



Effect of environmental variables on black flies (Simuliidae) in aquatic streams and rivers (India)

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Abstract

Amidst global warming and anthropogenic impact, sensitive aquatic organisms in different ecosystems are indispensable. For this reason, the two habitats succeeding (aquatic in larval life and terrestrial life) black fly is so important and some species of their female adult act as a potential vector for onchocerciasis disease to human. Hence, the present study is aimed to assess the black fly in tropical streams along with environmental factors. Twelve species were found from 42 sampled streams and rivers different region of Eastern and Western Ghats, Peninsular India. The individual black flies were highly abundance recorded in *Simulium ruficorne* species group of *S. (N.) kiliyurens* (32.7%). The active observation of PCA shows that F1 (53.86%) had the high value than F2 (11.34%). Squared cosines of the variables value clearly indicating lower site of Thirumoorthy falls (0.046) and the higher site of Kiliyur falls (0.028), where these tourist part had more individual black fly larvae. Spearman's r_s correlation coefficient, and this result indicated that water temperature, leaf litter and total dissolved solids had the significant value ($\gamma = 0.79058, 0.734682, 0.41112$) with abundance of black flies. The results of one-way ANOVA revealed that the relative abundance of black flies was significant ($F = 77.13, p = 3.3$) between the sampling sites. Environmental factors were influenced with larval distribution and abundance varied with stream air temperature, water temperature, conductivity, total dissolved solids and salinity observed in the present study.

Keywords: *Nevermannia*, environmental variables, black fly, abundance, India

Introduction

Effect of environmental variables on distribution of black fly larvae in the tropical streams and rivers of India. Black fly species in prepare breeding sites specified some physicochemical parameters for morphology changes and larval density. Environmental variables like temperature, the relationship between morphology and temperature was initially observed for endothermic animals and extended insects^[1,2], conductivity, salinity and total dissolved solids^[3] and other stream substrates are highly correlated to larval morphology of leaf litter^[4]. The several populations have unique features of the biology and ecology forming communities of different species along the streams and river. The black fly species distribution and abundance along water bodies are influenced by several factors, such as the stream size and width^[5], flow of the water courses^[6], stream substrates and substances dissolved in water^[7], food availability^[8], riparian vegetation^[9], altitude and anthropogenic impact interference in the stream sites^[10].

Oriental region has high species diversity for aquatic insects, as is the family Simuliidae. Since the discovery of fresh water insects comprise, >60% of total fresh water animals. Of these, Diptera is the major representative (43%) of fresh water insects. The family Simuliidae (Order: Diptera) is distributed with the highest diversity on Oriental region^[11]. *Simulium* species most widely distributed in the oriental region. *Simulium aureohirtum* Brenetti, the most widely distributed species in the Oriental Region, has been known to be able to develop the first batch of eggs without a blood meal^[12]. Although Western Ghats is the most important hotspots in the world, and many black fly studies were previously done so far. In total, 88 (14 unnamed and 74 named) black fly species have been recorded under six subgenera in the genus *Simulium* in India^[13,14]. Among the

88 species the majority of (84 species or 95.45%) were recorded in Western Ghats, and remaining (four species or 4.55%) in Eastern Ghats. But many unexplored regions present in Eastern Ghats. Yet the biology and ecology of black fly species is poorly known Eastern Ghats. The recent works were done in few parts of Eastern (19 sites) and Western Ghats (23 sites).

Hence, we made the first attempt on black fly distribution in 42 streams and rivers of Eastern and Western Ghats. In this study, we focused to analyse the distributional pattern of pre-imaginal black flies and effects of environmental parameters in streams and rivers of Eastern and Western Ghats, India.

Materials and methods

1. Study area

A total of 42 streams and rivers sampling were done between November 2016 and September 2019 in Eastern and Western Ghats, India (Figure 1). The study site between Lat 8°54' to 14°50' N and Lon 77°37' to 74°62' E. The elevational ranges from 54.5 m to 1532 m. Among the 42 sites located into 9 different hills situated in various places of India. The nine hills are split into two distinct hill range one is Eastern and another one is Western Ghats. The majority of streams and rivers of 23 were sampled in Western Ghats, and remaining 19 sampling sites were collected from Eastern Ghats. In each hills have an unexpected different variety of fauna and flora.

2. Sampling methods

42 streams and rivers in India's Eastern and Western Ghats were sampled as part of a pilot study between November 2016 and September 2019. Three samples were obtained from a 1m² region in each stream. Manual collection of

larvae and pupae was done from leaf litter, woody debris, and water-soaked polythene sheets. With the use of a fine brush and forceps, the immature pupa was identified and extracted from substrates for rearing. Pupae have been kept for one to two days on moist filter paper in a tiny plastic container before emerging as adults. The specimens were preserved in ethanol that was 80–90%. Using a portable water analysis tester (PCS Testr 35, Eutech Instruments, India), physicochemical parameters were determined. The Global Positioning System (GPS) was used to measure altitude, latitude, and longitude on the Garmin-12 maps.

3. Statistical analysis

Statistical analyses of Karl Pearson’ Correlation coefficient, One-way ANOVA, Principal Component Analysis (PCA) and Abundance measures were carried out by statistical software PAST version 3.15b, XLSTAT version 2022 and Microsoft Excel Standard 2016. Principal Component Analysis (PCA) was computed for measuring the relationship between blackfly abundance and physicochemical variables arriving during the 42 streams and rivers for different season.

Result and discussion

Twelve species were found from 42 sampled streams and rivers different region of Eastern and Western Ghats, Peninsular India. In total of 5383 larvae belongs to subgeneric level, *Gomphostilbia* (six species) was the largest subgenus in our collection, followed by *Simulium* (four species) and *Nevermannia* (two species) (Table 1) [15-22]. Total population of black fly larvae were high in Thirumoorthy falls (23.22%) and low Mancholai (0.03%). The current study also successfully revealed 12 out of the 88 species groups recorded in India. The *Simulium batoense* species group (six species) was the most abundant, followed by the *Simulium striatum* species group (four species), and *Simulium ruficorne* species group (two species). The individual black flies were highly abundance recorded in *Simulium ruficorne* species group of (*S. (N.) kiliyurens*

(32.7%) published in Ph.D thesis, Vijayan [15]. And the other hand *Simulium batoense* species group of *S. (G.) kumbakkaraiense* (0.74%) was individual lowest black flies were recorded (Table 1) [18].

Physicochemical properties were analysis in the Principal Component Analysis (PCA). Principal Component Analysis (PCA) indicated that air temperature, water temperature, conductivity, total dissolved solids, salinity, canopy, bedrock, boulders and leaf litters (Table 2). The active observation PCA shows that F1 (53.86%) had the high value than F2 (11.34%) Figure 2. The F1 and F2 values are clearly indicating that higher elevational site had conductivity, total dissolved solids and salinity; and lower elevational site have a canopy, air and water temperature. Microhabitat provided an excellent larvae population of density higher than lower for example: bedrock and boulders. And other hand substrate is a food sources for density of individual population; leaf litter have the richest sources than the other substrates (Table 2 & Figure 3). Squared cosines of the variables value clearly indicating lower site of Thirumoorthy falls (0.046) and the higher site of Kiliyur falls (0.028), where these tourist part had more individual black fly larvae (Table 3).

The PCA indicated parameters of temperature, water temperature, conductivity, total dissolved solids, salinity, canopy, bedrock, boulders and leaf litters were tested to the spearman’s rs correlation coefficient. And this result indicated that water temperature, leaf litter and total dissolved solids had the significant value ($\gamma = 0.79058, 0.734682, 0.41112$) with abundance of black flies (Table 5). The results of one-way ANOVA revealed that the relative abundance of black flies was significant ($F = 77.13, p = 3.3$) between the sampling sites. Cluster analysis was depicted that conductivity, salinity, air temperature and water temperature significant role of black abundance (Figure 4). This prediction showing conductivity and salinity was high supporting to black fly distribution and micro habitat of leaf litter provide more colonization than other stream substrate (Figure 5).

Table 1: Twelve species were present in the streams and rivers of India

Species/Subgenera	<i>Gomphostilbia</i> (%)	<i>Simulium</i> (%)	<i>Nevermannia</i> (%)	Authors
<i>S. (G.) alagarmalaiense</i>	3.77	0	0	Vijayan, 2021, PhD thesis [15]
<i>S. (G.) agasthyamalaiense</i>	4.16	0	0	Vijayan et al, 2019 [16]
<i>S. (G.) dinakarani</i>	5.02	0	0	Anbalagan et al, 2020a [17]
<i>S. (G.) krishnani</i>	3.53	0	0	Anbalagan et al, 2020a [17]
<i>S. (G.) kumbakkaraiense</i>	0.74	0	0	Anbalagan et al, 2019a [18]
<i>S. (G.) sirumalaiense</i>	5.00	0	0	Vijayan, 2021, PhD thesis [15]
<i>S. (S.) pothigaiense</i>	0	1.49	0	Anbalagan et al, 2018b [19]
<i>S. (S.) striatum</i>	0	3.29	0	Brunetti, 1912 [20]
<i>S. (S.) valparaiense</i>	0	30.30	0	Anbalagan et al, 2018b [19]
<i>S. (S.) yanaense</i>	0	2.67	0	Anbalagan et al, 2019b [21]
<i>S. (N.) karavalliense</i>	0	0	7.33	Anbalagan et al, 2020b [22]
<i>S. (N.) kiliyurens</i>	0	0	32.7	Vijayan, 2021, PhD thesis [15]

Table 2: PCA analysis indicating the environmental variables distribution of black flies.

	F1	F2	F3	F4	F5
Atmosphere Temperature (°C)	1.702	1.216	-0.287	-0.229	0.169
Water Temperature (°C)	0.793	0.769	-0.174	-0.217	0.066
pH	-2.331	-0.286	0.126	-0.235	-0.482
Conductivity (µsec)	16.372	-3.393	0.998	-0.091	0.349
Total dissolved solids (ppt)	10.794	-3.722	0.412	-0.381	-0.623
Salinity (ppm)	7.177	-1.833	0.097	0.080	-0.245
Dissolved oxygen (mgL ⁻¹)	-1.974	-0.340	0.086	-0.178	-0.503

Stream order	-3.219	-0.613	0.300	-0.241	-0.592
Stream width (m)	-2.619	-0.490	0.249	-0.160	-0.570
Water depth (cm)	-0.398	0.368	-0.009	-0.313	-0.231
Current velocity (sec/m)	-2.675	-0.355	0.127	-0.402	-0.601
Canopy cover (%)	6.705	6.092	-6.700	-1.194	-0.852
Bedrock (%)	3.055	5.640	5.154	-5.469	0.295
Boulders (%): >25.6cm	1.071	0.031	-0.595	1.408	1.283
Cobbles (%): >6.5-25.5 cm	-0.928	-1.505	-1.460	0.226	0.601
Pebbles (%): 0.2-6.4 cm	-0.951	-0.070	0.185	2.873	-0.194
Sand (%) 0.05-0.2 cm	-2.323	0.122	-1.099	0.149	-1.006
Mud/silt (%): <0.05 cm	-1.862	-0.610	-1.771	-0.754	-1.564
Riparian vegetation	-3.203	-0.585	0.160	-0.236	-0.651
Bedrock (no.)	-3.465	-0.603	0.507	0.214	-0.445
Boulders (no.)	-2.993	0.276	0.175	0.592	0.770
Cobbles (no.)	-3.237	-0.887	0.237	-0.155	-0.175
pebbles (no.)	-3.491	-0.777	0.246	-0.090	-0.576
Leaf litter (no.)	3.614	5.417	2.853	6.464	0.577
Woody debris (no.)	-2.815	-0.808	0.629	0.386	-0.646
Waste cloths (no.)	-3.326	-0.499	0.641	-0.300	-0.739
Polythene sheet (no.)	-2.471	-0.774	-1.416	-1.042	6.466
Snacks cover (no.)	-3.547	-0.731	0.047	-0.483	0.878
Others (no.)	-3.453	-1.051	0.281	-0.221	-0.758

Table 3: Squared cosines of the variables between sampling sites and black fly larvae distribution.

Name of sites	F1	F2	F3	F4	F5
Yana	0.837	0.031	0.025	0.088	0.001
Bene hole	0.629	0.002	0.002	0.008	0.002
Balur	0.205	0.140	0.013	0.488	0.000
Kathelekan	0.539	0.056	0.226	0.036	0.000
Thadagainachiyamman	0.334	0.069	0.005	0.014	0.007
Thirumoorthy falls	0.046	0.200	0.089	0.515	0.007
Monkey falls	0.180	0.019	0.113	0.010	0.078
Sirukundra	0.263	0.008	0.102	0.493	0.000
Nadumala	0.309	0.152	0.409	0.093	0.001
Pudhuthottam	0.510	0.058	0.055	0.186	0.076
Ayyan odai	0.649	0.074	0.025	0.000	0.012
Manimutharu	0.461	0.010	0.210	0.200	0.002
Manimutharu falls	0.041	0.002	0.045	0.079	0.739
Manjolai	0.818	0.019	0.000	0.136	0.002
Kakkachi 1	0.363	0.545	0.001	0.059	0.001
Kakkachi 2	0.136	0.205	0.240	0.024	0.036
Nalumukku	0.282	0.654	0.001	0.033	0.001
Oothu	0.313	0.336	0.263	0.069	0.002
Ayyan aruvi	0.857	0.102	0.000	0.003	0.000
Moolayar	0.622	0.060	0.084	0.120	0.004
Kurusadai	0.512	0.451	0.003	0.004	0.001
Silver cascade	0.851	0.051	0.000	0.028	0.000
Pampar	0.361	0.014	0.266	0.007	0.002
Kumbakarai	0.954	0.003	0.001	0.008	0.002
Mavoor	0.852	0.095	0.027	0.002	0.006
Adukkam	0.849	0.081	0.003	0.000	0.004
Kandan odai	0.072	0.109	0.030	0.112	0.012
Kozhikootu aaru	0.762	0.000	0.026	0.009	0.032
Periya thoppu	0.797	0.014	0.039	0.010	0.009
Uluppakkudi	0.835	0.055	0.012	0.004	0.000
Gundur	0.627	0.002	0.146	0.008	0.037
Kiliyur falls	0.028	0.017	0.029	0.009	0.733
Aathuppalam	0.746	0.164	0.023	0.017	0.003
Kaaravalli	0.606	0.159	0.007	0.021	0.000
Naachiyamman	0.284	0.134	0.370	0.001	0.032
Karumbur	0.509	0.110	0.001	0.034	0.009
Sitraruvi	0.719	0.015	0.088	0.020	0.020
Aagaayagangai	0.649	0.001	0.044	0.004	0.003
Kolinjipatti	0.747	0.218	0.014	0.001	0.000
Kombaikaattu	0.761	0.192	0.021	0.003	0.000
Athumedu	0.935	0.018	0.036	0.000	0.000
Palaiyur	0.775	0.120	0.052	0.036	0.003

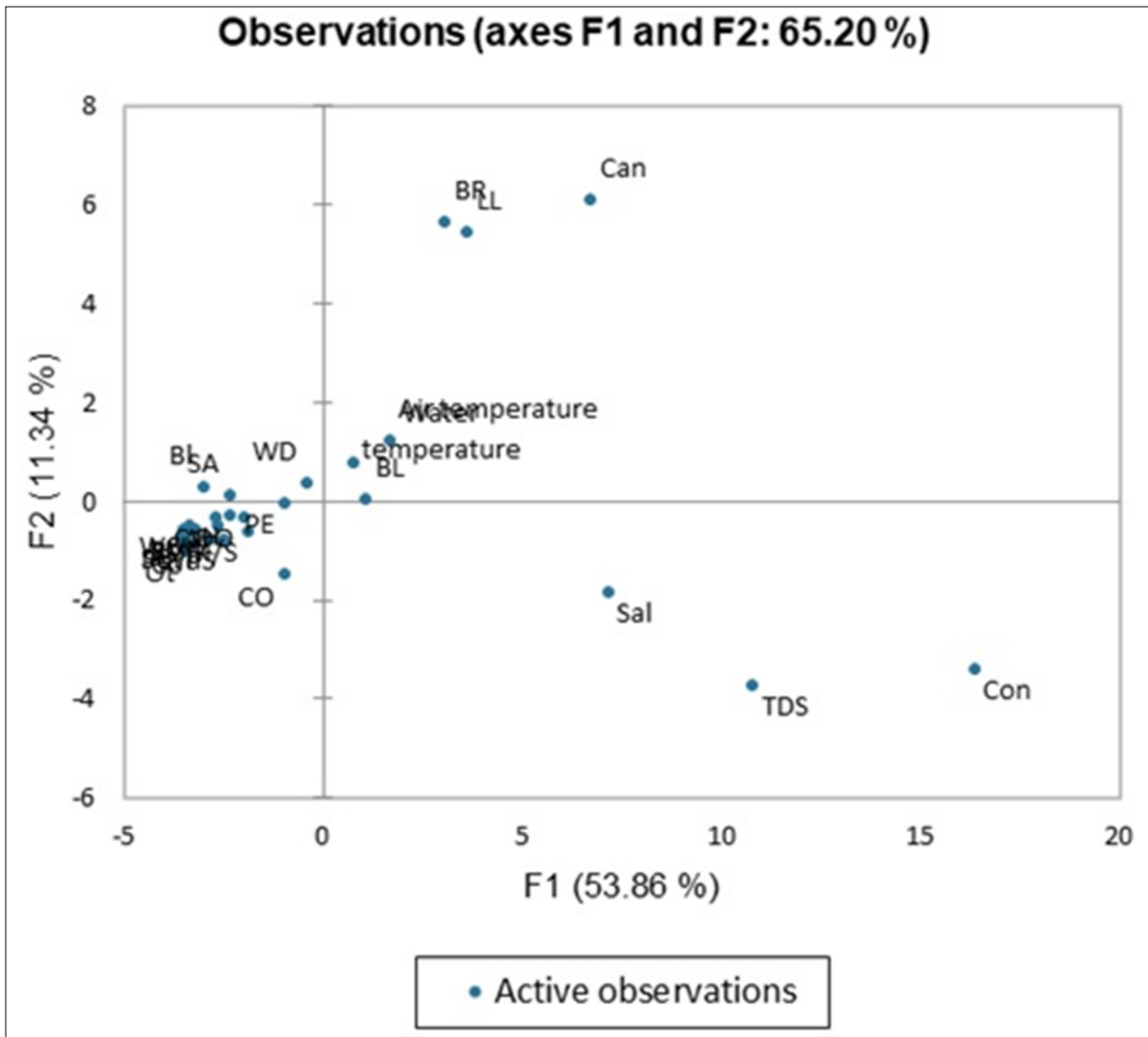


Fig 3: Active observation indicated F1 and F2 axis values among environmental variables and black fly species colonization.

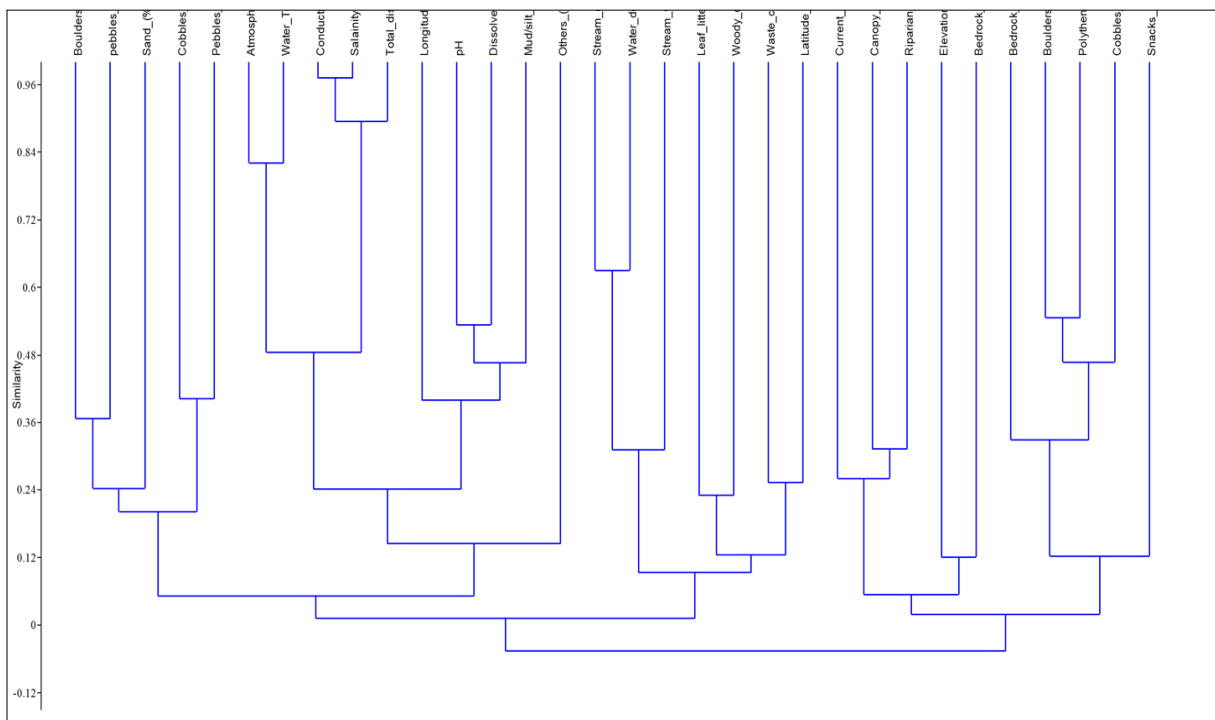


Fig 4: Cluster analysis illustrated the significant physicochemical parameters distributed of black flies.

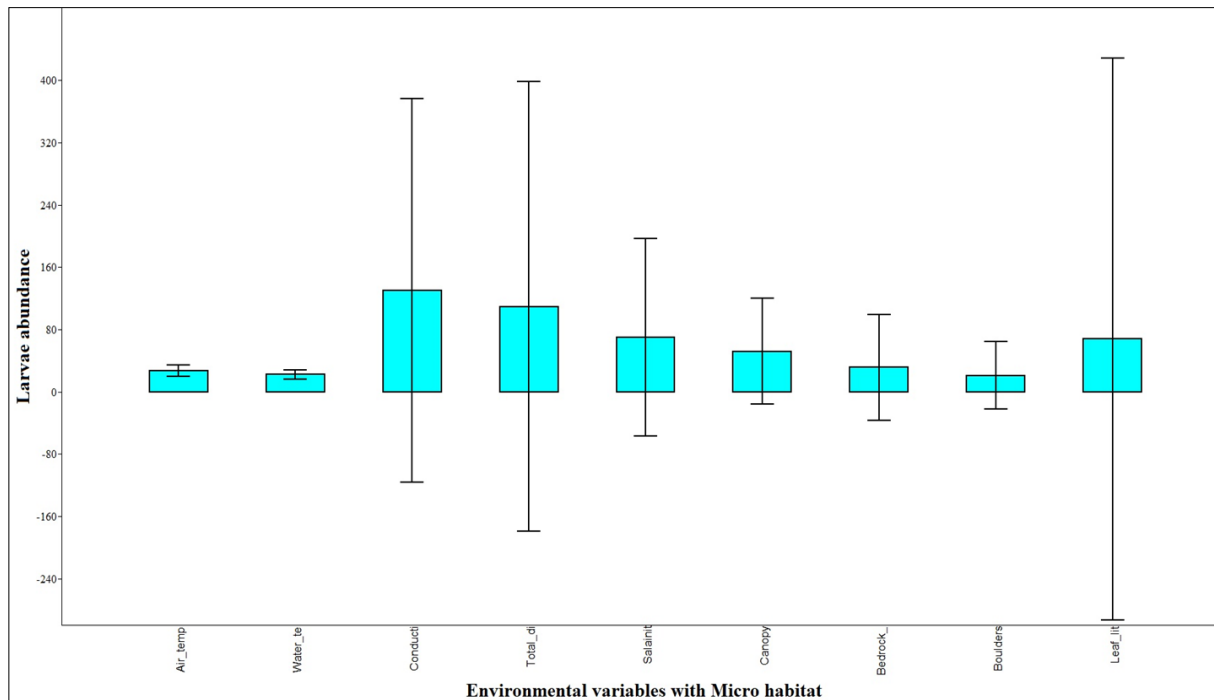


Fig 5: Bar chart analysis was used to depict the larval abundances in relation to environmental parameters and microhabitat.

Discussion

The present study yielded only 12 species for 42 sampling sites in Peninsular India, representing 13.63% of the total simuliids in India [13, 14]. The subgenus *Nevermannia* rare species group was discovered in our study. *Simulium* (*Nevermannia*) *ruficorne* species group of (*S. (N.) kiliyurensis* (32.7%) published in Ph.D thesis, Vijayan [15]. The higher abundance of black fly is found where the low to high anthropogenic impacted stream [4]. These findings indicated that all sampled streams are the natural breeding habitats for black flies. According to Dhiman *et al.* [23], reported biting black flies were found in the north-east India. In these regions, *S. (N.) aureum* biting species were recorded. However, there are no records of biting black flies in South India, and all known species are nectar feeders [14]. In addition, leaf litter contained more black flies than other substrates because its surface area and nutrients may attract black flies more than natural stream substrates. A similar pattern was previously observed by Anbalagan *et al.* [3]. The presence of allochthonous coarse particulate organic matter (leaf litter and woody debris) and associated microorganisms can have a significant impact on the population and distribution of stream insects [24]. Additional studies on aquatic insects' use of artificial and natural leaf litter have revealed that food value, but less than substrate, and microhabitat are the main factors influencing leaf litter consumption [25]. It is important to compare how insects live and use different types of leaf litter as food or microhabitats [26].

Black flies are abundant in the lower stream at Thirumoorthi falls. This is because Thirumoorthi falls is a well-known tourist destination and the stream has a lot of human waste. Similar report was found in aquatic insect distribution in south Indian streams [3]. However, the higher altitudinal site of Kiliyur falls had a large population of individual species group of *Nevermannia*. Evidently, *Nevermannia* has genetic diversity over the mountain ranges and is a bonafide high-mountain black fly species [27]. A high concordance between conductivity and salinity influenced both sites, with

the lower Thirumoorthi and higher Killiyur waterfalls increasing the number of black fly larvae. The fact that these two streams are popular tourist destinations in the Eastern and Western Ghats may also account for this trend.

The multivariate analysis of PCA highlights that air temperature, water temperature, conductivity, total dissolved solids, salinity, canopy, bedrock, boulders and leaf litters factors alter the abundance of black fly larvae in streams and rivers of Eastern and Western Ghats. Air and water temperature are important factor, temperature is an essential factor that influenced the aquatic insect distribution [28]; conductivity and total dissolved solids [29]; salinity [30]; canopy [31]; bedrock and boulders [32]; and leaf litter [3,25]. Further, two sites of Manimutharu river and Nachiamman odai has not been black fly species collection during field survey. It may seasonally affect or influences of flood.

Conclusion

Our results indicated that physicochemical parameters of the stream habitats that are associated with black fly distribution (e.g., air temperature, water temperature, conductivity, total dissolved solids and salinity) varied along an altitudinal and elevational gradient. The present study revealed that black fly species richness and abundance was high in lower study sites than higher site. However, the higher altitudinal site of Kiliyur falls had a large population of individual species group of *Nevermannia*. Evidently, *Nevermannia* has genetic diversity over the mountain ranges and is a bonafide high-mountain black fly species [27]. Nevertheless, the higher elevational areas showed evidence of genetic diversity and speciation. The findings of the present study indicate that anthropogenic impact, along with environmental factors are significant in the diversity and abundance of black fly larvae in streams and rivers of the Eastern and Western Ghats, Peninsular India.

Conflict of Interest

Author declares that there is no conflict of interest

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