

Understanding Zoobenthos Dynamics in Some Crenic Habitats of Kashmir Valley

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ABSTRACT

This research was carried out to have a better understanding of how various variables like water chemistry and the seasons influence Zoobenthos dynamics in freshwater springs of Kashmir Valley. In this regard, a detailed Zoobenthic assessment of six freshwater springs in the valley of Kashmir was carried out. A total number of 46 taxa of Zoobenthos were recorded representing Mollusca-4 (Gastropoda-3 and Bivalvia-1), Annelida-5 (Oligochaeta-3 and Hirudinae-2), and Arthropoda-37 (Crustacea-8, Arachnida-1, and Insecta-28). We observed that the Zoobenthic community was dominated by 28 insect forms while 18 non-insect forms were also represented. The non-insect community was comprised of 4 species of molluscs, 8 crustaceans, and 5 annelids. Insecta was represented by 8 different orders (Ephemeroptera-6, Hemiptera-2, Odonata-1, Coleoptera -1, Plecoptera-1, Megaloptera-1, Trichoptera-7 and Diptera-9). NMDS and ANOSIM results revealed that zoobenthos differed significantly among the springs, wherein alpha diversity metrics showed decreasing and increasing trend among the springs, with high Shannon's H' and Pielou's Evenness recorded at Achabal spring. Regression analysis did not have any significant relationship with the environmental variables while ANOVA displayed significant variations in Shannon's H' in Achabal springs compared to the other springs. In contrast, beta diversity patterns were significantly explained by variation in environmental factors, especially WT, pH, DO, FCO, Cl, TP, and PO₄³⁻. The similarity of percentage (SIMPER) identified zoobenthic taxa like *Tubifex tubifex*, *Chironomus* sp., *Branchiura sowerbyi*, *Gammarus pulex*, *Erpobdella octoculata*, *Baetis rhodani*, and *Drunella* sp. responsible for the average dissimilarity between the springs. Spatial and environmental factors played a significant role in explaining different aspects of zoobenthic assemblage characteristics compared to the temporal factors.

Keywords: Springs, Crenobiology, Diversity, Zoobenthos

INTRODUCTION

Springs being unique biotopes (Kurzweil *et al.*, 2021) and diverse in many facets (Cantonati *et al.*, 2020; Lone *et al.*, 2021), are present throughout the earth's landscape acting as windows into the earth. These crenic habitats contribute largely to aquatic biodiversity due to heterogeneous habitat complexity, peculiar physicochemical stability (Bhat and Pandit, 2009, 2010), high degree of individuality, and a zonal character and typologies (Cantonati *et al.*, 2006). Springs are species-rich and contain a larger

number of unique biodiversity, including many Red List taxa than other aquatic habitats (Cantonati *et al.*, 2020). From the conservation perspective, spring ecosystems hold a position of importance as study areas for the protection of their habitats (Cantonati *et al.*, 2006, 2010) that are out of proportion to their size and number (Van der Kamp, 1995). Due to their distinctive "mosaic" and diverse "ecotonal" organization, springs mark the boundary between two separate ecosystems, resulting in significant structural heterogeneity, metabolic rates, and functioning

(Bolpagni *et al.*, 2019). These characteristics, together with the abundance of suitable microhabitats, flow permanence, and water chemistry, among other factors, are major determinants of assemblage composition in spring compared to other freshwater environments (Hoffsten and Malmqvist, 2000; Sun *et al.*, 2019). Springs have been identified as an important and reliable supply of water for household use, agriculture, and livestock since prehistoric times (Bhat *et al.*, 2020) while crenic endemism has only lately been recognized at local and regional levels (Ferrington *et al.*, 1995). Springs provide habitat for specialized organisms adapted to the relatively stable environment (Thienemann, 1924; Odum 1971; Ellenberg, 1996). Springs are characterized by distinctive crenic biodiversity and marked heterogeneity of environmental conditions and communities (Bonettini and Cantonati, 1998). Hence, the species composition of springs differs from the adjacent ecosystems, in which the short-term and seasonal variability in the environment is more pronounced. Some benthic macroinvertebrates are only found in spring habitats (Erman and Erman, 1995). Macroinvertebrates are the most commonly used biological indicators, which are sensitive to environmental changes and are used to track changes in water quality (Pignata *et al.*, 2013). Spring environments are characterised by a mosaic-like substrate composition and a high level of uniqueness in macroinvertebrate assemblages (Cantonati *et al.*, 2006).

Groundwater fed springs provide a unique link between surface and groundwater ecosystems (Webb *et al.*, 1998), allowing researchers to investigate the interplay between epigeal and subterranean habitats. Due to their isolation and

geographical fragmentation, spring habitats, which are species-rich and complex, are typically small ecotones (Cantonati *et al.*, 2006), sensitive to climate change (Taxbock *et al.*, 2017). Despite the high degree of endemism often associated with crenic ecosystems from different parts of the world, their biodiversity value is poorly documented (Botosaneanu, 1998; Hinterlang and Lischewski, 1996; Di Sabatino *et al.*, 2003; Cantonati *et al.*, 2006). On the one hand, there seems to be enough information on the economic perspective of high-quality water from springs, including the hydrogeological interface. However, on the other hand, the ecological perspective of springs is missing, wherein only a few studies have comprehensively documented the crenic biodiversity (Cantonati *et al.*, 2006; Lone *et al.*, 2021). Despite having such importance, they are also insufficiently covered by protective legislation, often destroying their natural habitat (Cantonati *et al.*, 2012). Globally science of crenic habitats (Crenobiology) is catching renewed interest among researchers regarding the valuing of spring ecosystems and many efforts in this direction have been carried out (Fatchen, 2000; Hartnett, 2000; Sada *et al.*, 2001; Sada and Sharpe, 2004; Shrestha *et al.*, 2018). There is a growing understanding that crenic biodiversity is confronting various threats, the majority of which are anthropocentric (Williams and Williams, 1998; Lone, 2020; Bhat *et al.*, 2021). Also are there are growing calls to bring these vulnerable crenic environments to the attention of policymakers for conservation and management (Cantonati *et al.*, 2020).

Major focus on spring research in Indian Himalayan Region (IHR) (Stevens *et al.*, 2021) has been on hydrogeology (Valdiya and Bartarya,

1991; Negi and Joshi, 1996, Jeelani *et al.*, 2010; ACWADAM, 2011), water quality (Jeelani, 2005, 2007) while as ecosystem perspective including research on crenic biodiversity has been altogether missing. Springs across IHR are data deficient systems (Bhat and Pandit, 2018; Bhat *et al.*, 2020; Lone *et al.*, 2020) wherein very few studies have been carried out on crenic biodiversity (Bhat and Yousuf, 2002; Bhat and Pandit, 2009; Lone and Bhat, 2020; Poddar and Das, 2018; Mehetre *et al.*, 2018; Kumar *et al.*, 2020), threats to springs (Lone, 2020; Lone *et al.*, 2020) and conservation and management aspects (Bhat *et al.*, 2020). Nevertheless, efforts from various individual education and research institutions, community level, NGOs, and few Govt. Departments are in the swing to further the cause of spring research across IHR. The States which have dominated the spring research in IHR include Jammu and Kashmir, Sikkim, and Uttarakhand. It is pertinent to mention that such studies are inherently deficient in crenic biodiversity research because of wanting for expertise in such a research endeavor. Despite the importance of springs, they are much less studied than other aquatic ecosystems and national efforts in this direction are therefore urgently needed to bring attention to the importance of springs and conservation strategies thereof (Bhat and Pandit, 2020).

Kashmir is recognized for its widespread springs and spring-fed streams ranging from hundreds to thousands which are an appealing phenomenon, surrounded by a variety of landscapes from lofty mountains to low-land floodplains. The outstanding importance of these habitats for the conservation of freshwater biodiversity is slowly but increasingly being recognized (Stevens *et al.*,

2021). Extensive works on spring ecosystems from the hydrogeological and ecosystem perspective is yet to be carried although there are very few works available on water quality (Qadri and Yousuf, 1979; Bhat and Pandit, 2009a, b; Bhat *et al.*, 2010; Jeelani *et al.*, 2011; Bhat and Lone, 2015; Bhat and Pandit, 2018, 2020, Bhat *et al.*, 2020) macroinvertebrates (Yousuf *et al.*, 1983) and periphytic algae (Bhat and Yousuf, 2002; Lone *et al.*, 2011, Lone *et al.*, 2021). Given the above-noted scenario on crenobiology, it is evident that there exist large knowledge gaps across the regions especially from a checklist or inventory of comprehensive multi-taxon perspectives. These crenic systems are under-represented in the voluminous body of literature on inland waters. The existing knowledge gaps call for further in-depth studies on crenic habitats with an emphasis on ecosystem health, biodiversity, and ecosystem services, apart from the well-recognized role in the drinking water supply of the increasing population (Bhat and Pandit, 2010a, b, 2018; Bhat *et al.*, 2010; Hammed *et al.*, 2018) and to prioritize management actions for conservation (Bolpagni *et al.*, 2019) including policy initiatives on spring ecosystems and institutional mechanism to implement the same (Bhat *et al.*, 2020). It was in this background, this study was carried out to have some database on the zoobenthic composition of some freshwater springs of Kashmir Himalaya.

MATERIALS AND METHODS

Six freshwater springs fall in two Southern districts of the valley, namely Anantnag (Verinag, Kokernag, Achabal, and Sherebagh) and Pulwama (Indraznag and Dobinag) were selected for

zoobenthic study (Fig. 1) during 2004-2006, covering 8 seasons.

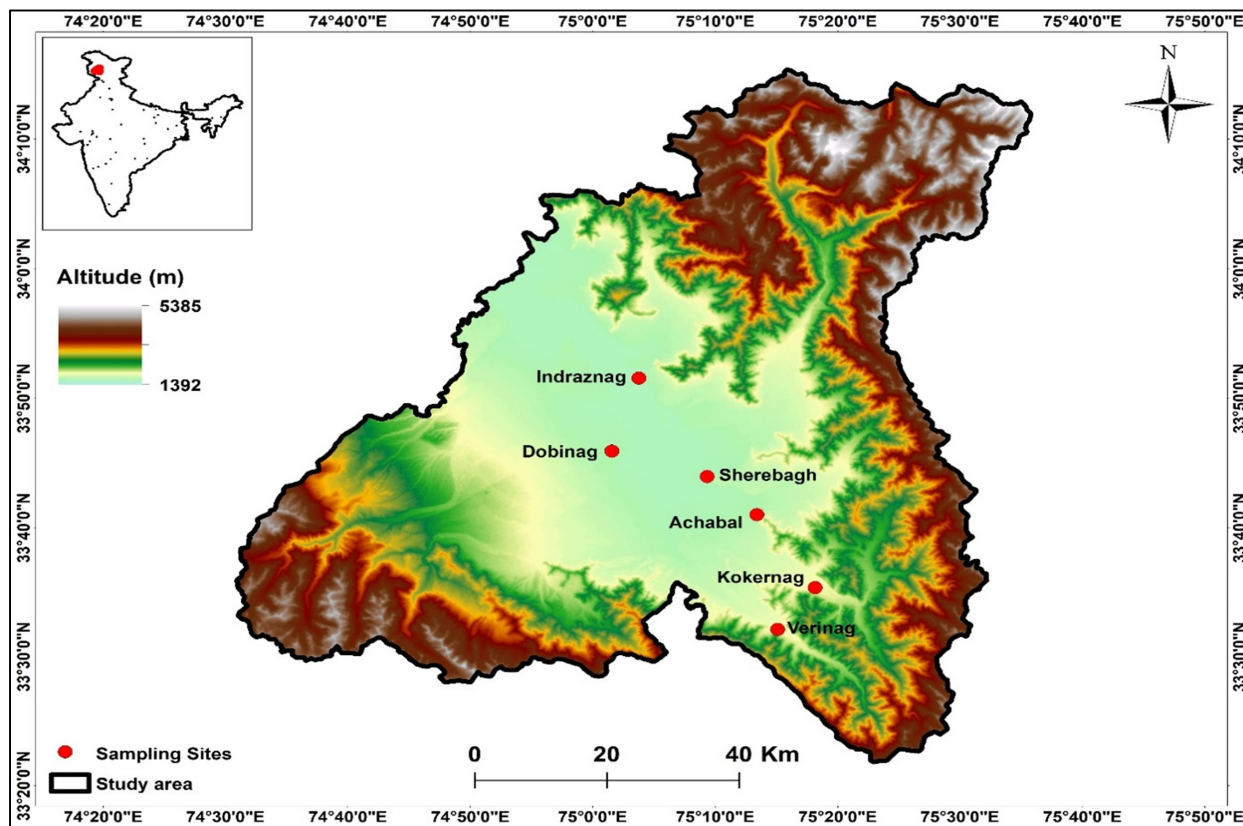


Fig. 1 Six freshwater springs in the Southern part of Kashmir Valley

Zoobenthos was collected using D-net (0.01 m^2) with 0.5mm mesh size (Cuffney *et al.*, 1993). The bottom substrate was (Paasivirta, 2005; Von Fumetti *et al.*, 2007) sampled to cover an area of 1 m^2 of the substratum. After removing the debris attached to the samples by the combination of washing, sieving, brushing, following standard methods (Borrer *et al.*, 1976; APHA, 2005), organisms were sorted out and preserved in 4% formalin (shelled) and 70% alcohol (soft-bodied) (Borrer *et al.*, 1976; Von Fumetti *et al.*, 2007). The Identifications were done to the lowest taxonomic level possible using standard keys (Borrer *et al.*, 1976; Cummins and Merritt, 1995; Rossetti *et al.*, 2006; Thorp and Covich, 2009). For analyzing the relationship between various

variables of water chemistry with zoobenthos, data was used from published works like Bhat and Pandit, 2018, 2020. Venn diagram was created using function "gg Venn Diagram" in R programming for statistical computing (R Core Team, 2017). To reduce the bias caused by the taxa, occurring in one or two springs with a relative abundance of <1% were excluded from the multivariate analysis.

Data processing and analysis

We performed non-metric multidimensional scaling (NMDS) using package "vegan" (Oksanen *et al.*, 2016), a robust ordination technique that produces an ordination based on similarity or dissimilarity among sites. Springs that are more

similar in terms of zoobenthic assemblages are ordinated together. NMDS ordination plot with low stress is considered a good fit (Shepard, 1962; Mahecha *et al.*, 2007). To understand the diversity and distribution of the zoobenthos community, and their interaction with another and their environment, Alpha diversity (Shannon's H' , Species Richness and Pielou's Evenness), the relationship between Alpha diversity and environmental factors, and analysis of variance (ANOVA) were employed. Shannon's index H' (Shannon and Weiner, 1949) takes into account the total number of species and how evenly distributed species abundance is at each site. Simple linear regression models were employed to understand the positive and negative relationships between the environmental factors and Shannon's H' . The ANOVA was used to understand the relationship between Shannon's H' and environmental factors. To explore the beta diversity, pairwise Bray-Curtis (Bray and Curtis, 1957; Legendre and Legendre, 1998) and Jaccard's Dissimilarity (Jaccard, 1912) metrics were employed to understand the structure of the zoobenthic community through spatio-temporal scale and the species/taxa which are driving these trends. Percentage of similarity (SIMPER) (Clarke, 1993; Warton *et al.*, 2012) test was executed to determine the contribution of most influential taxa/species to Bray-Curtis Dissimilarity between each pair sites. The principal coordinate analysis (PCoA) ordination technique (Zuur *et al.*, 2007) was further used to visualize the structure of communities in a two-dimensional space and to visualize underlying relationships. Finally, to establish the relationships between zoobenthos and environmental variables measured, distance-

based redundancy analysis (dbRDA) was employed (Anderson *et al.*, 2008). A forward selection process ($p < 0.05$) based on 9999 permutations was used for selecting environmental factors. Prior dbRDA, highly correlated (Pearson's $r > 0.80$) environmental variables (multi-collinearity) were excluded after calculating variance inflation factors (VIFs), while as included variables were transformed (z-transformation) accordingly, to fulfill the assumption of normality. Adjusted determination coefficients obtained for environmental variables from unconstrained and constrained ordination were included in the study owing to their unbiased nature (Meng *et al.*, 2018). Subsequently, environmental variables responsible for controlling the dynamic pattern of macro-invertebrate assemblages were identified accordingly. Various packages used in the present study for data analysis in R statistical computing include: "vegan package", "ggplot2", "viridis", and "cowplot" (R Core Team, 2017).

RESULTS AND DISCUSSION

A closer look at the data sets revealed that among 46 taxa reported here which is comparatively high as compared to previous studies from the same study area wherein 19 and 23 taxa are reported (Bhat and Pandit, 2009; Lone, 2020). This is the only one of the few detailed study conducted on the zoobenthic composition of Kashmir Himalayan springs. In this study five most common taxa found in all the six springs included *Tubifex tubifex*, *Erpobdella octoculata*, *Gammarus pulex*, *Baetis rhodani*, and *Simulium* sp. while there were fourteen taxa (*Alona* sp., *Paracyclops* sp., *Epeorus* sp., *Hydroptila* sp., *Diamesinae* sp., *Tendipes* sp., *Glossiphonia* sp., *Nigrobaetis*

gracilis, *Corixa* sp., *Gerris* sp., *Aeshna* sp., *Nemoura* sp., *Tabanus* sp., and *Culex* sp.) found only in one particular spring (Table 1 and Fig. 2). Certain taxa like *Gyraulus* sp., *Sida* sp., *Tyrellia* sp., *Caenis* sp., *Torleya* sp., *Corydalus* sp., *Rhyacophila yamanakensis*, and *Limnephilus* sp. were recorded only from two out of six springs, thereby reflecting the habitat diversity. Similarly taxa like *Limnodrilus* sp., *Branchiura sowerbyi*,

Cyclocypris sp., *Cypris* sp., *Grouvellinus* sp., *Hydroptila* sp., *Rhyacophila obscura*, *Stenopsyche* sp., *Atherix* sp., and *Tipula* sp. were found only in three springs. We also observed that springs relatively with high annual mean discharge and high habitat heterogeneity like Kokernag (26), Verinag (24), Achabal (22), and Sherebagh (15) were having more taxa (Table 1).

Table 1. Presence/absence of macroinvertebrate taxa at different springs

Taxa/Species	Verinag	Kokernag	Achabal	Sherebagh	Indraznag	Dobinag
<i>Radix ovate</i>	+	+	+	+	-	-
<i>Corbicula</i> sp.	+	+	+	+	-	+
<i>Erpobdella octoculata</i>	+	+	+	+	+	+
<i>Baetis rhodani</i>	+	+	+	+	+	+
<i>Gammarus pulex</i>	+	+	+	+	+	+
<i>Simulium</i> sp.	+	+	+	+	+	+
<i>Chironomus</i> sp.	+	+	-	+	+	+
<i>Tubifex tubifex</i>	+	+	+	+	+	+
<i>Cyclops cyclopoid</i>	+	+	+	+	-	-
<i>Grouvellinus</i> sp.	+	-	-	-	+	+
<i>Hydropsyche</i> sp.	+	+	+	-	-	-
<i>Drunella</i> sp.	+	+	+	-	+	-
<i>Rhyacophila obscura</i>	+	+	+	-	-	-
<i>Stenopsyche bergeri</i>	+	+	+	-	-	-
<i>Atherix</i> sp.	+	+	+	-	-	-
<i>Daphnia pulex</i>	-	+	+	+	-	+
<i>Tipula</i> sp.	+	+	+	-	-	-
<i>Choaborus</i> sp.	-	+	+	-	+	+
<i>Glossosoma</i> sp.	-	-	+	+	+	+
<i>Lymnaea auricular</i>	-	+	+	+	+	-
<i>Branchiura sowerbyi</i>	-	-	-	+	+	+
<i>Cypris</i> sp.	-	-	+	-	+	+
<i>Tendipes</i> sp.	-	-	-	+	-	-
<i>Diamesinae</i> sp.	-	-	+	-	-	-
<i>Gyraulus</i> sp.	+	+	-	-	-	-
<i>Corixa</i> sp.	-	-	-	-	+	-
<i>Gerris</i> sp.	-	+	-	-	-	-
<i>Sida</i> sp.	-	-	+	+	-	-
<i>Alona</i> sp.	+	-	-	-	-	-

<i>Nemoura</i> sp.	-	+	-	-	-	-
<i>Tyrrelia</i> sp.	+	+	-	-	-	-
<i>Torleya</i> sp.	+	+	-	-	-	-
<i>Nigrobaetis gracilis</i>	+	-	-	-	-	-
<i>Cyclocypris</i> sp.	+	+	-	-	-	-
<i>Aeshna</i> sp.	-	+	-	-	-	-
<i>Corydalis</i> sp.	+	+	-	-	-	-
<i>Caenis</i> sp.	+	-	+	-	-	-

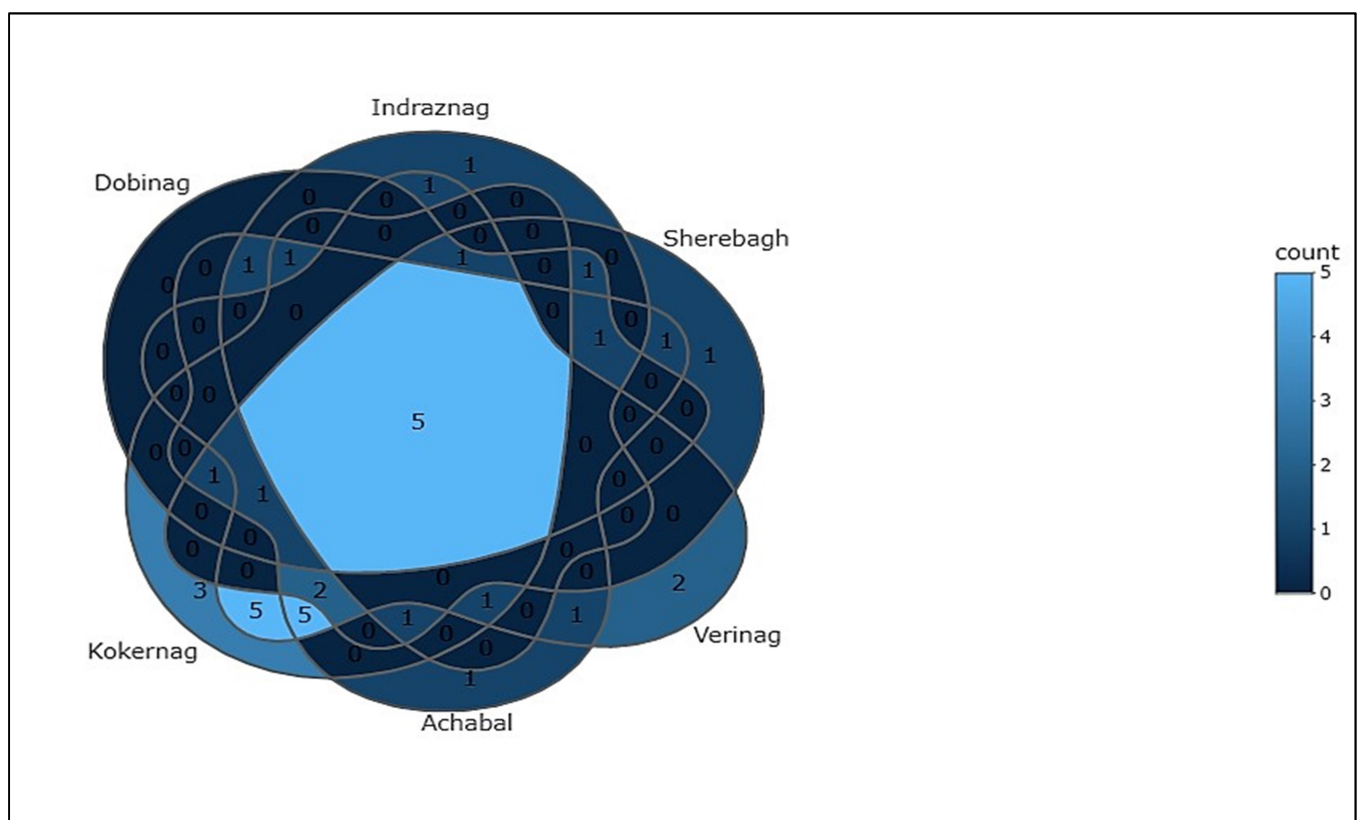


Fig. 2. Venn diagram depicting the taxa common between various springs.

The community composition analysis using NMDS plots (Stress = 0.07) and associated ANOSIM analysis showed that community composition was significantly different for the springs under investigation ($R = 0.889$, $p = 0.0001$), however, no significant variations were noticed for seasons ($R = -0.075$, $p = 0.849$) (Fig. 3). The R statistic value which indicates the degree of difference between

communities is higher for springs while the seasons showed insignificant p -value and very low R statistic value. Clements *et al.* (2016) while working on springs in the Australian alps have reported that difference in taxa in springs makes a distinct contribution to crenic biodiversity. The authors further believe that pH and Nitrate play an important role in distribution of

macroinvertebrates in spring ecosystems. These community composition distinctions based on springs are statistically driven by species which reflect the adaptation to such crenic environs (Fig. 2). The NMDS plot shows that the Zoobenthic community for Dobinag, Indraznag, and Sherebagh were dominated by *Chironomus* sp., *Branchiura sowerbyi*, *Simulium* sp., and *Glossosoma* sp. Verinag spring showed

dominance of *Grouvellinus* sp. and *Tubifex tubifex*. Achabal was characterized by the presence of *Caenis* sp., *Diamesinae* sp., *Drunella* sp., *Stenopsyche bergeri*, and *Lymnaea auricula*, Kokernag spring was predominated by *Gammarus pulex*, *Atherix* sp., *Neumora* sp., *Baetis rhodani*, *Tipula* sp., *Torleya* sp. and *Rhyacophila obscura* (Fig. 3).

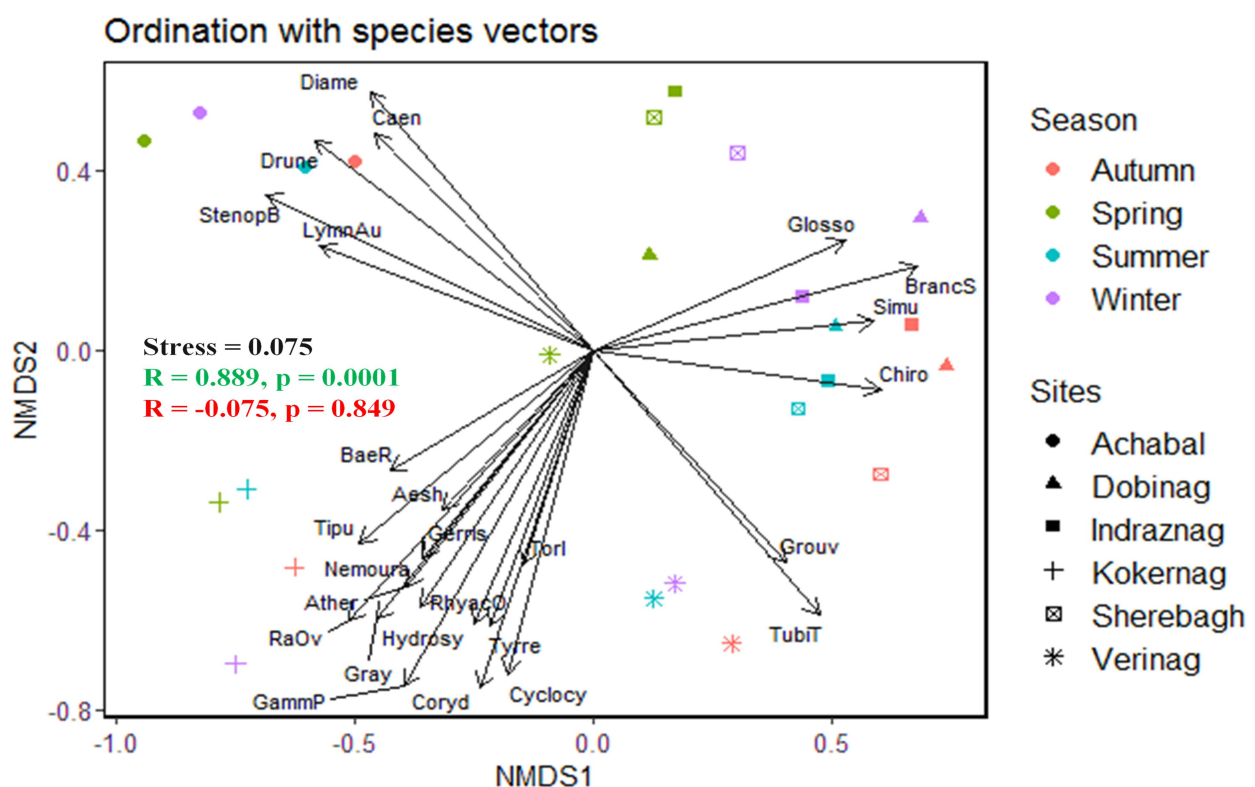


Fig. 3. NMDS plot showing community composition of macroinvertebrates from the Kashmir Himalaya, based on springs (site) and seasons. Stress value for 2-dimensional NMDS plot is displayed along with R-statistic and p-value yielded from the associated ANOSIM.

Alpha-diversity

In the present study, we explored diversity across multiple scales using the zoobenthic community from the spring habitats of Kashmir Himalaya, to understand the key concepts of the community ecology. We explored the classic measures of

alpha and beta diversity, using statistical techniques to understand how environmental factors play out between alpha diversity metrics and the relationship between Shannon's H' , Pielou's Evenness, and species richness (Fig. 4).

Kokernag has a large number of species (high species richness) but is highly uneven (low Pielou's Evenness) resulting in low Shannon's H' . Achabal spring, in contrast, has a moderate number of species but is highly even (high Pielou's Evenness) resulting in a high Shannon's

H' . From the alpha diversity metrics, it becomes clear that the Alpha diversity is structured differently at Kokernag spring compared to Achabal spring. Employing all the three alpha diversity metrics we were able to identify these differences.

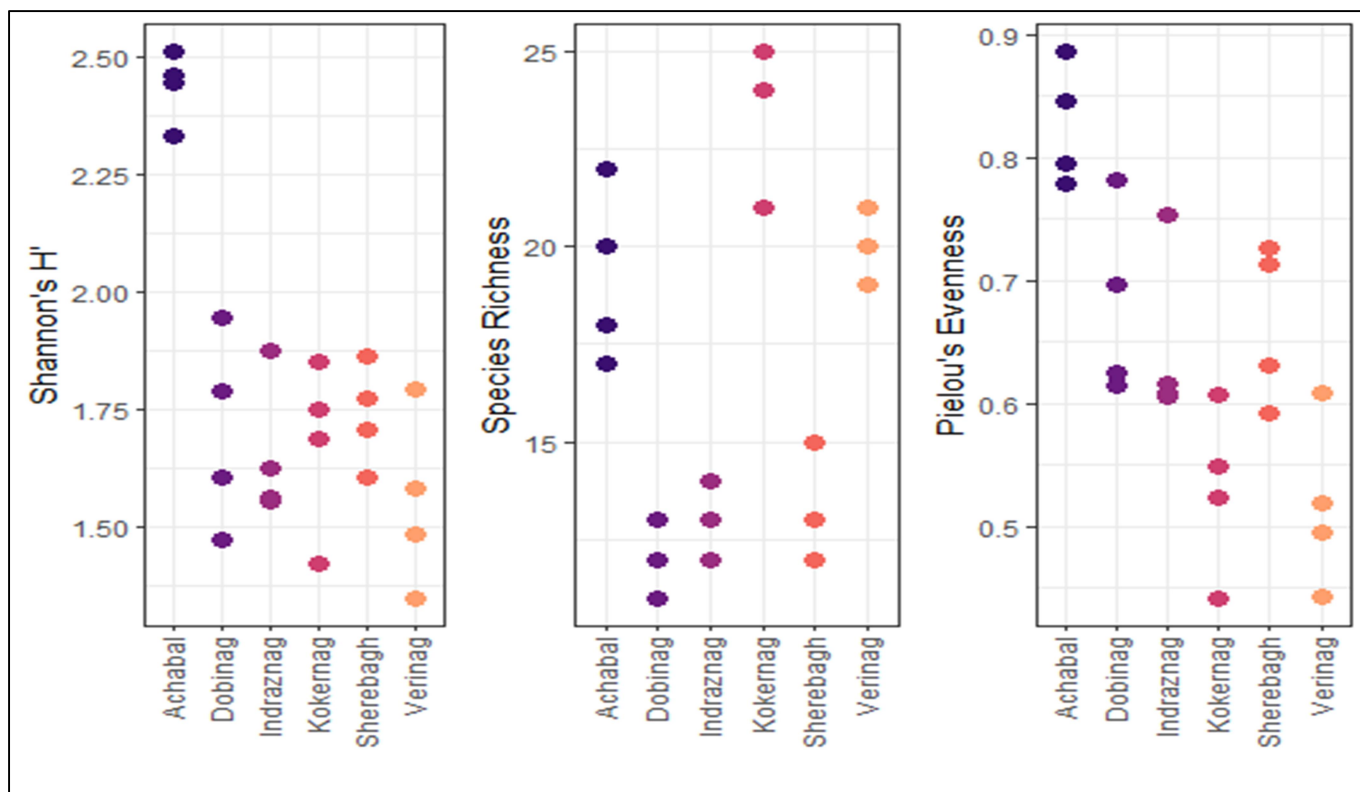


Fig. 4. Showing relationship and difference between alpha diversity metrics (Species richness, Shannon's H' and Pielou's Evenness).

This noticed difference seems to be driven by substrate composition and macrophytic growth which could influence community structure because the springs like Indraznag and Dobinag are dominated by watercress (*Nasturtium officinale*) and muddy substrate were found to support less number of insect taxa than non-insect communities (annelids). There have been also reports of insect dominated species followed by Hydracarina and Crustacea on *Sphagnum*

dominated alpine spring in Australia (Clements *et al.*, 2016). Results on zoobenthos of springs depict a greater number of taxa for high altitude except for Dobinag and relatively greater benthic diversity in rheocrene springs like Kokernag and Achabal (Bhat and Pandit, 2010). Sediment type and sediment organic content are known to have a pronounced effect on the spatial distribution of benthic organisms. With more benthic animals occurring in sandy sediment than in mud which

was also witnessed in this study wherein the number of taxa at Verinag, Kokernag, Achabal being dominated by sand, gravel, pebbles are relatively high than soft-bottom sediments at Sherebagh, Indraznag and Dobinag. However, higher benthic densities were recorded in the mud with high organic matter than in sand during

the present study. The mean zoobenthos density for two-year study at different springs were in the density ranges of 461-523 ind/m² (Verinag), 303-320 ind/m² (Kokernag), 179-197 ind/m² (Achabal), 329-436 ind/m² (Sherebagh), 332-346 ind/m² (Indraznag) and 495-555 ind/m² (Dobinag) (Table 2).

Table 2. Seasonal variations in density (ind.m⁻²) of total zoobenthic fauna at different springs from Mar. 2004-Feb. 2006

Spring Name	Year	Spring	Summer	Autumn	Winter	Mean	SD
Verinag	1	304	664	655	472	523	171
Verinag	2	170	458	612	607	461	207
Kokernag	1	324	205	329	356	303	67
Kokernag	2	195	319	374	395	320	150
Achabal	1	138	208	219	153	179	70
Achabal	2	173	171	227	218	197	108
Sherebagh	1	243	548	640	313	436	214
Sherebagh	2	185	350	335	447	329	108
Indraznag	1	165	386	502	272	332	186
Indraznag	2	177	446	371	390	346	180
Dobinag	1	274	647	642	657	555	266
Dobinag	2	205	477	735	565	495	270

When we look over the previous studies over here and elsewhere on macroinvertebrate density, it becomes abundantly clear the numbers range from hundreds to thousands and it depend upon the spring location besides other environmental attributes which are unique to a spring or a group of springs in a particular watershed or a region. For example, the density of macroinvertebrates from the same study area but with different springs range from 369-1190 ind/m² and 24 to 137 ind/m² respectively (Bhat and Pandit, 2009; Lone, 2020). Other studies on density reported elsewhere include Cantabrian springs- 6313-28615 ind/m² (Barquin and Death, 2009), karstic springs of Switzerland-(2250-14225

ind/m² (Fumetti *et al.*, 2007), Austrian alpine springs- 3880-9750 ind/m² (Staudacher and Fureder, 2007) and 68-125 ind/m² (Clements *et al.*, 2016), helocene springs in Denmark >70000 ind/m² (Lindegaard and Thorp, 1975; Thorp *et al.*, 1997) alluvial springs in Switzerland >200000 ind/m² (Zollhofer *et al.*, 2000).

The springs under study differed in substrate composition and discharge (Bhat and Pandit, 2018, 2020) which affects fine sediment transport and allochthonous material. Further, springs having large size and high discharge were found to harbor relatively more taxa as Verinag (842 L/s), Kokernag (1,371 L/s), and Achabal (1,012 L/s)

having comparatively maximum annual discharge were observed to support a greater number of zoobenthic taxa than others. The springs exhibiting the highest species diversity were the most stable springs throughout the entire study period and had (i) the least changes in water discharge, (ii) higher pH, and (iii) lower alkalinity (Bhat and Pandit, 2018, 2020). *Limnephilus* species was found to prefer low water currents and being observed in the intermittent springs with the least discharge which at times seems vulnerable to desiccation than other springs. *Baetis rhodani* was recorded at all springs. The typical representative of Plecoptera in studied springs is *Nemoura* sp. Its preference for cold waters is reflected by its restricted distribution at Kokernag having the lowest annual mean temperature of about 9.8°C (Bhat and Pandit, 2018). The population density of spring invertebrates varied

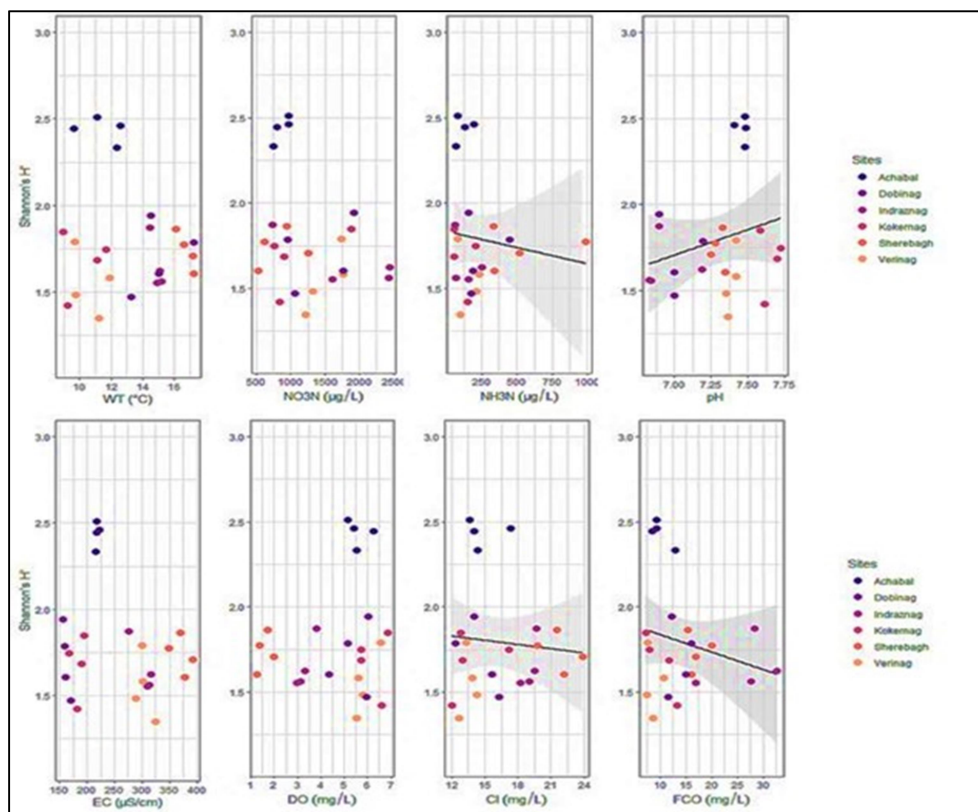
greatly and reached its highs in autumn-winter seasons and lows in spring-summer (Table 2).

Alpha-diversity and Environmental factors

The simple linear regression model outputs demonstrated that there are no significant positive and negative relationships between environmental factors and Shannon's H' across the studied springs (Fig. 5).

Analysis of variance (ANOVA)

The one-way ANOVA revealed a significant variation in Shannon's H' with environmental factors, as greater Pielou's Evenness, Shannon's H' and moderate species richness ($p < 0.05$) was observed in Achabal spring compared with the other springs throughout the area under investigation (Fig. 6).



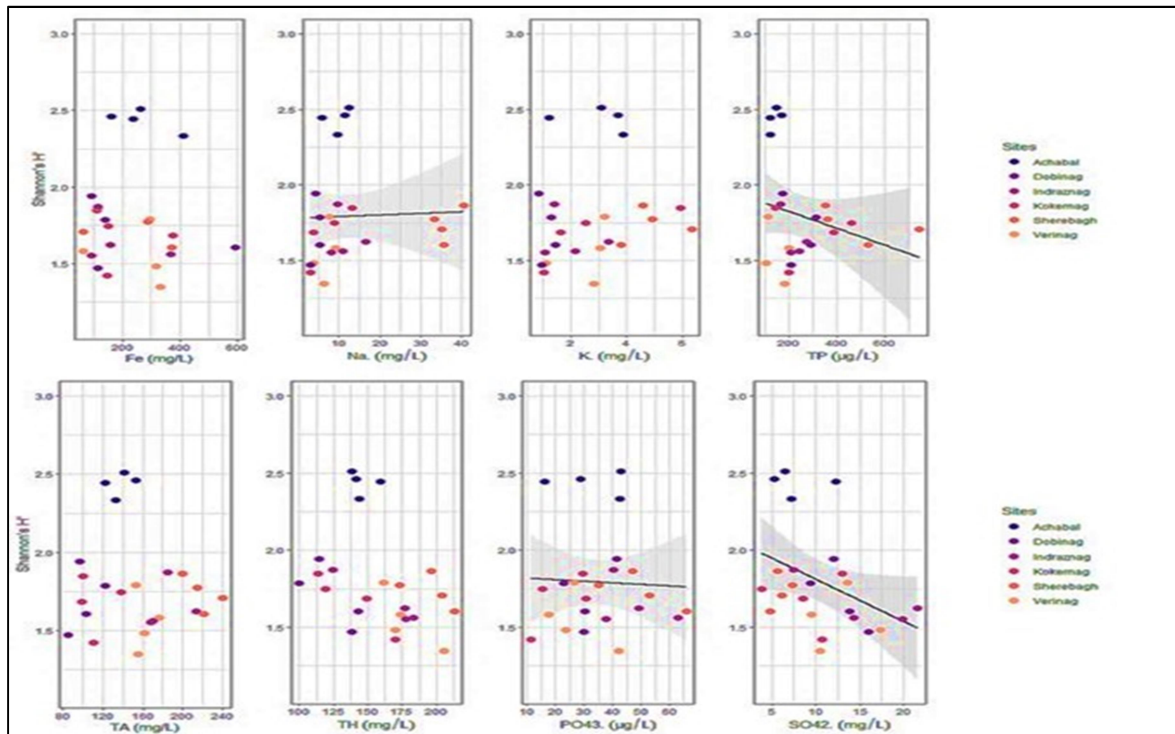
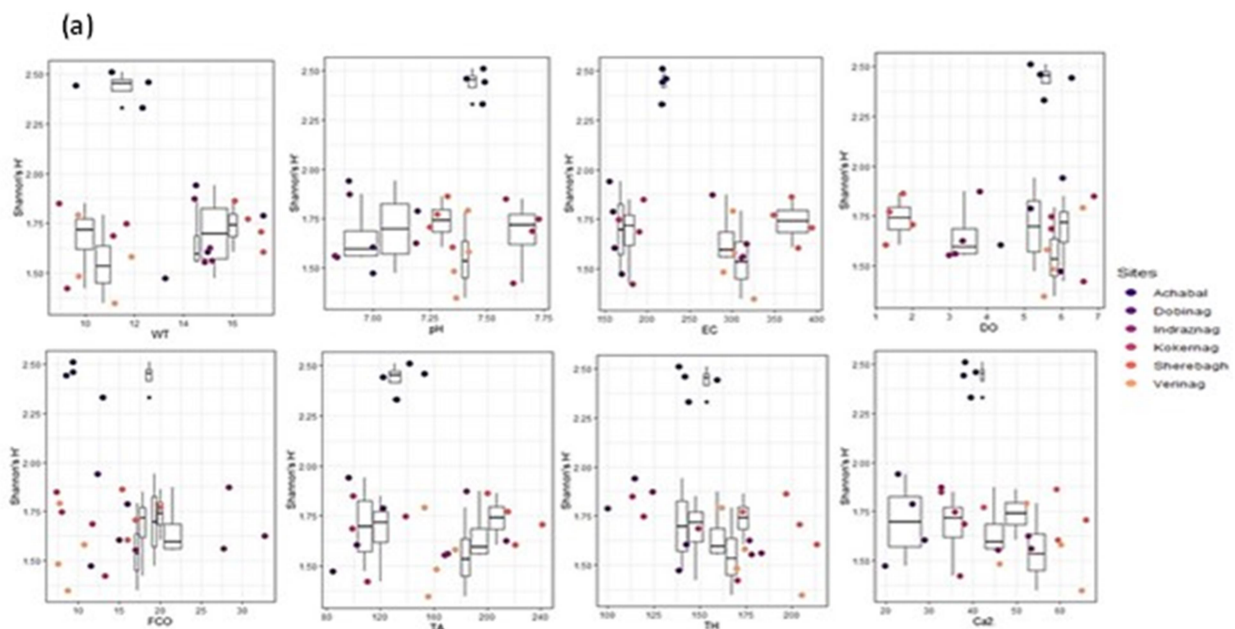


Fig. 5. Linear regression outputs showing a relationship between Shannon metrics and environmental factors.



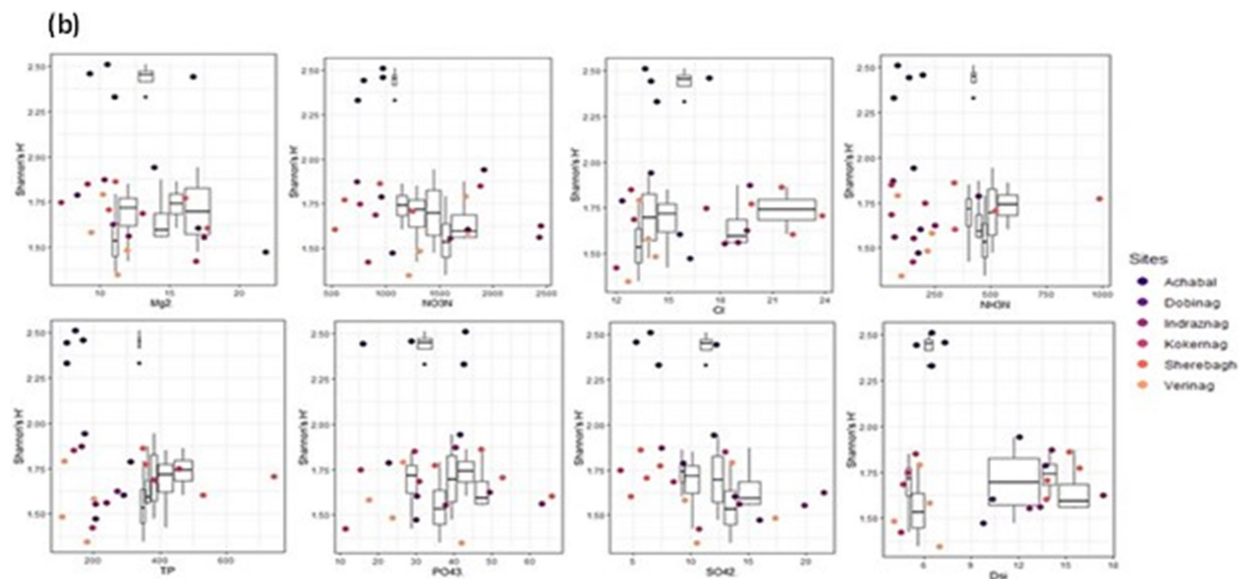


Fig. 6. ANOVA showing significant relationships between environmental factors and macroinvertebrates across studied springs from Kashmir Himalaya ($p < 0.05$).

Beta Diversity

To explore the differentiation between communities across the springs under investigation from Kashmir Himalaya, by comparing dissimilarity in community composition using Bray-Curtis similarity and Jaccard dissimilarity. Bray-Curtis dissimilarity which considers the abundance of species in each community differentiated between springs with the highest dissimilarity recorded for Verinag, Achabal, and Kokernag springs concerning Sherebagh, Indraznag, and Dobinag ranging from 69.12% to 72.8% while least dissimilarity was recorded Dobinag, Indraznag, and Sherebagh springs ranging from 23.3% to 42.1%. Jaccard dissimilarity, which merely considers the presence/absence of species differentiated between springs with the highest dissimilarity recorded for Verinag, Kokernag, and Achabal springs concerning Sherebagh, Indraznag, and Dobinag ranging from 60.0% to 73.3% while least

dissimilarity was recorded Dobinag, Indraznag and Sherebagh springs ranging from 31% to 55%. The results of Bray-Curtis and Jaccard dissimilarity metrics demonstrate that the Verinag, Achabal, and Kokernag are very much similar in terms of macroinvertebrate abundance and species/taxa type while Sherebagh, Indraznag, and Dobinag are very much similar in terms of macroinvertebrate abundance and species/taxa type (Table S1). Understanding community dissimilarity is important for determining the factors that structure the community spatio-temporal dimensions. It also becomes essential to consider the species/taxa driving these patterns across each pair of sites. SIMPER analysis identified the most abundant taxa/species which contributed most to the Bray-Curtis dissimilarity between springs. The species/taxa that contributed most to the Bray-Curtis dissimilarity metrics between each pair of springs included *Tubifex tubifex*, *Chironomus* sp., *Branchiura*

sowerbyi, *Gammarus pulex*, *Erpobdella octoculata*, *Baetis rhodani*, and *Drunella* sp. (Table S2). Principal coordinate analysis for both Bray-Curtis and Jaccard's Dissimilarity metrics helped us in visualization macroinvertebrate community structure and springs in a two-dimensional space, with underlying relationships. PCoA for Bray-Curtis explained 42.8% on PCoA 1 and 24.6% on PCoA 2, respectively while 55.8% on PCoA 1 and 14.9% on PCoA 2 for Jaccard's dissimilarity (Fig. 7).

Distance-Based Redundancy Analysis (dbRDA)

In the distance-based redundancy analysis (dbRDA), the environmental factors had an impact on the beta-diversity of the zoobenthic community. The relation between unconstrained and constrained variances tells us how well environmental factors describe the beta diversity of the investigated springs. While the measured environmental factors were not successful in describing Shannon's H' recorded throughout the studied springs.

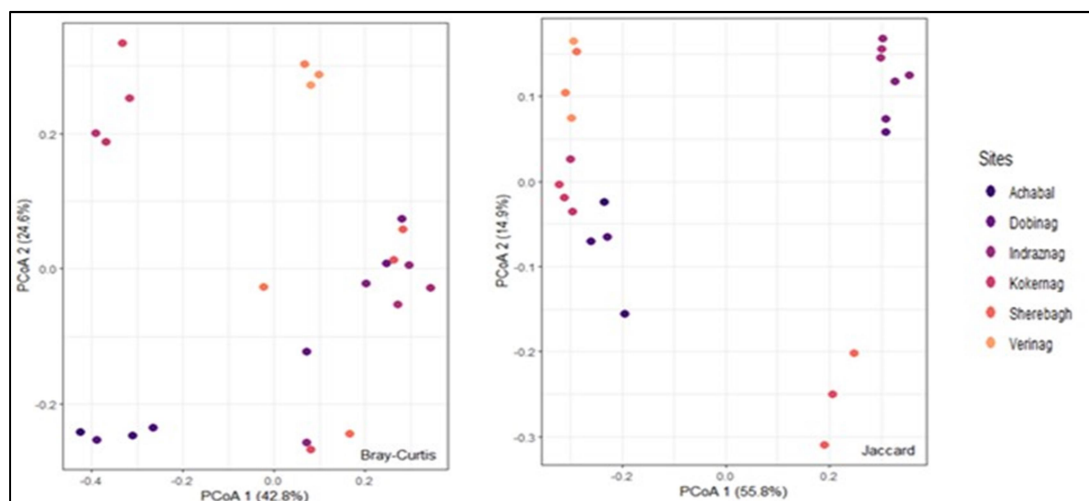


Fig. 7. Principal Coordinates Analysis (PCoA) showing the structure of Zoobenthos communities and their underlying relationships.

However, the environmental factors that we're able to explain a significant proportion of variance in beta diversity are WT, pH, DO, FCO, Cl, TP, and PO_4^{3-} (35.35% on RDA1 and 13.86% on RDA2) across the study area (Fig. 8). From the literature, we could find that different authors

have reported differently the strong relationship that emerges between water chemistry parameters and macroinvertebrate community like water temperature, ammonia, Chloride (Williams *et al.*, 1997), pH, and Nitrate (Clements *et al.*, 2016).

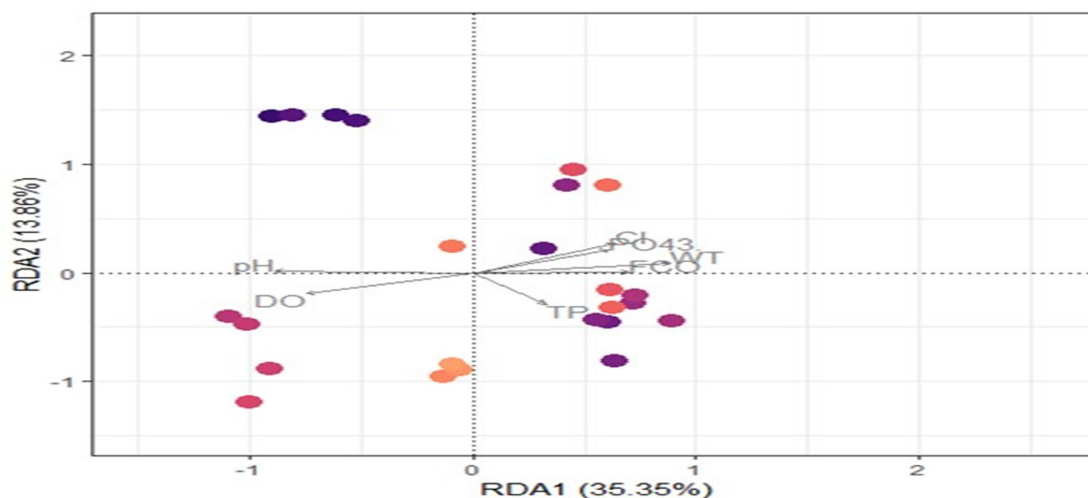


Fig. 8. Distance-Based Redundancy Analysis (dbRDA) plot showing the variance in macroinvertebrate community explained by environmental factors.

CONCLUSION

The central key message coming from this study is that spring attributes like Water temperature, pH, Dissolved Oxygen, Free carbon dioxide, Chloride, Ortho and Total Phosphorus play a vital role in the formation and establishment of the zoobenthic community in comparison to the seasons which have less influence. We also did not find any taxa from our study, which is different from those reported from the lakes, streams, and rivers of Kashmir Himalaya. However, considering the diversity of spring habitats in Kashmir, more comprehensive works on a large scale may keep this window open to expect some spring specialist species as has been documented from various parts of the world.

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