basic information on water mite assemblages and water quality. The present paper is part of this larger study, and describes the patterns in composition and diversity in lotic waters along an altitudinal gradient. We use this information to address three questions: (1) is there a difference in water mite diversity, abundance and assemblages between pre- and post-monsoon? (2) which physico-chemical parameters correlate with the presence and abundance of water mite genera? And (3) is there a gradient from a largely Oriental fauna to a more Palaeartic fauna rising from 500 m to 2,700 m?

MATERIAL AND METHODS

Study area

Mangde Chhu river basin has catchment area of 7,380 km², annual flow of 11,797 million m³, 1,173 high-altitude wetlands (such as brooks, lakes, marshy areas), and 287 glaciers (NEC 2016). Mangde Chhu River originates from Gangkhar Puensum, passes through Trongsa, and exits Bhutan through Zhemgang as Manas River after joining with Drangme Chhu River. Fifteen perennial tributary streams of Mangde Chhu River were selected for this study ranging in altitude from 500 m to 2,700 m (Figure 1). A multi-stage sampling method (Gascho-Landis & Stoeckel 2016) was adopted for classification of the study area into three groups along the altitudinal gradient to study the effect of altitude on the water mite composition, namely between 500–1,000 m (low), 1,001–1,999 m (mid) and above 2000 m (high). Ammonia concentration ranged 0.04±0.002–662±78.4, calcium hardness 40.1±5.12–1.66±0.12, magnesium hardness 30.2±6.49–8.46±0.12, total hardness 70.3±11.05–22.4±0.08, chloride 128.2±19.6–32.5±3.41, electrical conductivity 48.8±0.43–33.4±1.51, dissolved oxygen 15.7±0.66–103.5±0.42, pH 7.67±0.05–14±3.07, salinity 19.3±0.14–7.21±0.08, total dissolved solids 34.6±0.16–50.7±0.25, temperature 14.6±0.54–

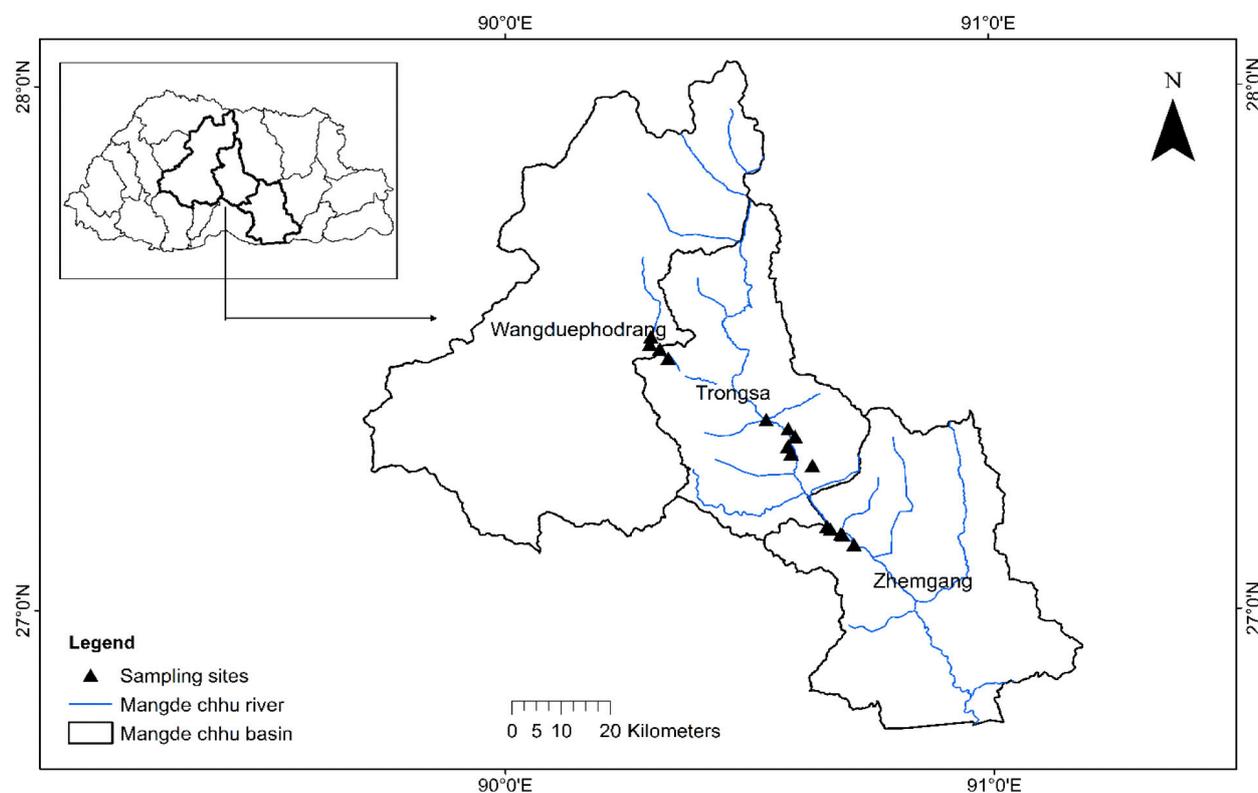


Figure 1. Streams under study in Mangde Chhu basin, central Bhutan.

44.6±0.64, and turbidity 0.62±0.20–46.5±3.41. Sampling was carried out in pre-monsoon (April–May) and post-monsoon (October–November), 2021.

Sampling sites and habitat description

MG1: Maidagang Chhu, Tingtibi, Zhemgang district, (27.12761°N, 90.71560°E, altitude 554 m, 24 April 2021; 20 October 2021) flows through a dense bushy vegetation and eventually drains into Mangde Chhu River. There is an agriculture field and a farm road above the confluence. Stream substrates mostly consist of cobbles with sand and rocks which often obstruct water forming small pools.

MG2: Berti Chhu, Berti, Zhemgang district (27.16264°N, 90.66003°E, altitude 590 m, 26 April 2021; 25 October 2021) flows through a narrow valley in deep forest. Before reaching the confluence, it passes the Berti community. The stream is adopted by the community for legal fishing in producing smoked fish (*Nyea Dhosem*). Berti Chhu substrates are predominantly composed of cobbles with sand. Riparian vegetation included ferns, climbers, and lowland grasses. Most of the sites had riffles, pools, and cascades. Water was heavily inhabited by fish fingerlings such as that of *Garra* spp. and *Danio rerio*.

MG3: Biggang Chhu, Berti-ecolodge, Zhemgang district (27.15729°N, 90.66721°E, altitude 586 m, 28 April 2021; 25 October 2021). Biggang Chhu flows along the mountain base parallel with the road connecting Tingtibi and Berti fishing community and it connects with Mangde Chhu River below Berti Eco-lodge camp. Stream riparian vegetation was covered by dense shrubs and grasses and the substrate was sandy with high debris content. The stream was inhabited abundantly by fishes. The natural water habitat was impacted due to frequent cleaning of the stream by the eco-lodge staff clearing the way for fish movement.

MG4: Takabi Chhu, Tingtibi, Zhemgang district (27.14782°N, 90.68833°E, altitude 543 m, 31 April 2021; 26 October 2021) stream flows through a steep mountain valley and has a high-water current. Riparian vegetation was mostly trees with underlying grasses. Substrate was mostly cobbles and sand. Rocks often obstruct the water forming pools and cascades.

MG5: Dakpay Chhu, Tingtibi, Zhemgang district (27.14621°N, 90.69220°E, altitude 539 m, 01 April 2021; 27 October 2021) flows through a thick vegetation with high water current. Water was murky. Substrate was rocky with cobbles and had less sand. Riparian vegetation had dense grasses under tall tree canopy. The submerged grasses along the streams formed a potential

habitat for mites.

MG6: Yumrung Chhu, Langthel, Trongsa district (27.36691°N, 90.53618°E, altitude 1,092 m, 02 May 2021; 28 October 2021) flows along abandoned mineral mining sites. Towards the confluence, the stream passes by the Mangde Chhu hydropower house and an automobile workshop. The stream was heavily disturbed due to dumping of gravel and soils from mining of the cliff. Downstream of the river was greatly impacted by the hydropower plant and an automobile workshop.

MG7: Wana Chhu, Langthel, Trongsa district (27.34964°N, 90.58144°E, altitude 1,139 m, 03 May 2021; 29 October 2021): this small stream flows through the thickly vegetated valley by the side of the paddy field. The water was murky, and stream substrate was sandy clay with debris. Riparian vegetation was dominated by *Ageratina adenophora* (invasive species), *Alnus nepalensis*, *Artemisia vulgaris* and climbers.

MG8: Dangdung Chhu, Langthel, Trongsa district (27.33461°N, 90.59562°E, altitude 1,039 m, 05 May 2021; 30 October 2021) is a fast-flowing montane stream with high water current. Sampling was conducted at the divergent point which had low current water flow. Substrate composed of mostly cobbles and sand.

MG9: Kartigang Chhu, Langthel, Trongsa district (27.27896°N, 90.63088°E, altitude 1,456 m, 07 May 2021; 01 November 2021) flows through a landslide washed stream beds with greyish sediments. Substrate was mostly pebbles and cobbles with minimum sand. Surrounding vegetation was sparse with trees having less undergrowth. Water was whitish in color due to white mud bed.

MG10: Chumpigang Chhu, Langthel, Trongsa district (27.31608°N, 90.58071°E, altitude 1,018 m, 08 May 2021; 02 November 2021) stream flows through a deep forest along a narrow valley. Rock obstructs the water flow creating falls, cascades and pools. Riparian vegetation had grasses and stream substrate was mostly cobbles with sand.

MG11: Waterfall stream (name unknown), Trongsa district (27.30171°N, 90.58711°E, altitude 1,195 m, 09 May 2021; 03 November 2021) flows through a mountain gorge forming water falls; the stream substrate mostly composed of cobbles and sand with high debris content.

MG12: Nika Chhu, Trongsa district (27.52601°N, 90.29947°E, altitude 2,609 m, 10 May 2021; 04 November 2021); water current was low despite the stream being quite large. Water temperature was lowest in this stream. Substrates mostly composed of cobbles and sand mixed with debris. Riparian vegetation was mostly shrubs with tall *Pinus* and hemlock tree canopies.

MG13: Rukhubji Chhu, Pelela, Trongsa district (27.51174°N, 90.29711°E, altitude 2,587 m, 12 May 2021; 05 November 2021) flows through a valley covered with thick high-altitude conifers and underlying shrubs, the color of the water is darker throughout the year. Stream substrate was rocky covered by layers of algae and mosses. The stream connects with Nika Chhu River nearby an old Chorten (Buddhist stupa).

MG14: Chuserbu, Trongsa district (27.50246°N, 90.31782°E, altitude 2,666 m, 13 May 2021; 06 November 2021) flows through dense riparian bamboo forest with tall tree canopies, substrate were mostly sand. The stream was pristine and there were fewer disturbances with no settlements upstream.

MG15: Khabab Chhu, Chendebji, Trongsa district (27.48492°N, 90.33490°E, altitude 2,500 m, 14 May 2021; 07 November 2021) flows through a gentle slope. Cobbles and pebbles mixed with sand and debris make up the stream substrate. Plastic waste was dumped along the stream and wastewater from the village is also discharged into the stream. In the post-monsoon, the stream was severely damaged by a flashflood, and there were huge depositions of sand and rocks washed from upstream.

Environmental characterization

At all localities, physico-chemical parameters of water were measured following APHA (2017) standards. Dissolved oxygen (mgL^{-1}), temperature ($^{\circ}\text{C}$), electrical conductivity (μScm^{-1}), pH, and total dissolved solid (mgL^{-1}) were analyzed on site using HANNA multiparameter digital probe (Code HI2004-02, S/N C05031A5). Water samples were collected and stored in freezer (at 5°C) and brought to lab for further analysis. Salinity was measured using salinity meter, turbidity (NTU) was measured following Nephelometric method, water hardness (mgL^{-1}) following EDTA method, Chloride (mgL^{-1}) following Argentometric method (Korkmaz 2001) and ammonia (mgL^{-1}) following Phenate method (Park et al. 2009).

Mites sample collection and preservation

Water mites were collected following quantitative approach as described by Gerecke et al. (2007) with a uniform sampling duration of 10 minutes on each station. In each stream, collections were carried out at four substations using D-frame kick net (mesh size $250\text{-}\mu\text{m}$ and frame size 30cm) with 100 m distance between the sampling substations. The stream bed was dislodged with foot and the materials carried by water current were collected by keeping the D-frame

net downstream ('kick-sampling'). Submerged aquatic plants along the periphery of the streams were disturbed mechanically and the material was collected downstream with the D-frame kick net. The material collected was transferred into a white tray, letting the substrate settle for a few minutes and the mobile mites were picked using a plastic pipette through visual observation. The specimens collected were preserved in Koenike-fluid (20% glacial acetic acid, 50% glycerin and 30% distilled water) for morphological study and a part of the material was also stored in ethanol (90%) for future molecular study.

Identification

Water mites were identified through morphological examination of specimens under a high-resolution microscope Olympus-829187. A Nikon D5600 DSLR-camera attached with NDPL-2(2X) converter was fixed on the eye piece of the microscope to take photographs of the specimens. Identification of the water mites were done using keys of Cook (1967) and Smit (2020). Following an identification by the first author, all identifications were confirmed by H. Smit.

Statistical test

The preliminary data processing was done using Microsoft Excel Professional Plus 2016. Genera composition curve was computed using PC-ORD v5.1 (Grandin 2006). Hydrachnidia genera diversity was examined using Shannon's diversity index, $H' = \sum(P_i) \ln(P_i)$ (where P_i is the proportion (n/N natural log), and \sum is the sum of the equations). Genera richness was calculated using the equation: $(S_r) = (S-1)/\text{Log}N$ (where, S is sum of the genera, N is sum of all genera). Genera evenness, and genera dominance (Simpson's index; D) was calculated using the formula, $D = \sum p_i^2$ (where, p_i is the proportion of genera in a community ($p_i = n/n$), \sum is sum of the equations). Means, standard deviations, total abundance and relative abundance values were calculated for all sites. Independent sample t -test was performed to compare the pre- and post-monsoon diversity indices. Before performing this test, Shapiro-Wilk's test was done in R-software to test if the data were normally distributed.

Principal component analysis (PCA) between environmental variables and the abundance of different water mite genera was performed using the distance measure of relative Sorensen (Bray-Curtis) method separately for both pre- and post-monsoon in PC-ORD v5.1 software. Pearson's correlation test was used to compute the relationship between the environmental variables and water mite assemblages.

RESULTS

A summary of environmental factors in the pre- and post-monsoon period is given in Table 1. In total of 802 water mites were collected belonging to 15 genera with an average of five genera with 26 specimens per location in pre-monsoon and five genera with 29 specimens per location in post-monsoon (Table 2). The mite genera accumulation curve for both (A) pre- and (B) post-monsoon suggests that the sampling efforts were adequate to characterize the water mite genera composition in the study area (Figure 2). Twelve genera, i.e., *Atractides*, *Aturus*, *Hygrobatas*, *Kongsbergia*, *Lebertia*, *Limnesia Monatractides*, *Piona*, *Protzia*, *Pseudotorrenticola*, *Sperchonopsis*, and *Woolastookia* (Image 1 & 2) from eight families (i.e., Aturidae, Hygrobatidae, Lebertiidae, Limnesiidae, Pionidae, Hydryphantidae, Sperchontidae, Torrenticolidae) are recorded new to Bhutan. Pre-monsoon (491) mites were more abundant than that of post-monsoon (311), dominated by *Monatractides* (162) and *Torrenticola* (109) in respective seasons (Table 2).

Diversity indices

Diversity indices such as genera richness, evenness, Shannon diversity index and dominance of 15 streams were calculated (Table 3) and compared (Figure 3) for two seasons. There was a significant positive correlation between pre- and post-monsoon genera diversity ($r = 0.693$, $p = 0.004$), evenness ($r = 0.704$, $p = 0.003$) and dominance ($r = 0.605$, $p = 0.017$) but genera richness ($r = 0.479$, $p = 0.71$) was not significantly correlated for the two seasons (Table 4). Independent sample *t*-test showed no significant differences between pre-monsoon and post-monsoon diversity indices. Genera richness for the pre- ($M = 4.71$, $SD = 1.72$) and post-monsoon ($M = 4.57$, $SD = 1.34$) was not significantly different ($t(26) = 0.244$, $p = .809$). Likewise, for genera evenness for pre- ($M = 0.800$, $SD = 0.139$) and post-monsoon ($M = 0.758$, $SD = 0.163$); ($t(26) = 0.735$, $p = 0.469$); Shannon diversity index for pre- ($M = 1.18$, $SD = 0.389$) and post-monsoon ($M = 1.14$, $SD = 0.366$); ($t(26) = 0.315$, $p = 0.755$) and dominance for pre- ($M = 0.573$, $SD = 0.217$) and post-monsoon ($M = 0.598$, $SD = 0.175$); ($t(26) = -0.335$, $p = 0.741$) were also not significantly different. In pre-monsoon stream MG13 had the highest genera diversity ($H' = 1.63$), however, in post-monsoon MG14 harbored maximum diversity ($H' = 1.62$). Further, genera abundance was also not significantly different between pre- ($M = 24.3$, $SD = 15.2$) and post-monsoon ($M = 29.1$, $SD = 11.4$); ($t(28) = -0.976$, $p = 0.330$).

Pre-monsoon correlations between assemblages and environmental factors

Principal Component Analysis (PCA) was performed between water mite assemblages and environmental factors of pre-monsoon (Figure 4). Axes with highest percentage of variance and Eigen values greater than broken-stick Eigen values were considered for the analysis.

Principal axis 1 (57%) and 2 (20%) explained 72% of the total variance. Temperature ($r = 0.821$) and salinity ($r = 0.511$), calcium hardness ($r = 0.405$), total hardness ($r = 0.470$), magnesium hardness ($r = 0.417$), electrical conductivity ($r = 0.435$), and total dissolved solids ($r = 0.430$) had strong to moderate positive correlation with Axis 1. However, chloride ($r = -0.617$), and ammonia ($r = -0.603$) had strong negative correlation with Axis 1. Similarly, *Hygrobatas* ($r = -0.755$), *Lebertia* ($r = -0.935$), *Sperchon* ($r = -0.910$), *Torrenticola* ($r = -0.730$), *Woolastookia* ($r = -0.544$) and *Monatractides* ($r = -0.490$) exhibited strong to moderate negative correlation with the first axis.

Ammonia ($r = 0.561$), turbidity ($r = 0.525$), chloride ($r = 0.442$), and altitude ($r = 0.434$) had strong to moderate positive correlation with the second principal axis, whereas magnesium hardness ($r = -0.649$), total hardness ($r = -0.509$), temperature ($r = -0.556$), salinity ($r = -0.553$), total dissolved solids ($r = -0.509$) and electrical conductivity ($r = -0.464$) had strong to moderate negative correlation. Similarly, *Atractides* ($r = -0.938$) had strong negative correlation with the second axis. However, *Lebertia* ($r = 0.567$), *Hygrobatas* ($r = 0.453$), *Protzia* ($r = 0.432$), and *Sperchon* ($r = 0.499$) exhibited strong to moderate positive correlation with the second principal axis.

Pearson's correlation test between environmental factors and water mite assemblages showed that *Atractides* ($r = 0.572$, $p = 0.033$) was positively correlated with magnesium hardness. *Hygrobatas* had strong negative correlation with temperature ($r = -0.600$, $p = 0.023$), and salinity ($r = -0.574$, $p = 0.032$). *Lebertia* was positively correlated with altitude ($r = 0.719$, $p = 0.004$), but negatively correlated with temperature ($r = -0.825$, $p = 0.002$), electrical conductivity ($r = -0.536$, $p = 0.048$), salinity ($r = -0.613$, $p = 0.020$) and total dissolved solids ($r = -0.534$, $p = 0.049$). *Sperchon* was positively correlated with altitude ($r = 0.672$, $p = 0.009$), but negatively correlated with temperature ($r = -0.746$, $p = 0.002$). *Torrenticola* was negatively correlated with total hardness ($r = -0.621$, $p = 0.018$) and temperature ($r = -0.633$, $p = 0.015$). *Woolastookia* was positively

Table 1. Summary of environmental characterization of pre- and post-monsoon, 2021.

ID	Season	Alt	NH4	Ca. H	Cl	E.C	DO	Mg. H	pH	Sal	TDS	Tem	TH	Tur
MG 1	Pre-	516±38.4	0.02±0.002	71.02±6.36	90.9±6.75	112.8±2.49	15.95±0.9	15.2±7.49	6.72±1.81	39.2±0.61	79.9±1.60	19.4±0.38	86.2±8.53	0.85±0.24
	Post-	516±38.42	1.38±0.08	13.1±0.85	20.6±7.56	71.2±1.25	10.55±0.20	24.7±5.12	7.87±0.22	43.7±6.48	35.7±5.29	17.9±0.33	37.8±5.97	0.40±0.24
MG 2	Pre-	613±21.4	0.03±0.01	56.2±5.02	93.05±12.4	207.2±2.06	10.7±0.64	18.9±4.35	8.14±0.04	57.3±12.4	104.2±41.6	22.9±0.20	75.2±9.32	0.80±0.12
	Post-	613±21.4	1.81±0.08	38.5±3.41	50.1±3.02	116.8±0.85	9.32±0.17	8.25±1.70	8.14±0.04	52.8±0.85	46.3±0.46	21.9±0.64	46.7±5.42	0.68±0.18
MG 3	Pre-	662±78.4	0.04±0.01	35.2±4.87	88.9±7.91	148.2±5.21	11.4±0.83	12.02±5.35	7.04±0.09	48.9±0.26	102.6±0.47	20.4±1.05	47.2±8.18	0.81±0.30
	Post-	662±78.4	1.66±0.12	32.5±3.41	33.4±1.51	103.5±0.42	8.46±0.12	14±3.07	7.21±0.08	50.7±0.25	44.6±0.64	22.4±0.08	46.5±3.41	0.50±0.30
MG 4	Pre-	575±46.4	0.03±0.005	54.05±7.4	103.3±19.6	80.1±0.71	10.1±1.05	20.5±5.90	7.51±0.10	30.2±0.08	57.06±0.61	19.9±0.12	74.6±6.42	1.22±0.25
	Post-	575±46.4	0.98±0.08	12.8±0.85	18.3±7.56	46.6±1.28	10.3±0.25	13±6.83	8.06±0.12	23.2±0.08	20.1±0.12	21.2±0.34	25.8±7.68	0.19±0.08
MG 5	Pre-	565±36.6	0.03±0.002	28.2±6.39	93.05±15.6	83.5±4.95	9.01±0.94	15.5±6.55	7.47±0.008	30.9±1.57	59.5±3.77	19.4±0.12	43.7±9.46	0.97±0.17
	Post-	565±36.6	1.4±0.20	18.2±0.49	44.1±1.51	38.2±0.5	7.88±0.21	13.5±3.41	7.66±0.22	18.7±0.54	16.3±0.40	17.8±0.21	31.5±3.41	0.19±0.06
MG 6	Pre-	1103±49.9	0.04±0.002	40.1±5.12	128.2±19.6	48.8±0.43	15.7±0.66	30.2±6.49	7.67±0.05	19.3±0.14	34.6±0.16	14.6±0.54	70.3±11.05	0.62±0.20
	Post-	1103±49.9	1.21±0.13	18.6±2.56	42.1±3.02	35.1±0.85	7.781±0.08	7.12±0.85	7.51±0.08	12.5±0.18	10.9±0.16	12.6±0.12	25.7±1.70	0.75±0.13
MG 7	Pre-	1118±102.4	0.04±0.009	44±4.54	130.2±19.5	215±0.81	14.01±0.77	16±8.28	7.15±0.012	72.3±3.58	153.3±1.24	18.3±0.26	60±12.7	0.40±0.06
	Post-	1118±102.4	1.25±0.12	19.3±2.56	118.5±11.51	59.4±0.42	7.93±0.12	22.7±0.95	7.58±0.08	28.8±0.25	24.9±0.12	18.6±0.17	41.3±2.56	0.80±0.08
MG 8	Pre-	1246±43.3	0.03±0.01	59±2.16	136.4±14.3	85.4±2.18	11.7±1.46	14.5±4.79	7.48±0.01	30.03±0.55	60.8±1.32	15.1±0.40	73.5±5.19	0.42±0.07
	Post-	1246±43.3	0.61±0.08	12.8±0.85	50.07±3.02	38.8±0.85	7.55±0.33	9.75±1.70	7.7±0.34	19.3±0.14	16.8±0.05	14.7±0.08	22.6±2.56	0.66±0.10
MG 9	Pre-	1470±76.4	0.04±0.009	50±5.22	128.2±4.77	151.6±4.90	13.6±0.77	11.5±2.38	7.65±0.08	49.5±1.34	108±2.94	14.8±0.35	61.5±7.54	0.37±0.03
	Post-	1470±76.4	0.36±0.09	45.3±24.7	54.2±4.54	69.4±0.42	8.55±0.34	15.8±7.68	8.07±0.17	32.7±0.59	28.1±0.55	15.3±0.25	61.2±32.4	0.84±0.16
MG 10	Pre-	1131±169.6	0.03±0.006	49±6.17	113.7±7.91	353±0.81	12.6±1.04	79.7±6.35	8.07±0.12	112.6±0.47	250.6±0.94	17.4±0.29	128.7±6.22	0.29±0.02
	Post-	1131±169.6	0.50±0.13	97.1±0.85	77.1±18.1	209.4±0.42	11.3±0.08	23.7±11.9	8.66±0.22	103.9±0.59	90.9±1.11	16.6±0.21	120.8±12.8	0.35±0.12
MG 11	Pre-	1163±212.8	0.04±0.003	54.6±1.20	150.9±29.7	160.9±0.63	14.9±1.33	17.7±3.57	8.31±0.19	52.6±0.16	114.6±0.47	15.4±0.12	72.4±3.57	1.10±0.01
	Post-	1163±212.8	0.61±0.08	35±6.83	72.8±1.51	89.25±0.5	9.32±0.17	14.7±5.12	7.89±0.08	44.2±0.12	38.3±0.17	16.1±0.08	49.7±1.70	0.34±0.107
MG 12	Pre-	2606±5.5	0.04±0.001	63.9±1.57	167.5±27.3	231.1±44.4	13.8±0.81	14.7±3.33	7.77±0.28	66.3±9.21	158±22.6	7.66±0.24	78.6±2.72	1.57±0.02
	Post-	2606±5.5	0.6±0.14	48.5±3.4	45.1±6.05	126.2±1.70	6.68±0.21	21.5±3.41	8.64±0.17	61.2±0.34	53.4±0.59	12.5±0.38	70.5±1	0.82±0.06
MG 13	Pre-	2588±15.5	0.04±0.002	47.2±2.62	148.8±13.5	133.5±12.1	14.4±0.78	8.72±3.37	8.35±0.02	40.2±0.89	88.8±2.46	11.4±0.21	56±0.99	0.76±0.06
	Post-	3271±31.1	0.21±0.32	31.7±1.70	54.5±9.08	46.3±9.39	6.71±0.08	39.3±2.56	7.77±0.51	23.1±4.39	20.05±3.65	13.36±0.42	71.1±0.85	0.58±0.17
MG 14	Pre-	2588±49.3	0.05±0.01	38.2±6.23	148.8±20.2	43.2±2.05	10.1±0.76	10.6±1.66	7.73±0.07	14.3±0.571	30.7±1.38	7.56±0.16	48.9±7.62	1.12±0.08
	Post-	2588±49.3	0.18±0.08	17.6±9.39	67.7±3.02	21.4±0.42	8.31±0.76	23.5±10.2	8.01±0.13	9.76±0.09	8.44±0.05	13.2±0.25	41.1±0.85	0.70±0.10
MG 15	Pre-	2494±26.1	0.05±0.006	35.3±1.26	173.7±30.2	62.6±0.91	12.2±0.60	9.50±0.71	7.57±0.04	19.9±0.32	44.6±0.62	7.93±0.18	44.8±1.08	0.89±0.03
	Post-	2494±26.1	0.24±0.23	44.3±4.26	45.6±3.02	30.8±4.69	7.73±0.18	2.87±0.85	8.11±0.21	12.2±0.49	10.5±0.19	13.4±0.25	47.2±5.42	0.48±0.11

Alt—Altitude | NH4—Ammonia | Ca. H—Calcium hardness | Cl—Chloride | EC—Electrical conductivity | DO—Dissolved Oxygen | Mg. H—Magnesium hardness | Sal—Salinity | TDS—Total dissolved solid | Tem—Temperature | TH—Total Hardness | Tur—Turbidity

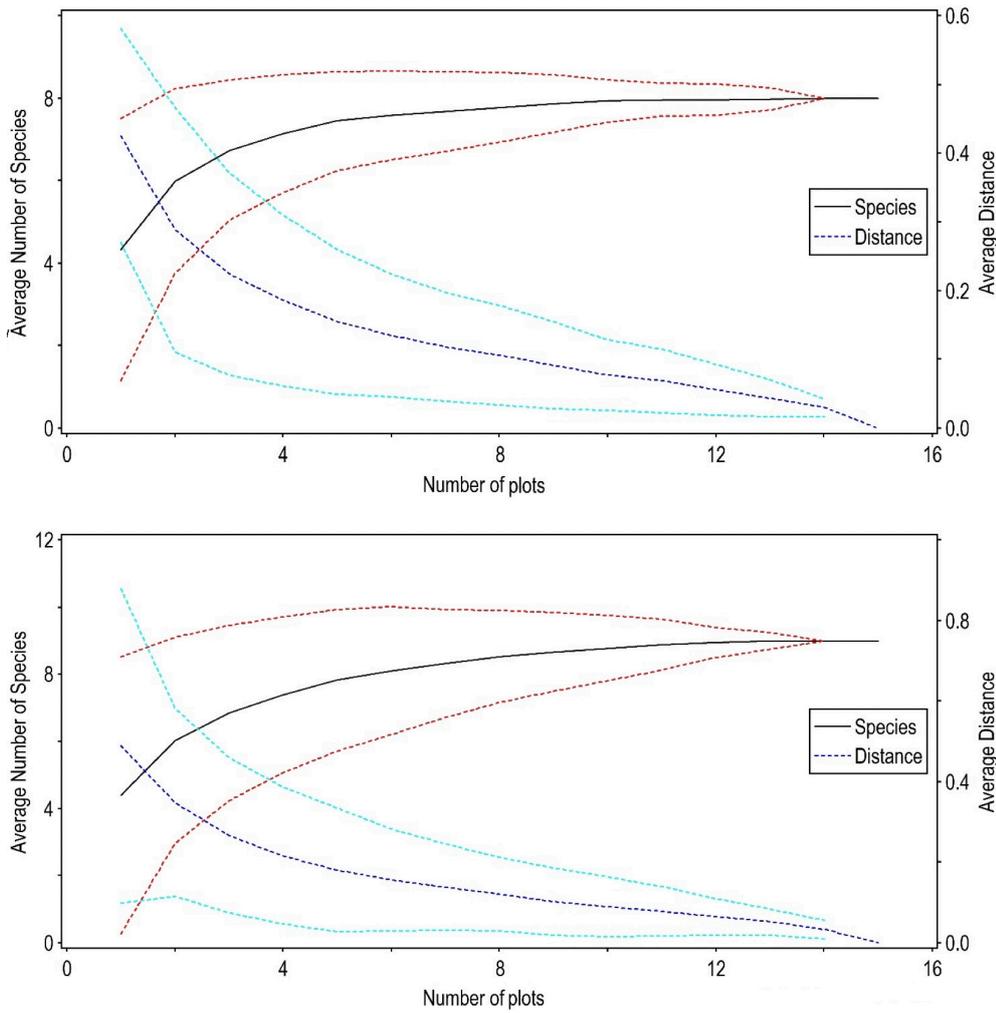


Figure 2. Water mites genera accumulation curve for (A) pre- and (B) post-monsoon seasons.

correlated with altitude ($r = 0.583, p = 0.029$) but negatively correlated with temperature ($r = -0.562, p = 0.037$).

Post-monsoon correlations between assemblages and environmental factors

Principal component analysis (PCA) was also performed between post-monsoon environmental factors and water mite assemblages (Figure 5). Principal axis 1 (53%) and 2 (24%) explained 77% of the total variability. Variables such as dissolved oxygen ($r = 0.410$), temperature ($r = 0.445$), electrical conductivity ($r = 0.435$), salinity ($r = 0.456$), and total dissolved solids ($r = 0.453$) had moderate positive correlation with first principal axis. Similarly, there was strong to moderate positive correlation between *Monatractides* ($r = 0.841$), *Torrenticola* ($r = -0.552$), *Aturus* ($r = -0.333$), and *Lebertia* ($r = -0.327$) with the first principal axis. Altitude ($r = 0.423$), total hardness ($r = 0.496$), and calcium hardness

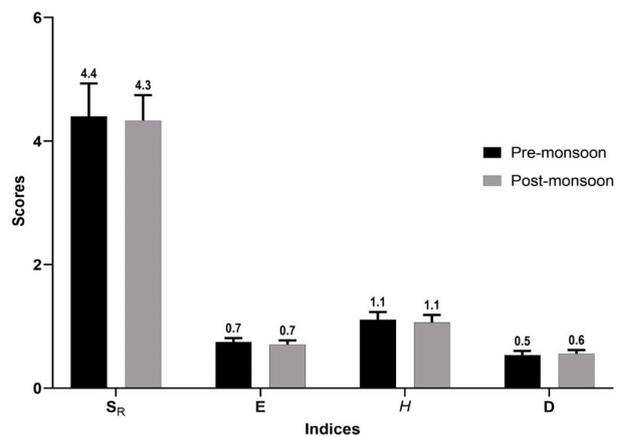


Figure 3. Pre-monsoon and post-monsoon mean diversity indices.

($r = 0.496$) had moderate positive correlation with the second axis. Furthermore, *Atractides* ($r = 0.724$), *Sperchon* ($r = 0.667$), and *Lebertia* ($r = 0.361$) also had

Table 2. Pre- and post-monsoon mite abundance at different elevations.

Genus	Season	500–1000 m						1,001–1,999 m						>2,000 m				
		MG 1	MG 2	MG 3	MG 4	MG 5	MG 6	MG 7	MG 8	MG 9	MG 10	MG 11	MG 12	MG 13	MG 14	MG 15		
1 <i>Atractides</i>	Pre	12	2	2	3	10	0	14	2	5	15	0	3	2	0	2		
	Post	1	4	16	1	4	0	5	1	0	23	0	12	6	9	6		
2 <i>Aturnus</i>	Post	0	0	0	1	0	0	0	1	0	0	0	0	1	1	0		
	Pre	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3		
3 <i>Hygrabates</i>	Post	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0		
	Post	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
4 <i>Kongsbergia</i>	Pre	1	0	0	0	0	0	3	0	1	0	0	5	12	17	20		
	Post	0	0	0	0	4	0	1	0	0	0	0	2	2	3	0		
5 <i>Lebertia</i>	Post	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Pre	3	3	15	3	1	0	0	0	5	1	9	2	2	1	1		
7 <i>Monatractides</i>	Post	19	5	16	12	4	8	33	12	23	12	17	0	0	1	0		
	Post	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0		
9 <i>Protzia</i>	Pre	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0		
	Post	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0		
10 <i>Pseudotorrenticola</i>	Post	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0		
	Pre	2	4	0	2	3	0	0	0	3	4	0	5	11	11	13		
11 <i>Sperchan</i>	Post	2	13	5	1	2	0	0	0	0	6	0	2	3	10	7		
	Pre	1	2	1	0	0	0	0	0	3	0	0	2	0	0	0		
12 <i>Sperchanopsis</i>	Post	5	2	4	1	0	0	0	3	1	0	0	4	0	2	0		
	Post	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0		
14 <i>Torrenticola</i>	Pre	0	3	9	4	14	0	9	3	10	2	1	18	9	10	17		
	Post	7	8	3	13	0	0	0	14	8	0	2	17	2	11	3		
15 <i>Woolastookia</i>	Pre	0	0	0	0	0	0	0	0	0	0	0	2	3	0	1		
	Total	19/34	14/33	27/44	12/30	28/14	0/8	26/39	5/33	27/34	22/42	10/20	37/37	40/15	41/38	47/16		

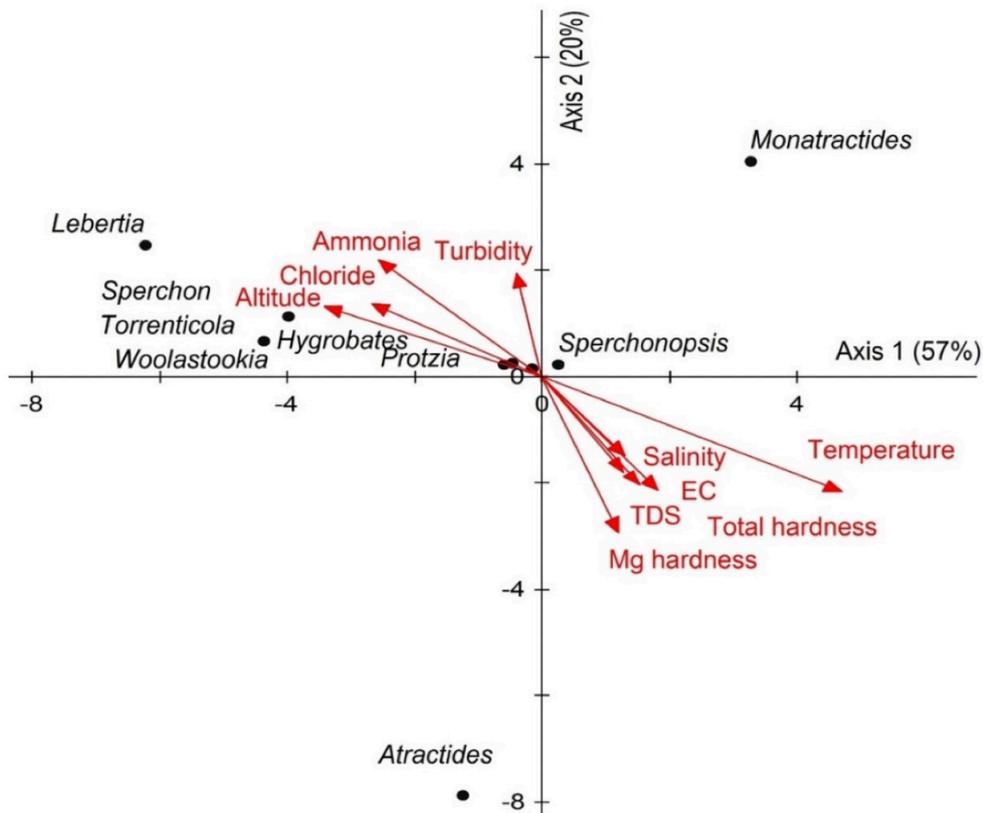


Figure 4. Principal component analysis (PCA) displaying dependence of water mites on environmental variables (pre-monsoon).

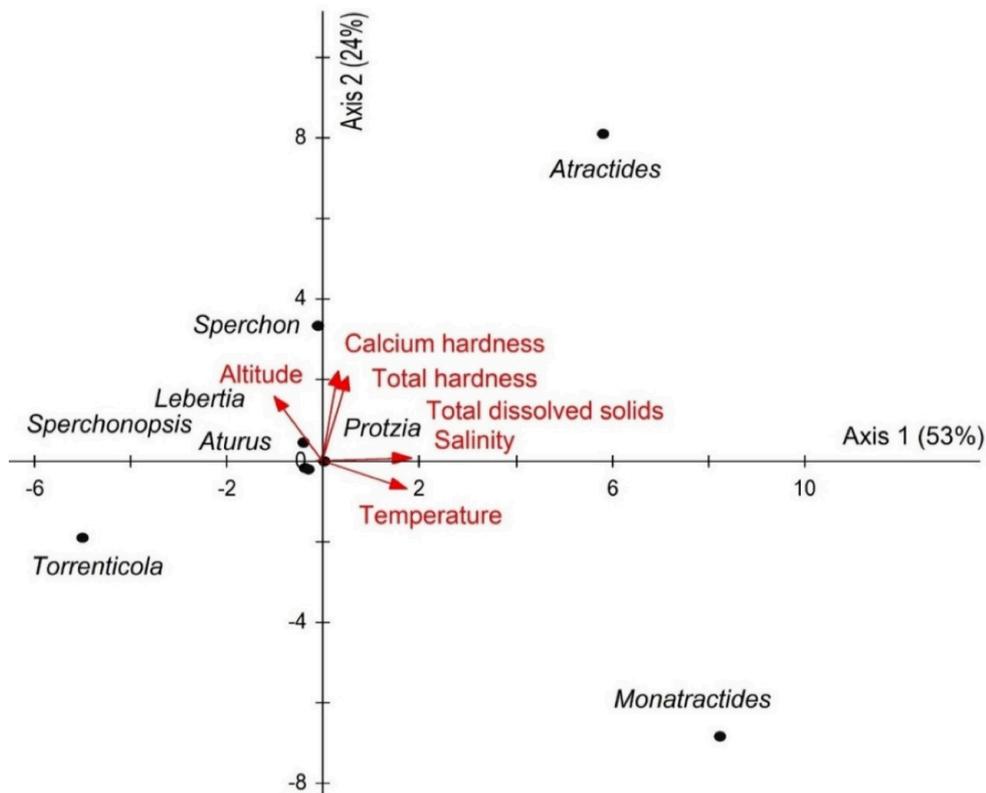


Figure 5. Principal component analysis (PCA) of association between water mites and environmental variables (post-monsoon).

Table 3. Summary of water mites genera diversity indices of two seasons in 15 streams. S_R —genera richness | E —evenness | H' —Shannon diversity index | D —dominance of (1) pre- and (2) post-monsoon.

Streams	Pre-monsoon				Post-monsoon			
	S_{R1}	E_1	H'_1	D_1	S_{R2}	E_2	H'_2	D_2
MG1	5	0.70	1.13	0.56	5	0.75	1.20	0.62
MG2	5	0.98	1.57	0.79	5	0.89	1.44	0.73
MG3	4	0.73	1.00	0.57	5	0.86	1.38	0.71
MG4	4	0.98	1.35	0.74	6	0.66	1.19	0.62
MG5	4	0.77	1.07	0.61	4	0.98	1.35	0.73
MG6	-	-	-	-	1	0	0	0
MG7	3	0.86	0.95	0.58	3	0.45	0.50	0.27
MG8	2	0.97	0.67	0.48	6	0.74	1.33	0.67
MG9	6	0.90	1.60	0.77	4	0.58	0.81	0.45
MG10	4	0.67	0.93	0.49	4	0.76	1.06	0.60
MG11	2	0.47	0.33	0.18	2	0.49	0.34	0.19
MG12	7	0.81	1.57	0.07	5	0.79	1.28	0.67
MG13	7	0.84	1.63	0.77	5	0.89	1.44	0.72
MG14	6	0.77	1.33	0.69	7	0.83	1.62	0.77
MG15	7	0.76	1.48	0.73	3	0.95	1.04	0.63
Average	4.4±2.06	0.75±0.25	1.11±0.48	0.53±0.26	4.33±1.60	0.71±0.25	1.06±0.46	0.56±0.23

Table 4. Correlation between pre- and post-monsoon mean diversity indices.

	S_{R2}	E_2	H'_2	D_2
S_{R1}	.479	.719**	.642**	.680**
E_1	.726**	.704**	.709**	.725**
H'_1	.587*	.713**	.693**	.724**
D_1	.515*	.609*	.580*	.605*

Correlation is significant at the 0.05 level (2-tailed). **—Correlation is significant at the 0.01 level (2-tailed) | S_R —genera richness | E —evenness | H' —Shannon diversity index | D —dominance of (1) pre-monsoon and (2) post-monsoon (2).

strong to moderate positive correlation with principal axis 1, whereas *Monatractides* ($r = -0.794$) had negative correlation.

Pearson's correlation test between mites genera abundance and environmental variables showed that *Atractides* had positive correlation with total hardness ($r = 0.671$, $p = 0.006$), calcium hardness ($r = 0.611$, $p = 0.015$), and electrical conductivity ($r = 0.541$, $p = 0.037$). *Sperchonopsis* had strong negative correlation with chloride concentration ($r = -0.545$, $p = 0.036$).

Zoogeographical aspects

All the 15 genera found are predominantly Palearctic in distribution. The most dominant genera, i.e., *Atractides*,

Lebertia, *Monatractides*, *Torrenticola* and *Sperchon* are also dominant genera in Palearctic streams (Di Sabatino et al. 2008). Oriental genera, such as *Nicalimnesia*, *Bharatonia*, *Khedacarus*, *Navamamersides*, *Nilgiriopsis*, *Paddelia*, and *Sinaxonopsis* were not found, also at lower altitudes (~500 m). It must be stated, however, that most of these genera occur in springs or in the hyporheic, which was not studied during this study. Moreover, the genus *Lebertia* is very rare in the Oriental region (Cook 1967; Di Sabatino et al. 2008).

DISCUSSION

There were no significant differences between indices of pre- and post-monsoon ($p < .05$). Further, genera abundance was also not significantly different ($t(28) = -0.976$, $p = 0.33$). This could be due to presence of dominant Palearctic genera and less observed variation in environmental variables in the two seasons. Similarly, Zawal et al. (2017), Pozojević et al. (2018), and Zawal et al. (2020) also suggested that there was no significant variation in seasonal abundance and assemblage of water mites in lotic habitats of Dinaric region of Croatia and ancient Lake Skadar basin in southern Europe respectively. This could be anticipated due to high degree microhabitat specialization by most genera (Di



Image 1. *Atractides* sp: A—Dorsal view, leg | B—Ventral view, leg. V. Pešić | C—*Atractides* sp 3. Dorsal view, leg. M.M. Gurung | D—*Limnesia* sp. Ventral view. © M.M. Gurung.

Sabatino et al. 2000).

Most water mite genera exhibited negative correlation with water parameters indicating sensitivity to pollution (Goldschmidt 2016; Savić et al. 2022). Abelho et al. (2021), who carried out an experiment

on the effect of salinity on aquatic macroinvertebrates, also suggested that genera diversity and abundance are negatively correlated with salinity as it impacts on the osmoregulation of aquatic insects and often hypertonicity can be lethal (Griffith 2017). Roberts



Image 2. A—*Monatractides* sp. Ventral view, leg. | B—*Sperchon* sp. Ventral view | C—*Torrenticola* sp. Ventral view | D—*Sperchonopsis* sp. Ventral view. © M.M. Gurung.

& Palmeiro (2008), Da Costa et al. (2014), Kent et al. (2014), and Delaune et al. (2021) suggested that exposure to high chloride concentration in water causes acute toxicity on macroinvertebrates and zooplankton. Since most of the mites collected were Palearctic genera, they exhibited positive correlation with altitude and negative correlation with temperature. Similarly, Young (1969) and Pozojević et al. (2020) also suggested that *Atractides*, *Lebertia*, *Sperchon*, and *Torrenticola*

genera are negatively correlated with temperature and are more abundant at higher elevations (>1,000 m). High ammonia content in water lowers the ability to excrete digestive waste for aquatic insects causing toxic built up in the tissues and the genera abundance declines gradually (Willingham et al. 2016; Wood 2019). Magnesium and chloride ions in the water increase water hardness, salinity, and electrical conductivity. These ions also contribute to chloride toxicity modification and

cause ameliorating impact on aquatic diversity (Soucek et al. 2011). Thus, diversity decreases with increase in water hardness, salinity, chloride, and TDS. During post-monsoon, *Atractides* (Hygrobatidae) exhibited a significant positive correlation with water hardness ($r = 0.671, p = 0.006$) and electrical conductivity ($r = 0.541, p = 0.037$). This genus was relatively abundant in hard water with higher electrical conductivity habitats. However, it should not be interpreted as affinity towards polluted environments but rather as higher tolerance against unfavorable conditions. Similarly, Goldschmidt (2016) indicated that genera from the family Hygrobatidae are relatively abundant in polluted environments, indicating a higher tolerance for pollutants.

The 15 streams were grouped in three altitudinal ranges, namely between 500–1,000 m (low), 1,001–1,999 m (mid) and above 2000 m (high) (Table 5, Figure 6). The abundance of water mites increased in higher altitudes. Some of the genera seem to show a preference for either lower or higher altitudinal ranges with *Woolastookia* being restricted to higher altitude, *Lebertia* preferring higher altitudes and *Monatractides* being largely absent at higher altitude. All 15 genera collected had a largely Palearctic distribution, and typical Oriental genera such as *Nicalimnesia*, *Bharatonia*, *Khedacarus*, *Navamamersides*, *Nilgiriopsis*, *Paddelia*, and *Sinaxonopsis* were not encountered. In general, the Bhutanese fauna below 1,000 m becomes increasingly dominated by Oriental genera (Rasaily et al. 2021), hence the absence of Oriental water mite taxa is surprising. It seems likely that the fast-flowing water bodies in which our sampling took place allows the Palearctic genera to occur at lower altitude and prevents the occurrence of Oriental genera. Furthermore, most mites we sampled were stream dwelling, unlike oriental genera which are more abundant in spring and standing water habitats (Pešić et al. 2012). Sampling of standing waters at lower altitude or smaller brooks fed by local sources will probably show that such habitats are at least inhabited and may be even dominated by Oriental genera. Although there is an increasing abundance of mites along the altitudinal gradient, there is no change in genera diversity. There was a uniform faunal composition throughout the altitude dominated by Palearctic genera.

Ethical standards

The research project with permit no. 17703966556045F2C2BFD63 was approved by Ugyen Wangchuck Institute for Conservation and Environmental Research (UWICER), Lamaigoenpa, Bumthang Bhutan. Specimen collections and preservation were done

following standard protocols as detailed in the methods section.

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