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Investigating the Diversity and Taxonomy of Aquatic Insects in Surface Drinking Water Sources of Pithoragarh, Uttarakhand, India

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Abstract: Aquatic macro-invertebrates, being small organisms visible to the naked eye and primarily dwelling in freshwater ecosystems, play crucial roles in indicating environmental health. The objective of this study was to investigate the diversity and taxonomy of aquatic insects. Monthly sampling was performed from various surface drinking water sources in Pithoragarh, Uttarakhand, India over a period of two years using the quadrate method. Samples were classified by taxonomic keys, while alpha and beta diversity indices were calculated seasonally. The findings of this study revealed the presence of eight orders and 35 families within the phylum Arthropoda, resulting in the identification of 59 genera and 2476 individuals in total. Monthly abundance data indicates a peak in January, contrasting with a low in July. March displayed the highest taxonomic richness, while September and October exhibited the lowest. Ephemeroptera and Diptera consistently showed high abundance, followed by Trichoptera and Coleoptera, whereas Plecoptera, Odonata, Hemiptera, and Lepidoptera were present in very low numbers across all sites. Significant fluctuations in aquatic insect diversity across different seasons were observed, with July exhibiting the highest Shannon wiener and Simpson diversity index, while November recorded the lowest diversity indices. Beta diversity analysis further elucidated substantial differences in community composition among the sampling periods. These results underscore the dynamic nature of macroinvertebrate diversity and emphasize its importance in environmental conservation and monitoring efforts, providing valuable insights into the implications of ecological diversity for effective management and conservation strategies.

Keywords: Alpha diversity, Beta diversity, Aquatic insects, Classification, Macroinvertebrates, Simpson diversity index, Shannon-Wiener evenness, Diptera, Ephemeroptera, Trichoptera, Coleoptera, Plecoptera, Odonata

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Introduction

Organisms large enough to be kept on a sieve with a mesh size of 250–1000µ or caught with a net are classified as macroinvertebrates (Ziglio *et al.*, 2006). They can be either benthic, living on substrates like sediments, trash, logs, pelagic, moving freely in the water column (Ghosh *et al*., 2015). This group includes insects, crustaceans, mollusks, worms, and other invertebrates that inhabit freshwater environments, such as rivers, streams, lakes, ponds, and wetlands. The biological roles that macroinvertebrates play in freshwater ecosystems, such as nutrient cycling, organic matter decomposition, and the aquatic food web, make qualitative and quantitative studies of their diversity extremely important (Yuen *et al*., 2016; Allan *et al*., 2021). Moreover, they are widely employed in the ecological assessment of aquatic systems, since one of the often-employed protocols for standard water management through water quality indicators is the employment of macroinvertebrates (Dar *et al*., 2010; Fierro *et al*., 2017).

To examine the diversity of aquatic insects alpha and beta diversity analysis is performed. The assessment of variations in the biodiversity values of insects in aquatic ecosystems has historically been done using various diversity indices (Magurran, 2004). Alpha diversity, or variety at the habitat level, is the most often utilized factor in community classification. It consists of two parts: the equitability index and species richness. The number of individuals per unit area (such as a quadrat) and the species content of the area are both quantified by species richness. Equitability indices provide the relative number of various species within a population based on how evenly they are distributed (Simons *et al*., 2019). Beta diversity quantifies the degree of change in species composition as one moves from one habitat or location to another within a given area. Beta diversity is important in ecology because it helps researchers understand how species are distributed across different habitats, how they interact with each other, and how they respond to environmental gradients and disturbances (Whittaker, 1960; Baselga, 2010).

Our study investigates the richness and taxonomy of aquatic insects found in Pithoragarh City's various surface water sources. The main objectives of this study were to investigate the taxonomy of the insect species found and to quantify the diversity of the species present in the chosen water sources. This research holds a lot of significance, as understanding the distribution and composition of macroinvertebrate communities facilitates the identification of key habitats, guiding targeted conservation strategies and sustainable management practices.

Materials and Methods

Study site:

At 1645 metres above sea level, Pithoragarh is a city in Uttarakhand that is situated between 29.4° and 30.3° latitude and 80° and 81° longitude along the eastern and southern regions of the central Himalayas. Three distinct surface water sources in Pithoragarh city were selected for this study: Mahadev, Panda, and Madh (Table 1). These locations have been widely used as sources of water by the surrounding residents.

Collection of samples:

Sampling to obtain data on aquatic insects was undertaken monthly at 3 sampling sites during a period of two years, i.e., from January 2022 to December 2023. Macroinvertebrates were collected in morning hours from around the water source in 1ft^2 quadrats by using a serber sampler and picked with the help of a brush and forceps (Kumar *et al*., 2012; Mamgain *et al*., 2021). Following the collection procedure macro-invertebrates were immediately placed in 70% alcohol and were brought to the lab for identification and enumeration (Subramanian and Sivaramakrishanan, 2007).

Identification and classification of samples:

An individual sample was taken at a time and identified by examining under a dissection or stereo zoom microscope (10X and above) (Feldman *et al*., 2006). The samples were classified to the lowest possible practical taxonomic level by using standard taxonomic keys as designed by Feldman *et al.* (2006) and Oscoz and Galicia (2011) and by referring to online sites

Table 1: Sampling sites with geological information of the sampling spot

S. No.	Sampling sites	GPS Coordinates of spots		
		Latitude	Longitude	Altitude
Site 1	Mahadev	29°34′34″N	80°12′9″E	1540m
Site 2	Panda	29°36′7″N	80°13′54″E	1510m
Site 3	Madh	29°36′37″N	80°12′58″E	1591m

(https://www.macroinvertebrates.org/).

Diversity indices calculation:

A variety of alpha diversity indices were used to assess the distribution and diversity between sampling sites, including dominance, Simpson diversity index, Shannon-Wiener evenness, Brillouin, Menhinick, Margalef, equitability, Fisher-alpha, and Berger-Parker (Odum 1971; Simpson 1949; Shannon and Wiener 1949; Pielou 1966; Brillouin 2013; Menhinick 1964; Margalef 1957; Fisher 1943; Berger and Parker 1970). The beta diversity was calculated using quantification of the degree of difference in species composition between various communities (Whittaker, 1972). The PAST software (Hammer, 2008) and Microsoft Office Excel 2019 (Davis *et al.*, 2018) were used for all statistical analysis.

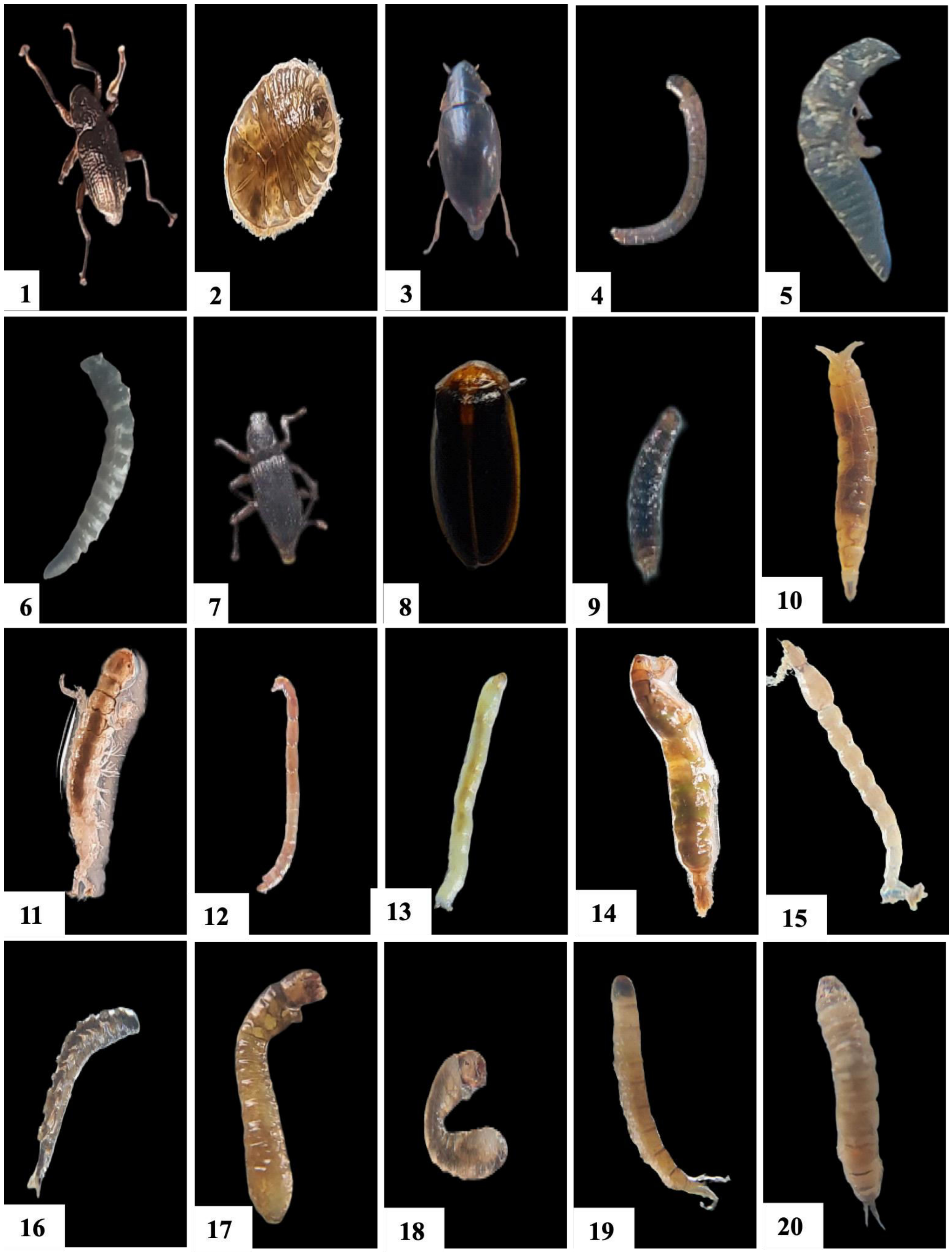
Results

The population dynamics of insects in the water sources were mainly studied to determine their composition and diversity. To analyze their diversity, both alpha and beta diversity indexes were computed.

Species composition and diversity:

The purpose of the study was to assess the variety and density of class Insecta in Pithoragarh City's surface water sources, which were sampled monthly for two years (Fig. 1). A total of 2476 individuals, comprising 59 genera overall and representing 8 orders and 37 families of class insecta were recorded at all three sampling sites (Table 2). The order Ephemeroptera had the greatest diversity, with 15 genera covering 6 families. The most common and populous family, Heptageniidae, consists of five genera. The order Trichoptera, which includes 9 families and 14 genera, had the second-highest diversity**.** The order Coleoptera recorded 9 genera belonging to 6 families. 13 genera were recorded in order Diptera, representing 10 families. Order Odonata and Hemiptera consist of two genera each representing two families. Order Lepidoptera recorded a single genus representing family Pyralidae and order Plecoptera consists of 3 representative genera of family Perlidae. Odonata and Hemiptera consist of two genera each representing two different families.

The mean monthly abundance of individuals collected over a span of two years is detailed in Table 3. Examination of the data revealed a peak in individual counts during January, with 325 individuals recorded, contrasting notably with the lowest counts observed in July, totalling 125 individuals. Notably, March exhibited the highest taxonomic richness, with 27 distinct taxa documented, whereas September and October registered the lowest taxonomic diversity, with only 15 taxa detected. Consistently high abundances were observed throughout the year for Ephemeroptera and Diptera, with 981 and 764 individuals, respectively, followed by Trichoptera (463 individuals) and Coleoptera (216 individuals). Conversely, the remaining taxonomic orders, including Plecoptera (15 individuals), Odonata (8 individuals), Hemiptera (7 individuals), and Lepidoptera (5 individuals), were consistently scarce across all three sampling sites throughout the entirety of the observational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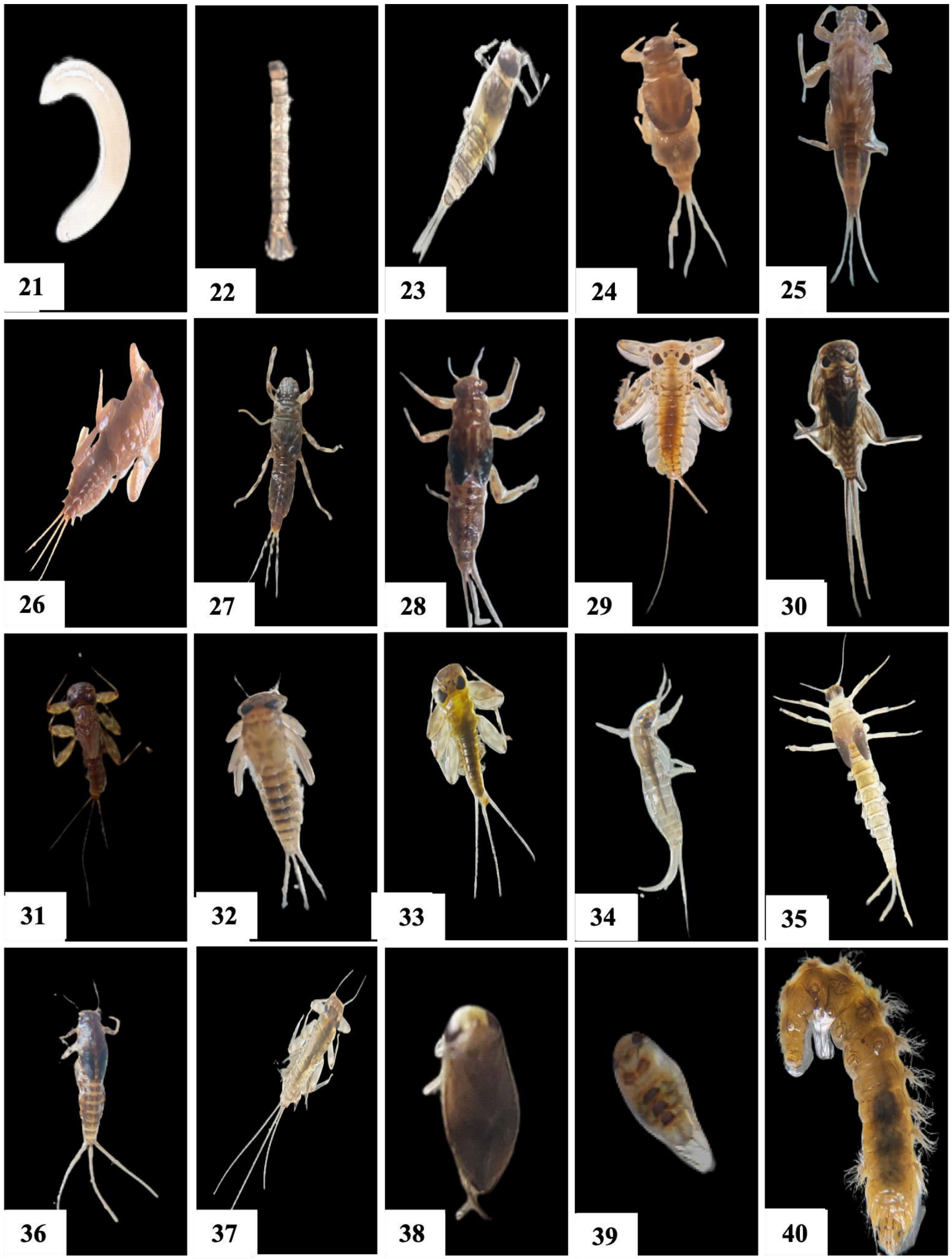
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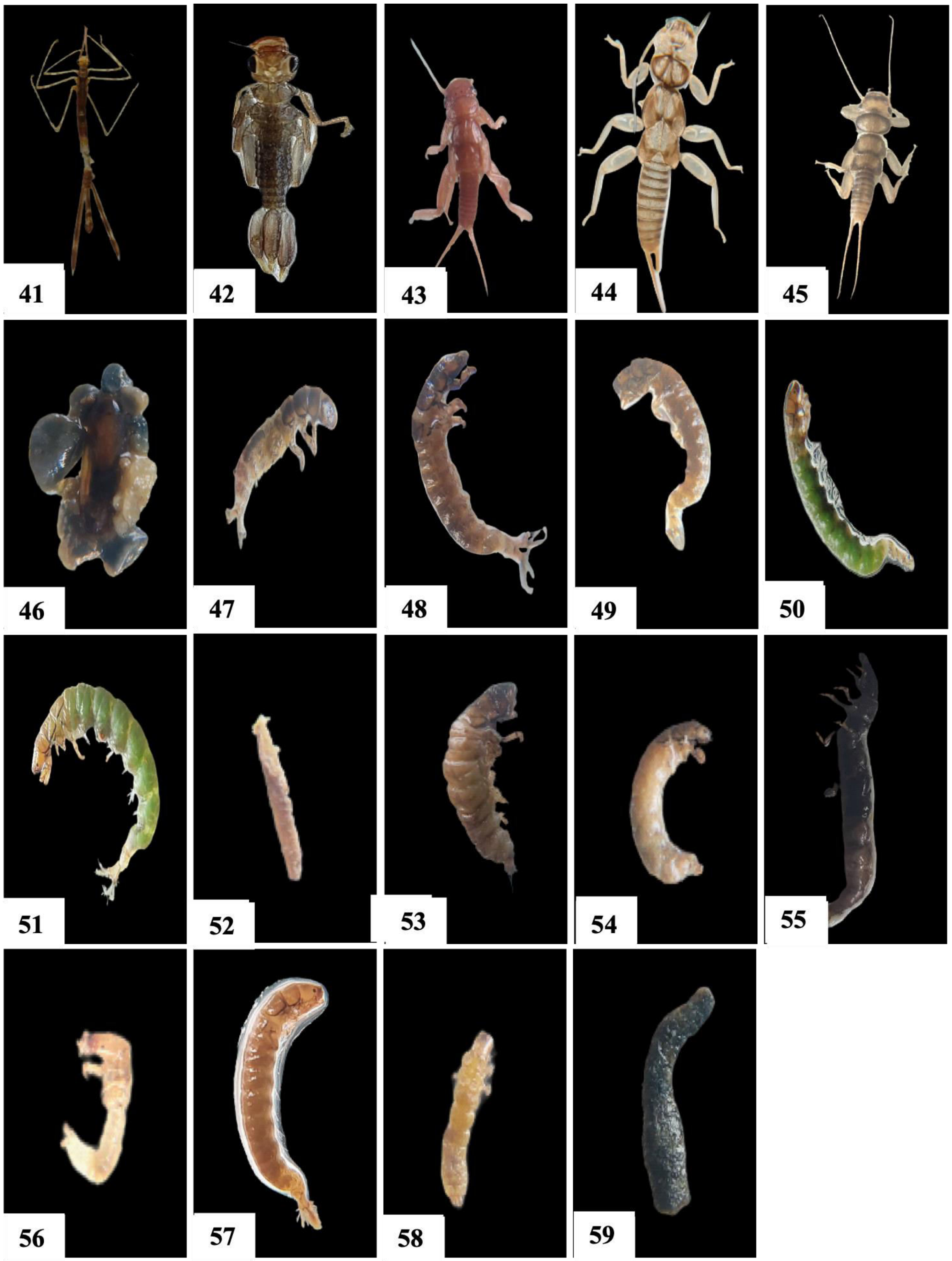

Fig 1: Photographs of aquatic insects collected from all sampling sites.

Table 2: Classification of benthic organism categorization up to the level of genus

PHYLUM	CLASS	ORDER	FAMILY	GENUS	COMMON NAME
Arthropoda	Insecta	Coleoptera	Dryopidae	*Helichus*	Long-toed water beetles
			Psephenidae	*Psephenus*	Water pennies
			Dytiscidae	*Agabus*	Predacious diving beetles
			Ptilodactylidae	*Anchytarsus*	Toe-winged beetle larvae
			Elmidae	*Ancyronyx variegatus*	Riffle beetle larvae
				Macronychus glabratus	Riffle beetle larvae
				Microcylloepus	Riffle beetles
			Hydrophilidae	*Tropisternus adult*	Water scavenger beetle
				Tropisternus larva	Water scavenger beetle larvae
		Diptera	Athericidae	*Atherix*	Watersnipe Flies
			Culicidae	*Aedes*	Mosquitoes
			Chironomidae	*Diamesa*	Non-Bitting Midges
				Chironomus	Non-Bitting Midges
				Orthocladius	Non-Bitting Midges
			Ceratopogonidae	*Probezzia*	Bitting Midges
			Psychodidae	*Pericomaina*	Moth flies
			Simullidae	*Simulium*	Black flies
				Prosimulium	Black flies
			Limoniidae	*Antocha*	Limoniid crane flies
			Pediciidae	*Dicranota*	Hairy-eyed Crane flies
			Muscidae	*Musca*	House flies
			Dixidae	*Dixa*	Meniscus Midges
		Ephemeroptera	Baetidae	*Baetis*	Small minnow mayflies
			Caenidae	*Caenis*	Small Square-gilled mayflies
			Ephemerellidae	*Drunella*	Spiny crawler mayflies
				Ephemerella	Spiny crawler mayflies
				Eurylophella	Spiny CrawlerMayflies
				Serratella	Spiny crawler mayflies
			Heptageniidae	*Epeorus*	Flat-headed mayflies
				Heptagenia	Flat-headed mayflies
				Leucrocuta	Flat-headed mayflies
				Rhithrogena	Flat-headed mayflies
				Stenacron	Flat-headed mayflies
			Isonychiidae	*Isonychia*	Brush-legged mayflies
			Leptophlebiidae	*Habrophlebia*	Prong-gilled mayflies

				Leptophlebia	Prong-gilled mayflies
				Neoleptophlebia	Prong-gilled mayflies
		Hemiptera	Corixidae	*Hesperocorixa*	Water Boatmen
			Micronectidae	*Micronecta*	Lesser Water Boatmen
		Lepidoptera	Pyralidae	*Petrophila*	Snout Moths
		Odonata	Calopterygidae	*Calopteryx*	Jewel wings
			Coenagronidae	*Argia*	Dancers
		Plecoptera	Perlidae	*Acroneuria*	Common stoneflies
				Neoperla	Common stoneflies
				Paragnetina	Common stoneflies
		Trichoptera	Glossosomatidae	*Glossosoma*	Saddle casemaker caddisflies
			Hydropsychidae	*Arctopsyche*	Seine-net weaver caddisflies
				Cheumatopsyche	Seine-net weaver caddisflies
				Diplectrona	Seine-net weaver caddisflies
				Hydropsyche	Seine-net weaver caddisflies
				Macrostemum	Seine-net weaver caddisflies
			Leptoceridae	*Nectopsyche*	Long- horned caddisflies
			Limnephilidae	*Ironoquia*	Northern Casemaker Caddisflies
			Odontoceridae	*Psilotreta*	Strong casemaker caddisflies
			Philopotamidae	*Chimarra*	Finger-net Caddisflies
				Dolophilodes	Finger-net Caddisflies
			Polycentropodidae	*Nyctiophylax*	Trumpet net, Tube maker caddisflies
			Psychomyiidae	*Psychomyia*	Net tube caddisflies
			Sericostomatidae	*Agarodes*	Bush- tailed caddisflies

period.

Diversity analysis:

Seasonal compositions and diversity indices of macroinvertebrate occurrences are presented in Table 4. The values of the Simpson diversity index varied from 0.88 to 0.93, with January and July exhibited the most diversity and November the lowest. The Shannon Wiener (H') index, which has a value ranging from 2.38 to 2.95, showed a similar monthly trend. Furthermore, Benthos Evenness Index values varied from 0.61 to 0.86. October represented the highest level of evenness, while May the lowest. In addition, the macro-invertebrate species richness Margalef Index varied from 2.57 to 4.87, peaking in March and falling to its lowest in October. Finally, the study's Benthic Fauna's Equitability Index values varied from 0.84 to 0.94, with May showing the lowest equitability while July and October showing the greatest. These diversity indices offer important information about the distribution patterns among macrobenthos in the studied area.

Whittaker's beta diversity analysis, which compared various habitats pair-wise in order to assess variations in community composition, is shown in Table 5. Mahadev and Panda are the groups that resemble each other the most,

Table 3: Enumeration of benthic organisms at all three sampling sites

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Helichus	-	-	-	-	-	-	-	2	6	5	-	-
Psephenus	29	11	26	22	6	6	10	6	17	9	12	22
Agabus	-	-	-	-	1	3	3	-	-	-	-	-
Anchytarsus	-	-	2	-	1	1	-	-	-	-	-	-
Ancyronyx variegatus	-	1	1	-	2	-	-	-	-	-	-	-
Macronychus glabratus	-	-	1`	2	1	-	-	-	-	-	-	-
Microcylloepus	-	-	-	1	-	-	1	1	-	-	-	-
Tropisternus adult	-	-	-	-	-	-	-	2	-	-	-	-
Tropisternus larva	-	-	2	1	-	-	-	-	-	-	-	-
Atherix	-	-	-	-	1	1	3	-	-	-	-	-
Aedes	-	3	-	4	-	-	-	-	6	-	-	-
Diamesa	-	15	16	6	14	4	5	-	4	-	-	-
Chironomus	15	25	34	13	31	22	12	24	-	-	38	44
Orthocladius	-	1	1	-	-	2	-	-	-	-	-	-
Probezzia	12	14	8	-	-	-	9	7	-	12	-	21
Pericomania	-	-	-	-	2	-	-	-	-	-	-	3
Simulium	40	11	-	-	28	15	7	35	-	-	22	48
Prosimulium	19	6	5	13	-	4	-	5	-	-	6	18
Antocha	-	6	12	4	5	5	-	-	-	-	-	-
Dicranota	-	-	5	-	3	-	-	-	-	-	-	-
Musca	-	-	-	1	-	3	-	-	-	-	-	-
Dixa	-	-	-	-	-	-	1	3	-	2	-	-
Baetis	7	12	8	22	10	9	12	12	-	19	17	18
Caenis	11	17	14	4	17	4	13	14	4	27	-	18

Drunella	2	-	5	2	-	-	-	-	-	-	2	-
Ephemerella	8	10	-	-	-	-	-	-	-	-	-	-
Eurylophella	6	-	-	-	-	5	5	-	-	12	-	-
Serratella	5	4	-	6	2	-	-	-	-	-	-	-
Epeorus	20	-	-	-	-	-	-	-	12	13	-	-
Heptagenia	8	-	-	-	-	-	5	-	9	18	-	8
Leucrocuta	35	8	14	25	-	8	5	22	26	24	14	12
Rhithrogena	2	4	1	1	-	-	2	-	-	-	3	-
Stenacron	-	-	-	-	-	-	3	-	7	4	-	-
Isonychia	-	-	-	1	-	2	-	-	-	-	-	-
Habrophlebia	-	-	-	-	13	11	-	3	-	-	9	-
Leptophlebia	-	-	-	8	-	-	4	3	-	-	-	-
Neoleptophlebia	28	42	10	24	-	15	-	19	13	26	53	38
Hesperocorixa	-	-	-	-	-	-	1	3	-	-	-	-
Micronecta	-	-	-	-	-	-	2	1	-	-	-	-
Petrophila	-	-	-	2	-	-	-	-	3	-	-	-
Calopteryx	-	1	-	-	-	-	-	-	-	-	1	-
Argia	4	-	-	-	-	-	-	-	-	-	-	2
Acroneuria	3	-	1	-	-	-	-	-	-	-	-	-
Neoperla	-	-	-	-	-	-	-	-	4	-	2	-
Paragnetina	-	-	-	-	1	1	-	3	-	-	-	-
Glossosoma	-	-	-	2	2	-	-	-	-	-	-	-
Arctopsyche	30	-	12	-	-	-	-	-	-	-	-	14
Cheumatopsyche	-	-	4	-	6	5	-	-	-	-	-	-
Diplectrona	-	-	1	-	-	-	-	-	2	6	3	-
Hydropsyche	16	14	6	9	5	7	3	4	18	26	15	14

Macrostemum	-	34	12	-	17	-	14	14	-	25	28	15
Nectopsyche	2	1	-	-	-	-	-	-	-	-	-	-
Ironoquia	-	4	-	-	3	-	-	-	-	-	-	-
Psilotreta	-	1	-	3	2	-	-	-	-	-	-	-
Chimarra	18	-	-	-	-	-	4	2	10	-	-	-
Dolophilodes	-	-	2	-	-	2	1	-	-	-	3	-
Nyctiophylax	1	-	2	5	1	-	-	-	-	-	-	-
Psychomyia	1	-	2	2	-	-	-	-	-	-	-	-
Agarodes	3	2	-	-	-	-	-	-	-	-	2	1
Taxa_S	**25**	**24**	**27**	**25**	**24**	**22**	**23**	**21**	**15**	**15**	**17**	**16**
Individuals	**325**	**247**	**207**	**183**	**174**	**135**	**125**	**185**	**141**	**228**	**230**	**296**

Table 4: Tabular representation presenting the Alpha diversity indices of macroinvertebrates

	JAN	**FEB**	**MAR**	**APR**	**MAY**	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**DEC**
Taxa_S	25	24	27	25	24	22	23	21	15	15	17	16
Individuals	325	247	207	183	174	135	125	185	141	228	230	296
Dominance_D	0.06	0.08	0.07	0.07	0.09	0.07	0.06	0.09	0.09	0.08	0.11	0.09
Simpson_1-D	0.93	0.91	0.92	0.92	0.90	0.92	0.93	0.90	0.90	0.91	0.88	0.90
Shannon_H	2.87	2.78	2.90	2.80	2.69	2.85	2.95	2.64	2.53	2.55	2.38	2.52
Evenness_e^H/S	0.71	0.67	0.67	0.66	0.61	0.78	0.83	0.66	0.84	0.86	0.63	0.78
Brillouin	2.70	2.57	2.63	2.52	2.42	2.52	2.58	2.40	2.30	2.40	2.22	2.39
Menhinick	1.38	1.52	1.87	1.84	1.81	1.89	2.05	1.54	1.26	0.99	1.12	0.93
Margalef	4.15	4.17	4.87	4.60	4.45	4.28	4.55	3.83	2.82	2.57	2.94	2.63
Equitability_J	0.89	0.87	0.88	0.87	0.84	0.92	0.94	0.86	0.93	0.94	0.84	0.91
Fisher_alpha	6.31	6.57	8.29	7.82	7.54	7.45	8.27	6.09	4.24	3.60	4.23	3.62
Berger-Parker	0.12	0.17	0.16	0.13	0.17	0.16	0.11	0.18	0.18	0.11	0.23	0.16

Table 5: Whittaker beta diversity pair wise comparison matrices between different sites

S. No.		Mahadev	Panda	Madh
1	**Mahadev**	0	0.24675	0.35802
2	**Panda**	0.24675	0	0.31707
3	**Madh**	0.35802	0.31707	0

according to the results, with Whittaker's index value of 0.24675 being the lowest. Conversely, the greatest Whittaker's beta diversity value of 0.35802 showed the highest degree of dissimilarity in community composition between Mahadev and Madh.

Discussion

The presence of insects from eight orders and thirty-seven families in our study is consistent with the findings of numerous previous investigations. For example, a study of the variety of macroinvertebrate taxa along the Alaknanda River in Uttarakhand by Rana *et al*. (2019) revealed insects from 52 families and 9 orders. In Siddharthnagar (Uttar Pradesh, India), Prakash and Verma (2018) did another study that found the presence of 20 distinct species from 6 orders and 19 families. According to our findings, the order Ephemeroptera has the most diversity, which is in accordance with other findings from other studies. Consistent with our findings, Kumar *et al*. (2012) demonstrated that mayflies were a diversified and abundant component in the Tamirabarani River in the Southern Western Ghats, India. Kubendran *et al*. (2016) also documented that Ephemeroptera, Plecoptera, and Trichoptera were the most prevalent groups of macroinvertebrates collected in freshwater streams in southern western ghats, India. Similar observations were made by Abhijna *et al*. (2013) in Vellayani lake in Kerala. Our study reported higher macroinvertebrate population in January and lowest in July which holds similarity to the observation made by Mamgain *et al*. (2021) in Rudraprayag District, Garhwal, Uttarakhand.

In our study, the Shannon-Wiener and Simpson diversity indices had relatively maximum values in the months of July and lowest values in the month of November due to unfavorable weather. These results are in line with earlier research done in the Garhwal region's Kyunja gad stream by Bahuguna and Negi (2018). Macroinvertebrates in Assamese oxbow lakes were found to have nearly the same Shannon Weiner diversity index values (2.00 to 2.38) (Doley *et al*., 2014). Our study's evenness value, which varied from 0.61 to 0.86, is consistent with that of Ghosh and Biswas (2015) investigation of an eastern Indian lake environment. The observed trend in Margalef's Index in our study is consistent with the findings of Yazdian *et al.* (2014) who have established a relationship between benthic macroinvertebrates bio-indices and surrounding physicochemical parameters. Whittaker's beta diversity approach, which examined different habitats pairwise to evaluate changes in community composition, was incorporated into our study. The three study sites yielded beta diversity values of 0.24675 and 0.35802. In a related work with similar findings, McCreadie *et al.* (2018) computed beta diversity patterns across a broad category of aquatic insects found throughout North America to access dissimilarity for all paired site comparisons.

Conclusion

In conclusion, this study underscores the importance of investigating the diversity and taxonomy of aquatic insects in freshwater ecosystems. The findings highlight the richness of macroinvertebrate communities and the fluctuation in diversity indices across seasons. The significant differences in community composition revealed by beta diversity analysis emphasise the dynamic nature of these ecosystems. By enhancing our understanding of aquatic macroinvertebrates, this research contributes valuable insights for environmental conservation and monitoring efforts. Moving forward, integrating these findings into management and conservation strategies will be crucial for safeguarding these fragile ecosystems and ensuring their long-term sustainability.

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