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Density and Diversity of Benthic may Fly Larvae in a Torrential Hill Stream of Mid Himalaya, Solan, Himachal Pradesh, India

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ABSTRACT: The present study was conducted for two years from June 2013 to May 2015 to investigate distribution and diversity of mayfly larvae in relation to diverse substrata within a sub-Himalayan stream. The study revealed that substratum plays an important role in may fly distribution patterns. *Caenis* species was most abundant at site 2 consisting of debris stone and mud and at site 4 with sand cobbles and mud. *Epeorus* species was found only at site-4 with sand cobbles and mud and absent all other sites.

Key words: Mayflies, density, diversity, Western Himalaya, India

INTRODUCTION

Himachal Pradesh in the northwest region of the Himalayan biodiversity hotspot is bestowed with an intricate network of thousands of freshwater torrential streams, which constitute an important source of fisheries as well as source of water for irrigation and drinking purposes (Jhingran, 1975; Julka *et al,* 1999). The overall health of a stream is related to the diversity, distribution pattern and population of its benthic fauna (Callisto *et al,* 2007), which are also excellent indicators of water pollution (Gaufin and Tarzwell, 1952), (Sharma and Chowdhary 2011), (Ikhlas *et al* 2014).

Human interference by way of urbanization, extensive use of pesticides in orchards and agriculture fields, deforestation, grazing, etc, are known to affect stream biodiversity (Scrimgeour and Kendall, 2003; Ibemenuga and Inyang, 2006). Regular bio-monitoring and bio-assessment are useful tools for ecosystem management programmes, restoration and maintenance of physico-chemical and biological integrity of freshwater streams (Chatzinikolaou, 2006; Sharma and Chowdhary, 2011).

Mayfly larvae are one of the most important groups of benthic organisms in both tropical and temperate freshwater streams (Elliot *et al* 1983). Larvae of most mayfly species are very sensitive to pollution. They are, therefore, employed in developing water indices on the basis of their occurrence and abundance at a particular location in a stream or river (Baurenfeind and Moog, 2000). Despite the importance of ephemeropterans in lotic waters, virtually not much work has been carried out on the ecology of these organisms in India, particularly in Himachal Pradesh.

At the best some fragmented information is available on the species (Sivaramakrishnan and Job, 1981; Gupta and Michael, 1992; Julka *et al,* 1999; Kumar *et al,* 2012; Ishak and Khan, 2013; Barman and Gupta 2015). The present studies were, therefore, undertaken to investigate distribution and diversity of mayfly larvae in relation to diverse substrata within a sub-Himalayan stream.

MATERIAL AND METHODS

A. Study Area

The study area is located in the vicinity of Shoolini University campus (altitude1189 meters; 30.8644° N, 77.1187° E) at village Bajhol is a part of Giri Hills in district Solan, Himachal Pradesh (Fig. 1). It has four distinct seasons: spring (late February to April), summer (May to August), autumn (September to middle of November) and winter (middle of November to middle of February). It is located at about 14 km from Solan town on Kumarhatti state highway. A perennial stream, Talheri Khad, drains the area and passes by Shoolini University campus Bajhol village to discharge into the river Giri of Yamuna river system. It is fringed with riparian vegetation comprising of *Pinus roxburghii*, *Rubus ellipticus*, *Populus deltoides*, *Pyrus communis*, *Callistemon* species and herbs of *Mentha balsamea* and *Salvia* species.

The stream is exposed to various activities like bathing, road construction, disposal of organic wastes and cattle grazing and agriculture. Talheri stream flows through a narrow valley and is largely exposed to sunlight. Its substrate comprises of sand, mud, cobbles, and boulders. Based on the substrate type, 5 sites were selected for the present study (Fig. 2). The stations are approximately 200 metres apart from each other.

Fig. 1. Overview of Talheri stream, Bajhol near Solan, Himachal Pradesh.

Fig. 2. Various sampling sites; A- pebbles+ mud; B- debris+ stones+ mud ; C- pool area; D- sand + cobbles + mud; E- rocky area.

B. Macroinvertebrate sampling

Mayflies were sampled for a period of two years June 2013 to May 2015. Quantitative samples were taken using Surber's sampler (25×25cm, 625cm^2 area; mesh opening of cloth 0.4mm), which was operated by the method as given by Welch (1948), Barbour *et al* (1994). On each occasion, five replicates of samples were obtained which are considered satisfactory for normal quantitative purposes (Dudgeon and Richardson, 1988). Mayflies were preserved in 4-8% formalin. They were sorted and identified up to species/generic/family level. The identification was carried out using the keys as given by Ward and Whipple (1992), Kapoor and Kriplani (1963), Ueno(1955), Subramanian and Sivaramakrishnan (2007), Webb and McCafferty (2008). Water samples were also analyzed for physicochemical parameters. Water surface temperature was taken with standard mercury Celsius thermometer, pH with pH meter and conductivity with conductivity meter. Current velocity was determined by timing a float in midcurrent (Harreslon *et al* 1994), water depth with meter scale. Dissolved oxygen, free carbon dioxide and total alkalinity were estimated by methods given by Welch (1948), APHA, 1998). Diversity was estimated using Shannon diversity index calculations .Species richness calculated by Margalef richness index and species evenness by Equitability Index.

RESULTS

A. Physicochemical parameters

Summary of Physico chemical parameters at five selected sites of Talheri khadd is given in the Table 1 and 2.

Table 1: Variations in physicochemical parameters of water during 2013-15 at Talheri stream, Bajhol, Solan, Himachal Pradesh.

	2013-14		2014-15	
Parameter	**Range**	**Mean**	**Range**	**Mean**
Temperature(°C)	8.0-25.00	15.25±2.10	8.0-26.00	18.0±1.85
pH	6.88-8.00	7.44±0.11	6.9-7.71	7.29±0.08
DO(mgl^{-1})	2.00-5.97	3.74±0.43	3.40-6.37	4.94±0.30
Total Alkalinity(mgl^{-1})	81.00-147.00	107.58±7.07	80.0-171.0	129.66±9.60
Electrical Conductivity(µ mhos/cm)	31.00-79.00	52.1±5.76	25.0-76.0	56.91±5.30

Table 2: Variations in physicochemical parameters of water during 2013-15 at Talheri stream, Bajhol, Solan, Himachal Pradesh.

	2013-14		2014-15	
	Range	**Average**	**Range**	**Average**
CPOM(gm^{-2})				
Site I (Pebbles + mud)	0.04-4.27	1.7±0.41	0.25-3.20	0.90±0.33
Site II (Debris + stones + mud)	0.05-2.49	1.04±0.24	0.27-2.02	0.77±0.18
Site III (Pool Area)	0.01-4.60	1.65±0.56	0.06-4.00	1.10±0.32
Site IV (sand + cobbles + mud)	0.06-4.64	0.93±0.40	0.19-5.22	1.96±0.51
Site V (Rocky Area)	0.24-4.09	0.79±0.32	0.01-0.99	0.30±0.09
FPOM(gm^{-2})				
Site I (Pebbles + mud)	0.02-2.05	0.52±0.19	0.11-2.38	0.50±0.18
Site II (Debris + stones + mud)	0.10-3.29	1.13±0.32	0.03-4.19	0.98±0.34
Site III (Pool Area)	0.04-2.39	0.9±0.22	0.003-2.01	0.55±0.19
Site IV (sand + cobbles + mud)	0.01-7.47	1.52±0.71	0.10-1.40	0.61±0.46
Site V (Rocky Area)	0.01-2.89	0.69±0.26	0.01-2.88	0.62±0.31
Velocity(ms^{-1})				
Site I (Pebbles + mud)	0.14-0.42	0.25±0.02	0.16-0.39	0.25±0.02
Site II (Debris + stones + mud)	0.16-0.48	0.30±0.02	0.10-0.54	0.28±0.03
Site III (Pool Area)	0.03-0.08	0.04±0.20	0.03-0.09	0.05±0.03
Site IV (sand + cobbles + mud)	0.09-0.37	0.20±0.02	0.07-0.38	0.17±0.02
Site V (Rocky Area)	0.25-1.58	0.70±0.08	0.48-1.20	0.75±0.05

The water temperature ranges minimum 8°C to maximum 25°C during the year 2013-14, and 8°C to 26°C in the year 2014-15. The average pH during first year is 7.44 and during the year 2014-15 is 7.29. The average dissolved oxygen is 3.74 mg l^{-1} and 4.94 mg l^{-1} during both years respectively. It is more during 2014-15 as compared to the 2013-14. The water velocity is much higher at rocky substrate as compared to the other substrates (0.75 ms^{-1}). The average CPOM is greatest at site-1 in the year 2013-14, while it is increased at site 5 having rocky substrate in the year 2014-15. During the year 2013-14 the average FPOM moreor less same at all the five sites as compared to the year 2014-15. In between the sites the site 4 with sand cobbles and mud and site 2 with debris stones and mud having the largest amount of FPOM during the both years respectively.

B. Species composition diversity of mayflies

During the period of two years from June 2013 to May 2015 nine species belonging to 6genera from 4 families of Ephemeroptera were collected from talehri khadd. The relative densities of species at five different sites are given in table 4. *Caenis* and *Baetis* sp.1 were the major species found at all five sites, followed by *Baetis* sp.2 and *Ecdyonurus* species. *Epeorus* species is found at site 1 and site 4 only during 20114-15, 2013-14 respectively. The Ephemeroptera species were represented by *Baetis* species 1, *Baetis* species 2, *Baetiella* species, *Ecdyonurus* species 1, *Ecdyonurus* species 2, *Epeorus* species, *Ephemerella* species 1, *Ephemerella* species 2, and *Caenis* species. The maximum relative densities were found high in the month of January in winter season and in the month of May during pre monsoon. However it is lowest in the month of February and April.

C. Species diversity indices

Maximum Shannon diversity index (H´) and Margalef index were recorded in the month of October 2013 while minimum in the month of March 2014. Dominance Index and Equitability Index were maximum in the month February 2014 and minimum in the month of March 2014 in the first year of study. Maximum Shannon diversity index (H´) was recorded in the month of December 2014 and minimum in the month of April 2015. Dominance Index was maximum in the month of December 2014 and was minimum in the month of April 2015. Margalef index value was maximum in the month of October 2014 while minimum in the month of April 2015. Maximum Equitability Index was maximum in the month of March 2015 while minimum in the month of April 2015 in the second year of study (Table 3).

Table 3: Monthly variations in diversity indices during study period 2013-15.

	Shannon Index	Margalef Index	Dominance Index	Equitability Index
June 2013	0.69	0.58	0.40	0.49
July 2013	1.04	1.44	0.83	0.94
August 2013	1.14	1.36	0.69	0.82
September 2013	0.63	0.45	0.44	0.91
October 2013	0.77	1.18	0.34	0.39
November 2013	0.14	0.24	0.06	0.21
December 2013	0.59	0.49	0.31	0.43
January 2014	0.89	0.60	0.54	0.55
February 2014	0.69	1.44	1	1
March 2014	0.02	0.17	0.006	0.03
April 2014	0.2	0.27	0.09	0.29
May 2014	0.45	0.18	0.27	0.65
June 2014	0.98	0.51	0.514	0.70
July 2014	0.82	0.80	0.53	0.75
August 2014	0.37	0.48	0.25	0.54
September 2014	0.37	0.48	0.25	0.54
October 2014	1.40	1.36	0.69	0.72
November 2014	0.17	0.33	0.06	0.15
December 2014	1.05	0.56	0.62	0.76
January 2015	0.94	0.78	0.55	0.52
February 2015	0.69	0.59	0.44	0.49
March 2015	0.61	0.43	0.46	0.88
April 2015	0	0	0	0
May 2015	0.52	0.33	0.32	0.47

D. Relative density of mayflies

Variability between sites. *Caenis* species was the most abundant at all the five sites. It was maximum at station 4 in both the years (2013-14, 2014-15). *Baetis* sp.1 was the most abundant at the rocky site in both the years. *Baetis* sp.2 was the most abundant at the site1 having pebbles and mud in both the years. *Baetiella sp*. was maximum at site 1 in both of years and was absent at site 1 & 4 in the first year (2013-14) of study and 3, 4 and 5 in the second year (2014-15). *Ecdyonurus* sp.1was max at site 2 and 3 in the first year (2013-14) and maximum at site 5 in the second year (2014-15) and was absent at site 1 in both the years (2013-14, 2014-15). *Ecdyonurus* sp. 2 was maximum at site 1 in both the years (2013-14, 2014-15) and was absent at all the stations except site 4 in the first year. *Epeorus* was found only at site 4 in the year 1 and was absent at all other stations. *Ephemerella* sp.1 was maximum at Site 4 in first year (2013-14) and site 3 in second year (2014-15). *Ephemerella* sp. 2 was present at site 1 in second year (2014-15) and was absent elsewhere (Table 4).

Table 4: Relative Density of recorded mayfly Species (Ephemeroptera) at five different sites in fresh water steam at Bajhol (H.P.) during 2013-15.

Taxa	Site I (Pebbles + mud)		Site II (Debris + stones + mud)		Site III (Pool Area)		Site IV(sand+ cobbles+ mud)		Site V (Rocky Area)	
	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
Baetis sp.1	24.89	42	24.12	36.73	38.26	53.43	21.65	18.33	39.88	61.87
Baetis sp.2	6.62	17.72	3.56	5.26	2.99	9.09	1.84	7.78	2.31	13.64
Baetiella species	0.68	1.7	0	0.12	0.14	0	0	0	0.29	0
Ecdyonurus sp.1	0	0.49	0.55	0.93	1.22	0.93	1.4	0.56	7.52	0.76
Ecdyonurus sp.2	6.4	1.45	0	0	0	0	0	0.33	0	0
Epeorus species	0	0	0	0	0	0	0.46	0	0	0
Ephemerella sp.1	0.68	1.45	0.55	1.63	0.27	4.45	2.30	0	0.29	1.51
Ephemerella sp.2	0	1.21	0	0	0	0	0	0	0	0
Caenis species	60.73	33.98	71.23	55.32	57.12	32.10	72.35	70	49.71	22.22

Variability between the months. Monthly relative density of mayflies for the year 2013-14 and 2014-15 is given in the Table 5. Relative density was maximum in the month of January in the both years. It was found to be minimum in February in the year 2013-14 and in the month of April in 2014-15. Species diversity was recorded maximum in August and December in the both years respectively, and was minimum in April in both of the years. As given in the Table 5 most dominant species for the year 2013-14 was *Caenis* species however it was *Baetis* sp. I for the year 2014-15. *Epeorus* was found only in the first year having minimum density.

Correlation between may fly larvae and physicochemical parameters. Population of mayflies was positively correlated to pH, dissolved oxygen, total alkalinity and electrical conductivity, and negatively correlated to water temperature and rainfall however it was not significant. Among various physicochemical parameters pH was negatively correlated to dissolved oxygen, water temperature and rainfall and was highly significant at 0.05 level of significance. Dissolved oxygen was also negatively correlated to water temperature and was highly significant, it is also negatively correlated to rainfall. Temperature was found to be negatively correlated with dissolved oxygen. The population of *Caenis* species was found to be positively correlated with pH, DO, total alkalinity and conductivity and was negatively correlated with temperature (Table 6).

Table 5: Relative Density of mayflies during different months in a fresh water steam at Bajhol (H.P.) in the year 2013-15.

	Relative Density (%)	
	2013-14	**2014-15**
June	7.75	14.27
July	0.19	0.50
August	0.43	0.34
September	0.43	0.34
October	7.51	11.21
November	2.85	15.78
December	20.11	8.65
January	33.95	24.98
February	0.1	6.51
March	14.55	0.429
April	1.85	0.08
May	10.27	17.00

Table 6: Pearson's coefficient of correlation between mayflies and physicochemical parameters.

	pH	**DO**	**TA**	**EC**	**Temp**	**Rainfall**
Ephemeroptera	0.318	0.377	0.258	0.158	-0.375	-0.390
pH		0.478*	0.175	0.141	-0.604**	-0.546**
DO			0.333	0.107	-0.698**	-0.426*
TA				.610**	-.036	-.279
EC					-.091	-.200
Temp						.473*

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION

The population of Caenis species was found to be positively correlated with pH ($r = 0.45$, $p < 0.05$) and was negatively correlated with temperature ($r = -0.601$, $p<0.01$). Sharma *et al* (2012) also found Ephemeroptera with an Inverse relation with temperature, when the difference in winter and summer temperature is small at high altitudes, of river Beas but at lower altitudes, where the differences are greater, the relation is direct. Imtiyaz *et al* (2013) reported the positive correlation between Ephemeroptera and temperature. Ephemeroptera larvae are common and occur everywhere in bodies of water, from fresh to brackish water, still to fast water flows, and clean to relatively organically polluted water (Ikhlas and Ilias (2014), Accordingly they are found at all five sites despite of different substrate.

Caenis were most abundant at site 2 consisting of debris stone and mud and at site 4 with sand cobbles and mud. Nymphs of *Caenis* are adapted to burrowing in coarse particles, sand and silt, and possess a large second pair of gills which are operculate and protect succeeding gills from becoming clogged with silt .Their abundance at sites with debris, mud, sand and cobbles is due to their adaptation to live in silt and coarse sand particles deposited between the stones and cobbles which provides them a sheltered microhabitat. Julka *et al* (1999), Cummins and lauf (1996) also found the *Caenis* species association with microhabitat preference with coarse sand particles. Various physico-chemical parameters of the water are known to affect directly the population of benthic organisms both in tropical and temperate streams (Townsend *et al,* 1987; Wood *et al,* 2001; Smith *et al,* 2003; Dudgeon, 1999). Maximum Density was found in the month of December and January. Seasonal changes in species diversity were evident both the years. Low values of diversity index during late winters in the period of study due to the presence of only one species that is also in very few numbers. Unfavorable climatic conditions probably caused depressed early summer values, Julka *et al* also observed low diversity values in Northwest Himalayan stream but with small variation in seasonal changes in species diversity.

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