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ARTICLE

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Stream macro-invertebrate diversity of the Phobjikha Valley, Bhutan

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Abstract: Macro-invertebrates form an integral part of aquatic systems, and assessment of macro-invertebrate communities is one of the best ways to monitor the health of a stream. Despite this, stream monitoring of macro-invertebrates has been extremely limited in Bhutan. A study was conducted to generate baseline information on macro-invertebrate diversity in the Phobjikha valley, covering community composition and seasonal variation in diversity during post and pre-monsoon seasons. From a total of 244 units sampled in streams, 50 families from 13 orders were recorded. The dominant order overall was Ephemeroptera (31%), which also dominated the pre-monsoon assessment (33%), while Trichoptera (39%) dominated the post-monsoon assessment. The pre-monsoon assessment recorded two additional orders, Caenogastropoda and Veneroida. There was no significant difference in diversity between the post-monsoon and pre-monsoon assessments ($p>0.3$), but a significant difference in species diversity between seasons was observed in pools and riffles. Physicochemical parameters indicated that pH, electrical conductivity and salinity were within favorable ranges for macro-invertebrates. Further studies of other habitats in different seasons will produce a more comprehensive understanding of macro-invertebrate diversity.

Keywords: Environmental variables, macro-invertebrate diversity, pools, post-monsoon, pre-monsoon, riffles, wetland.

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Author Contribution: JW - Principal investigator on this research findings. Field sampling, data collection, data analysis and preparation of manuscript were main contribution to this paper. Also served as a corresponding author to this paper.
KD - Assisted during the field data collection and development of manuscript. Equally involved that of first author in reviewing the manuscript and edition.

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INTRODUCTION

Freshwater constitutes only 0.01% of the world's water, yet it is host to high percentage of aquatic biodiversity (Dudgeon et al. 2006). Macro-invertebrates are vitally important to freshwater ecosystems, converting plant matter and detritus into major food resources for higher trophic levels in aquatic and terrestrial communities (Wallace & Webster 1996; Covich et al. 1999). Their role in decomposition of organic detritus, nutrient cycling, and water purification are crucial for the existence of stream organisms. Aquatic macro-invertebrates also constitute valuable natural resources for economic, cultural, aesthetic, ecological, scientific and educational purposes (Naiman et al. 2006). Across the globe, about 126,000 freshwater species are reported, and experts have projected the number of species to surpass one million as aquatic biodiversity assessment continues (Darwall 2010). Specifically, over 6,500 species of Odonata (Trueman & Rowe 2008), over 3,141 species of Ephemeroptera (Truffer et al. 2003), over 3,400 species of Plecoptera (Yang & Yang 2006), and 12,627 species of Trichoptera (Moor & Ivanov 2008) are known. From a total of 70,000 known species of Mollusca (Haszprunar 2001) 5,000 are freshwater species, of which 4,000 are Gastropoda and 1,000 are Bivalvia (Balian et al. 2008).

Bhutan is in the early stages of documenting freshwater biodiversity (Gurung et al. 2013; Dorji 2014; Gurung & Thoni 2015). Since 2004 documentation of freshwater biodiversity, particularly macro-invertebrates, was initiated by the National Environment Commission (NEC) in collaboration with Hindu-Kush Himalayan (HKH) experts. Their work reported 166 species of Trichoptera (Malicky et al. 2008), one species of Plecoptera (Stark & Sivec 2010), five species of Acari (Pešić & Smit 2007) and 13 species of Hemiptera (Zettel & Duc 2007; Zettel 2007,2013). Today the HKH catalog comprises 8,044 taxa (Schmidt-Kloiber & Brabec 2007), including Bhutan. Prior to the HKH project, a Swiss expedition in 1975 recorded few species from Bhutan (Malicky et al. 2008). Given the limitation of national experts, local studies have reported taxa of macro-invertebrates, mostly at the order and family level. Recently, several studies reported taxa including headwater streams of Toeberongchhu (Gurung 2013; Dorji 2014), Wangchuck Centennial Park (WCP 2012), Bumthang (Wangchuk & Eby 2013), high altitude wetlands (Wangdi et al. 2013), Threlpang, in Trongsa and Kawangjangsa in Thimphu (Dorji et al. 2014) and Samtse (Dorji 2016).

Phobjikha valley in Bhutan is an important conservation area and also a Ramsar site managed by the Royal Society for Protection of Nature (RSPN). Cultivation of potato, extraction of timber and fuel wood, non-timber forest products (NTFPs), and tourism are some of the major livelihood activities in the valley (Neves 2011). Streams in the study area are supported by wetlands, covered partially by shallow pools of water and grassland. Wetland plays an important role in sedimentation, filtering out pollution and reducing flood risk in downstream communities supporting terrestrial and aquatic biodiversity (Husain & Bhatnagar 2011). The stream network and wetland in Phobjikha Valley support over 396 Black-necked Cranes (*Grus nigricollis)* during winter (RSPN 2015). Although the area is designated as an international Ramsar site for conservation, macro-invertebrate diversity assessment has not been conducted to this current study. The increasing human population (over 5,000 people) has led to the expansion of agricultural areas, roads, and rapid development of private and government workplaces contribute immense threats to aquatic ecosystem (RSPN 2015). As a result, need to establish baseline information on macro-invertebrates diversity is crucial to mitigate the human-induced problem and devise appropriate future conservation management plans. The main purpose of the study was to establish baseline information that will gain impetus at par with terrestrial fauna and flora and provide data to the decision-making bodies to formulate well-informed biodiversity conservation plans.

MATERIALS AND METHODS

A quantitative assessment of macro-invertebrates, performed for measuring the health of the streams and biodiversity (Sharma & Rawat 2009) includes two collection times, pre-monsoon and post-monsoon seasons. At this time of the year insects attain bigger size that can be best identified (Hill et al. 2016). The study of macro-invertebrates in different seasons is essential for estimating gross diversity, as species are well adapted to the seasonal cycle of flood and drought (Li et al. 2012). The present study area falls in temperate streams, where egg stages may last from August to March while larval stages ranging from March to June, and attains adulthood in June or July (Voshell & Reese 2002). The presence or absence of macro-invertebrates species is determined by physical factors such as temperature, velocity, availability of substrates and energy flow (Sharma & Rawat 2009).

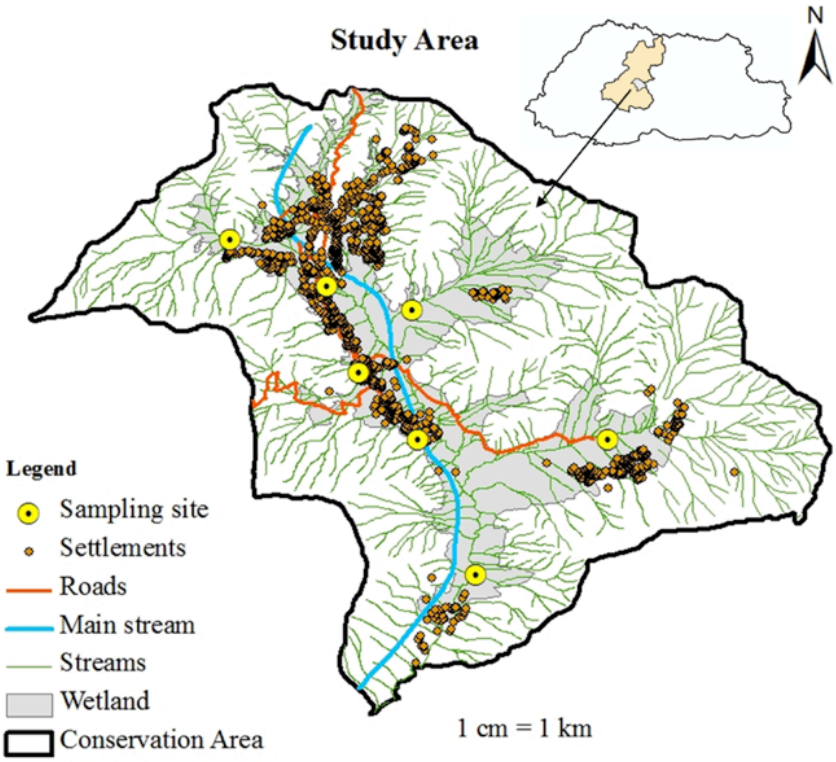

Figure 1. Study area showing the sampling sites

Macro-invertebrates adapt to temperature; warmer temperature increases metabolism, while colder temperatures have the opposite effect. Headwaters are important sources of nutrient and energy flow from upstream to downstream. Because of these factors, our assessment was conducted during post-monsoon, 2014 and pre-monsoon, 2015 in Phobjikha Valley. Seasonal streams were excluded from the sampling, and seven perennial streams were assessed (Table 1). Samples were collected twice during the pre-monsoon and post-monsoon season from each sampling site (Fig. 1). Each sampling reach was 100m in length delineated along the confluence of the stream. From every sampling site, 16 replicates (8 pools and 8 riffles) were collected. A total of 32 replicates were collected from each sampling site (16 during the post-monsoon and 16 pre-monsoon seasons).

a) Macro-invertebrate sampling

Macro-invertebrates were sampled using a square kick net (500µm mesh size) measuring 25cm X 25cm. Rocks measuring over 5mm in diameter were picked and the macro-invertebrates were gently rubbed into the net. Samples were collected starting from downstream to upstream in order to avoid the recapture of insects. The samples were rinsed in a bucket and enumerated, then identified in the laboratory using a dissecting scope. Different identification keys from HKH region on Plecoptera (Graf et al. 2006b), Diptera (Janeček 2006), Trichoptera (Graf et al. 2006a), Coleoptera (Huber et al. 2006), keys from Mongolia (Bouchard 2012), freshwater macro-invertebrates of North America (Thorp & Rogers 2011), and online keys from Alberta were used for identification of macro-invertebrates. Specimens retained in the laboratory were preserved in 70% ethanol.

b) Physicochemical properties of streams and other physical attributes

The pH, temperature, conductivity and salinity were measured using a multi-parameter tester (35 series; Oakton, Vernon Hills, IL 60061, USA). The width and depth of streams were measured using the calibrated measuring stick and a measuring tape. Global Positioning System (GPS; Garmin etrex Vista HCX) was used to map the sampling sites and record altitude.

c) Data analysis

Both descriptive and inferential data were analyzed, using Statistical Package for the Social Sciences (SPSS) version 16.0 and Microsoft Excel 2007. The Wilcoxon Signed-Ranked test was performed to compare the

Table 1. Location of sampling site, altitude and aspect of study the area

Stream name	Site code	Latitude	Longitude	Altitude (m)	Aspect
Zezychhu	ZZ	27.405083	90.202833	2819	west
Taphuchhu	TT	27.404000	90.235056	3009	north-west
Gangchhu	GC	27.438250	90.188556	2883	north-east
Gomphuchhu	GP	27.470389	90.187167	2871	north-west
Tangchhu	TB	27.471667	90.174056	2915	north-east
Aekorchhu	AK	27.487639	90.142389	2972	south-east
Phobjichhu	PC	27.476000	90.166361	2872	south

significance of diversity between post-monsoon and pre-monsoon. The Kruskal Wallis test was used to compare the diversity indices (Shannon diversity, richness, evenness) and density among the streams. Kendall's tau was performed to measure the relationship between environmental variables and diversity indices. ArcGIS 9.3.1 was used for generating the map of the study area. The Shannon Diversity Index (H' = - ΣPi * Ln Pi), the Margalef's Index, species richness and evenness were calculated for the macro-invertebrate diversity in each reach. Evenness (*E*) = H / Ln S where, H' = Shannon diversity index and S = number of species in the sample. Richness (*D*) = S / vN where, S = number of species in the sample, N = number of individuals in the sample. Ecologists have been increasingly using Shannon Diversity Index. Some critics, however, have described it as a questionable index. This is because H' does not reflect dominant/rare community composition and ranges of limits, particularly sample sizes are not clearly defined. Ecologists suggest the use of H' should be restricted to same taxa group (Washington 1984) which is limited to this report. Türkmen & Nilgün (2010), however, suggested this index can be used for determining the habitat qualities of a stream, as results correspond to Simpson Diversity Index, McIntosh Diversity Index and Margalef diversity Index. Thus, this ecological index has been used as it provides both abundance and richness in an area of given time. HKH biotic score and Family Biotic Index were also used to assess water quality.

RESULTS AND DISCUSSIONS

a) Macro-invertebrate diversity in streams

In this study, 11,336 individuals belonging to 50 families from 13 orders were collected. From a total of 224 sampling units, 112 represent riffles and pools for post-monsoon in 2014 and 112 pre-monsoon in 2015. Post-monsoon had 41 families from 12 orders and 6,435 individuals while pre-monsoon had 48 families from 12 orders and 4,901 individuals. Taxa Hydracarina and Ostracoda were reported at the order level; the former was observed in both seasons while latter was observed in post-monsoon. One of the taxon (Caenogastropoda: Amnicolidae) examined to the species level with the help of Naturalis from Netherland, was described new to science as *Erhaia wangchuki* (Gittenberger et al. 2017) named in honor of this first author. This species is known from the water source site GC Gangchhu (refer to site code in Table 1) emerging from an underground spring surrounded by Blue Pine *Pinus wallichina*. The stream substrates mostly covered by dark-green algae houses about 19 other families. The occurrence of this species in the spring source indicates the good health of the water. The highest richness at the family level belonged to the family Trichoptera (Fig. 2). The highest taxa at order level were recorded at TB while the lowest were in ZZ, TT and AK (Table 1). The additional order Veneroida was recorded during the pre-monsoon from TB, GC and PC.

There was no difference in overall mean diversity between post-monsoon *H'* (2.72±0.36) and pre-monsoon H' (2.81±0.18). Species richness and evenness showed similar results between seasons. But post-monsoon had

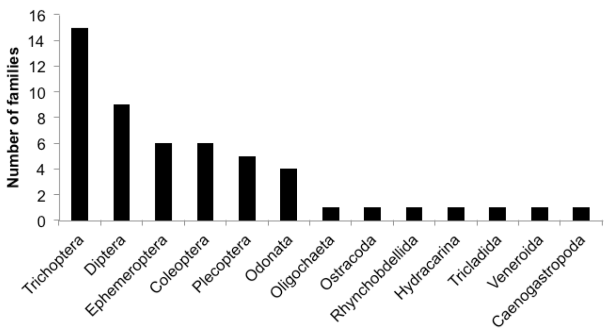

Figure 2. Number of families in each taxonomic group

higher density than the pre-monsoon season (Table 3). The pre-monsoon survey had seven additional taxa at family level. The taxa Staphylidae, Ostracoda and Dolichopodidae were found only during post-monsoon (Table 2). The highest density of individuals in pool to riffle ratio was at AK and PC during post-monsoon, while the lowest ratio was at TB. There was a higher ratio at GC, GP and ZZ while lower at PC and TB during pre-monsoon, which were lower than the post-monsoon (Table 2). This indicated that most larvae attain adult by pre-monsoon.

b) Percent composition of macro-invertebrates assemblage

The overall composition was dominated by Ephemeroptera (31.44%, n = 3564). Post-monsoon assessment in the stream was dominated by Trichoptera (38.94%, n = 2506) while pre-monsoon was dominated by Ephemeroptera (32.94%, n = 1614). Post-monsoon had one individual belonging to Ostracoda. Veneroida was recorded only during pre-monsoon (Table 4). There was a higher discharge attributed to higher depth (30.17±10.79 cm) and width (3.14±1.54 m) during post-monsoon and lower discharge with a lower depth (24.85±7.98 cm) and width (2.40±1.47 m) during pre-monsoon which may have affected the composition between the seasons. Higher representation of Ephemeroptera was reported by Dorji et al. (2014) at Threlpang stream indicating a healthy ecosystem. Ephemeroptera, Plecoptera, and Trichoptera are sensitive to environmental perturbations and occur generally in clean and well-oxygenated streams. Their presence in all the sampling sites infer good indicators of water health (Rosenberg & Resh 1993; Hamid & Rawi 2014).

c) Diversity indices and density between post and pre-monsoon

Non-parametric Wilcoxon Signed-Ranked Test was used to determine the differences in diversity indices between seasons. The test indicated that Shannon Diversity Index during the post-monsoon (mdn = 1.97, SD = .51) was not significantly different from pre-monsoon (mdn = 1.88, SD = 0.45); T = 631, p = 0.289, r = -0.09. The mean diversity was 2.82±0.18 and 2.72±0.36 between the post and pre-monsoon respectively (Table 5). The insignificant differences between the seasons were attributed to minimal changes in substrates and other environmental variables (Table 5). A similar result was reported by Dorji (2014). Brewin et al. (2000) found that the seasonal effect of monsoon climate on the abundance of macro-invertebrates in mountain streams of Nepal had no change from post-monsoon to pre-monsoon where geophysical condition seems to be similar to the present study area. Brachycentridae, Heptageniidae and Limnephilidae dominated the post-monsoon season, while Ameletidae, Hydrobiosidae, Leuctridae and Perlide were represented by a single individual each. Pre-monsoon was dominated by Chironomidae, Heptageniidae and Emidae while the least dominant were Dytiscidae, Epiophlebiidae, Leuctridae, and Psychomyiidae.

There was also insignificant species richness during post-monsoon (mdn = 1.73, SD = 0.52) than pre-monsoon (mdn = 1.70, SD = 0.47); T = 736, p = 0.045, r = -0.18. The density showed significant differences between post-monsoon and pre-monsoon. Mean density was higher during the post-monsoon (57.41±15.38) than pre-monsoon (43±7.18). The decline in density during pre-monsoon was reported (Brewin et al. 2000) and differences in richness between the seasons were determined by temporal changes on environmental variables and physicochemical variation (Scheibler et al. 2014).

d) Diversity, evenness, and richness among the streams

Among the streams Shannon Diversity Index H (6) = 16.19, p = 0.013; abundance H (6) = 19.24, p = 0.004; and richness H (6) = 20.43, p = 0.002 had significant differences in macro-invertebrate diversity (Table 6). Dorji (2014) reported similar results on diversity differences among the streams. Camara et al. (2012) have also reported differences in evenness from Benco stream in Africa. The significant differences in the diversity index among streams result from habitat disturbances by anthropogenic activities (Death & Winterbourn 1995), substrate (Rae 1985; Maston 2007) and energy supply (Kohler 1992) (Rios & Bailey 2006). Sites proximity to the settlements and disturbances were observed with lower diversity indices. This would have been influenced by the site selection, sampling methods, sample size and depth of the sampling (Washington 1984)

e) Diversity indices in riffles and pools between the seasons

Virtually, all taxa were more dominant in riffles than in pools. Significant differences in diversity were found in pool (mdn = 1.68) and riffle (mdn = 2.13) habitats respectively; T = 1012, p < 0.001, r = -0.41. There was higher diversity in riffles (2.06 ± 0.31) than in pools (1.64 ± 0.52). Pairwise comparison of riffles and pools during pre-monsoon and post-monsoon showed significant

Table 2. Number of family representatives under different taxonomic order between seasons

	Family	Post	Pre	Sno	Family	Post	Pre
1	Aeshnidae[6]	*	*	27	Lampyridae[1]	—	*
2	Amnicolidae[4]	*	*	28	Lepidostomatidae[11]	*	*
3	Athericidae[2]	*	*	29	Leptoceridae[11]	*	*
4	Baetidae[3]	*	*	30	Leptophlebiidae[3]	*	*
5	Brachycentridae[11]	*	*	31	Leuctridae[9]	—	*
6	Ceratopogonidae[2]	*	*	32	Limnephilidae[11]	*	*
7	Chironomidae[2]	*	*	33	Limoniidae[2]	*	*
8	Chloroperlidae[9]	*	*	34	Lumbriculidae[7]	*	*
9	Cordulegastridae[6]	—	*	35	Nemouridae[9]	*	*
10	Deuterophlebiidae[2]	*	*	36	Odontoceridae[11]	*	*
11	Dolichopodidae [2]	*	—	37	***Ostracoda***	*	—
12	Dytiscidae[1]	—	*	38	Perlidae[9]	*	*
13	Elmidae[1]	*	*	39	Perlodidae[9]	*	*
14	Emphididae[2]	—	*	40	Philopotomidae[11]	*	*
15	Ephemerellidae[3]	*	*	41	Phryganeidae[11]	*	*
16	Ephemeridae[3]	*	*	42	Planariidae[12]	*	*
17	Epiophlebiidae[6]	*	*	43	Polycentropodidae[11]	*	*
18	Glossiphoniidae[10]	*	*	44	Psychomyiidae[11]	*	*
19	Glossosomatidae[11]	*	*	45	Rhyacophilidae [11]	*	*
20	Gomphidae[6]	*	*	46	Scirtidae[1]	*	*
21	Heptageniidae[3]	*	*	47	Simuliidae[2]	*	*
22	***Hydracarina***	*	*	48	Siphlonuridae[3]	*	*
23	Hydrobiosidae[11]	—	*	49	Sphaeriidae[13]	—	*
24	Hydrophilidae[1]	—	*	50	Staphylinidae[1]	*	—
25	Hydropsychidae[11]	*	*	51	Stenopsychidae[11]	*	*
26	Hydroptilidae[11]	—	*	52	Tipulidae[2]	*	*

	Order	No. of families	Post	Pre
1	Coleoptera	6	*	*
2	Diptera	9	*	*
3	Ephemeroptera	6	*	*
4	Caenogastropoda	1	*	*
5	Hydracarina	1	*	*
6	Odonata	4	*	*
7	Oligochaeta	1	*	*
8	Ostracoda	1	*	—
9	Plecoptera	5	*	*
10	Rhynchobdellida	1	*	*
11	Trichoptera	15	*	*
12	Tricladida	1	*	*
13	Veneroida	1	—	*

(*)Present, (—) Absent, (1-13) Families under different orders

Table 3. Mean, standard deviation on diversity indices, ratio, number of taxa and density in different streams between seasons

Site	Season	Order	Family	Ratio	Individuals	H'	Richness	Evenness	Density
ZZ	Post	8	21	1:2	1121	2.89	1.16	0.79	70
	Pre	8	26	1:3	658	3.00	1.44	0.83	41
TT	Post	8	31	1:2	889	2.93	1.71	0.74	56
	Pre	8	28	1:1	735	2.68	1.43	0.73	41
GC	Post	7	27	1:5	974	2.89	1.15	0.80	61
	Pre	9	20	1:3	586	2.86	1.28	0.83	37
GP	Post	10	26	1:4	855	2.94	1.37	0.79	53
	Pre	10	26	1:3	778	2.85	1.14	0.82	49
TB	Post	9	26	1:3	427	2.97	1.69	0.83	27
	Pre	11	27	1:1	605	2.77	1.26	0.74	38
AK	Post	8	29	1:6	1178	2.36	1.36	0.61	74
	Pre	8	29	1:2	630	3.04	1.79	0.80	40
PC	Post	10	26	1:6	991	2.06	1.3	0.55	62
	Pre	9	31	1:1	909	2.52	1.26	0.69	57
Mean	Post	8.57	26.57		919	2.72	1.39	0.73	57
	Pre	9.00	26.71		700	2.81	1.37	0.78	43
SD	Post	1.13	3.49		246.1	0.36	0.22	0.1	15.38
	Pre	1.15	2.88		114.88	0.18	0.21	0.05	7.18

Table 4. Percent composition of different taxa between to post- and pre-monsoon

	Post (%)	Pre (%)	Overall (%)
Ephemeroptera	30.3	32.93	31.44
Trichoptera	38.94	17.53	29.68
Plecoptera	9.9	6.49	8.42
Diptera	10.78	21.93	15.61
Coleoptera	4.68	10.63	7.25
Odonata	0.48	1.27	0.82
Tricladida	4.12	3.04	3.65
Oligochaeta	0.51	0.55	0.53
Hydracarina	0.2	0.31	0.25
Caenogastropoda	0.65	0.85	0.65
Rhynchobdellida	0.06	0.67	0.33
Veneroida	-	3.14	1.36
Ostracoda	0.02	-	0.01
	N = 6435	N = 4901	N = 11336

differences in diversity (Riffle: mdn = 2.14; Pools: mdn = 1.60, T = 54, $p < .001$, r = -0.57); (Riffles: mdn = 1.80, Pools mdn = 1.59, T = 371, p = 0.003, r = -0.32) respectively. The diversity indices in pools between post and pre-monsoon had significant differences except for evenness (Table 8). The major differences between the habitats and seasons could be attributed to the levels of oxygen, intact substrate and energy flow (Callisto et al. 2005; Hamid & Rawi 2014; Scheibler et al. 2014).

f) Biotic index of macro-invertebrates and health of the streams

Macro-invertebrate composition in headwater streams differs largely contributed by physical and chemical attributes (Hamid & Rawi 2014). Aquatic organisms respond differently to changing habitats (Clarke et al. 2008), some are more tolerant while some are more sensitive depending on an array of environmental disturbances (Yule & Yong 2004). Family level diversity was highest from site TT (Table 3) and PC. The most abundant taxa were Chironomidae (n = 871), followed by Heptageniidae (n = 927) and Elmidae (n = 328) during the pre-monsoon season while Brachycentridae (n = 829), followed by Heptageniidae (n = 747) and Limnephellidae (n = 558) were abundant in the post-monsoon. The composition of families during the post and pre-monsoon appears to be similar (Table 1). Data indicated that the health of the streams had no differences between seasons. The HKH biotic score (Ofenböck et al. 2010), indicated that water quality values were similar across all streams. Here the organisms are assigned a tolerance number from 1–10 pertaining to that group's known sensitivity to organic

Table 5. Diversity indices and density between post- and pre-monsoon

	H'	D	E	Density
Z	-1.061	-2.006	-.956	-7.323
Asymp. Sig. (2-tailed)	0.289	0.045	0.339	<0.001
H': Shannon Diversity Index, D: Richness, E: Evenness, Density per unit area (.25 m2)				

Table 6. Diversity indices and density among streams

	Shannon Index	Richness	Evenness	Density
Chi-Square	16.19	20.43	19.24	10.15
df	6	6	6	6
Asymp. Sig. (2 tailed)	.013	.002	.004	.118

Table 7. HKHbios and Family Biotic Index between the seasons across different streams

Site	Season	HKHbios	FBI
ZZ	Post	6.7	2.41
	Pre	7.28	2.95
TT	Post	7.68	3.3
	Pre	7.37	3.7
GC	Post	6.58	3.42
	Pre	6.88	3.52
GP	Post	6.59	3.07
	Pre	6.62	3.43
TB	Post	6.73	2.3
	Pre	6.5	4.42
AK	Post	7.37	1.61
	Pre	7.06	3.17
PC	Post	6.62	2.14
	Pre	6.88	3.97
Mean	Post	6.82	2.6
	Pre	6.97	3.59
SD	Post	0.29	0.6
	Pre	0.3	0.45

Table 8. Diversity indices on habitat between and within the seasons

	H'	D	E
Riffle to pool (post and pre-monsoon) *z*	-6.16	-1.76	-1.90
p	<0.001	0.081	0.064
Riffle to pool (pre-monsoon) *z*	-6.06	-3.48	-0.34
p	0.002	<0.001	0.735
Riffle to pool (post-monsoon) *z*	-5.53	-2.18	-0.89
p	0.003	0.032	0.376
Pool to pool (post and pre-monsoon) *z*	-2.30	-2.94	-1.71
p	0.021	0.004	0.091
Riffle to Riffle (post and pre-monsoon)*z*	-0.88	-1.96	-2.06
p	0.384	0.051	0.047
H': Shannon diversity Index of pool and riffle, D: Species Richness of pool and riffle and E: evenness of pool and riffle			

pollutants; one being most tolerant, 10 being most sensitive. All the streams showed, the ecological class boundary between good/moderate (≥ 6.3) to reference/good (≥ 7.7) (Ofenböck et al. 2010). The HKH biotic score for water quality (Hartmann et al. 2007) revealed from different sites showed the results as expected. The highest impairment was observed at the site close to the settlements. Some families, however, were not assigned scores in the list, suggesting a need to improve the HKH scoring system. They are excluded from the calculation of the index, which affects the results. These families include Gomphidae, Staphylinidae, Siplonuridae, Amniccolidae and Lampyridae between good/moderate (≥ 6.3) to reference/good (≥ 7.7) (Ofenböck et al. 2010). Hilsenhoff (1988) Family Level Biotic Index was also performed to compare with HKH biotic score. Here the organisms are assigned tolerance number from 0–10; 0 being most sensitive and 10 being most tolerant. The FBI showed similar results that of HKH biotic score that ranges from 1.61 to 4.42, values indicating water quality within the categories good (4.26–5); very good (3.76–4.25); and excellent (0.00–3.75). Unlike HKH biotic score, Hilsenhoff (1988) Family Biotic Index had tolerance values of all families found in the area except for Amnicolidae and Lampyridae.

g) Physicochemical variable and relationship with diversity Indices

i. Temperature, pH, conductivity and salinity of streams

The mean temperature during the post-monsoon was 8.23±1.43 ^{0}C. The lowest temperature, 6.20^{0}C was recorded at GC, while the highest 10.1^{0}C was recorded at TT. During pre-monsoon, mean temperature was high 9.67±1.36. The highest was recorded at GP (12.43^{0}C), while the lowest was at GC (6.76^{0}C). The variation of temperature in the area could have been attributed to altitude, vegetation, aspects, time, and weather (Sharma & Rawat 2009) as it was measured at different time of the day. The temperature at GC was much lower than at other sites. This was because of higher density of vegetation, and because the sampling reach was close to the source.

Mean pH was slightly alkaline, during the post-monsoon (7.20 ± 0.33) and pre-monsoon (7.19 ± 0.42). According to National Environment Commission (2001) and Giri & Singh (2013) the pH of streams in Bhutan was found slightly alkaline. Both seasons had the lowest pH was at TT, while the highest was at GP. The pH was within acceptable limits for aquatic life. The largest varieties of aquatic life prefer the healthy range of pH from 6.8–8 (Robertson 2004), and outside this range the diversity decreases.

Conductivity during post-monsoon was 18.45 ± 4.65 µS/cm (micro-Siemens per centimeter), while there was a slight increase in conductivity during pre-monsoon to 20.27 ± 5.37 µS/cm. Conductivity can be affected by the temperature and concentration of dissolved ions. Increase in temperature and dissolved ions increases the conductivity (Kefford 1998). The variability of conductivity has been shown to affect macro-invertebrate diversity. Chama & Siachoono (2015) and Kefford (1998) showed that the diversity of macro-invertebrates reduces with the increasing conductivity, a pattern consistent with our results between post and pre-monsoon. There was a slight increase in salinity from post-monsoon (11.20 ± 2.22 ppt) to pre-monsoon (12.82 ± 2.60 ppt). Increase in salinity could be due to the dilution of water by the addition of freshwater from headwater streams (Satpathy 1996). Freshwater macro-invertebrates can tolerate up to a salinity of 1000 ppt (Rutherford & Kefford 2005).

ii. Relationship between physicochemical environmental variable and diversity indices

There was a significant relationship between the diversity indices of species richness to pH, and depth to evenness (Table 8). The significant relationship between pH and species richness were negatively correlated (τ = -0.55, p = 0.002). With increasing pH, richness decreases. This indicates an increase in pH can decrease the species in a freshwater system as few species can tolerate changing environment. Similarly, the relationship between water depth and evenness were negatively correlated (τ = -0.46, p = 0.021). Species evenness in this study decreases with increasing depth as Li et al. (2012) reported the level of oxygen in the stream decreases with the increasing depth.

CONCLUSION

Lack of previous studies in the important roosting ground for Blacked-necked Crane and community's lifeline, this study presents baseline information that will enable future studies addressing the growing anthropogenic pressure. The current study revealed no differences in diversity between seasons. Significant differences in diversity indices were apparent among the streams. Trichoptera, Ephemeroptera and Plecoptera were generally recorded from riffles, while Diptera and Veneroida were recorded from the pool. The most dominant taxa were Trichoptera followed by Diptera, Ephemeroptera and Coleoptera. Interesting findings of this study accounts new species described to science, *Erhaia Wangchuki.* Taxonomic expert in the country has constrained this far and likely if all the specimens are identified at the species level, may encounter many newer species to describe in the future.

Apart from taxonomic limitations, expending the sampling intensity across habitats and land use types has a high probability to record additional taxa from the study area. Streams appears healthy that can thrive macro-invertebrates across all the sites in spite of the expanding pressure from anthropogenic activities. The HKH biotic score for water quality classification derived from different sites showed the pattern as expected. The highest impairment was observed at the site close to the settlements. The interception capability of wetland vegetation may have helped to deplete the pollutant that drained into streams. Scientific management of conservation area has to acquire data from a number of studies for consecutive years to obtain the accuracy and preciseness of the diversity in the area. Achieving planned management of conservation areas and establishing a comprehensive list of macro-invertebrates, the study has to cover during winter and summer to get diversity for all-round year by increasing the sampling intensity and distribution. The record of diversity in this report have been limited to family level and two habitats types, where taxa identification at the species level; different land use types; and habitats were not considered. The current study being done for two seasons within few selected habitats will not have recorded all species present in the study area, and we suggest more studies to focus different land use and forest types that would account relative diversity of macro-invertebrates in the area.

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Appendix 1. Macroinvertebrate diversity from the study area

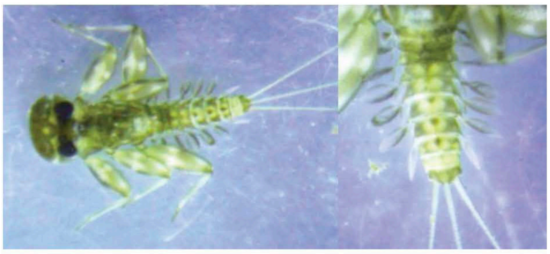
EPHEMEROPTERA (May Flies)

(Baetidae)

(Baetidae)

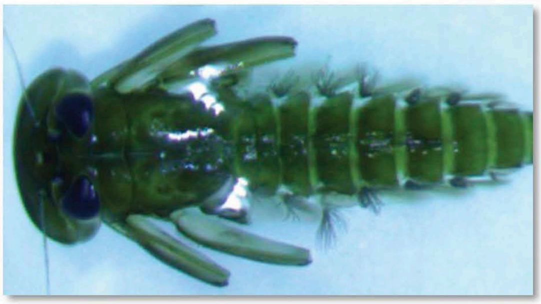
(Ameletidae)

(Leptophlebiidae)



(Heptageniidae)

(Heptageniidae)



(Heptageniidae)

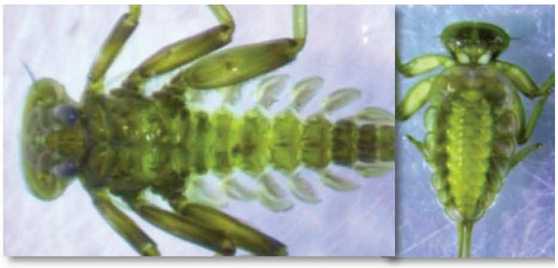
(Heptageniidae)

(Heptageniidae)

(Heptageniidae)

Images shown here are of low resolution but included for general readers.

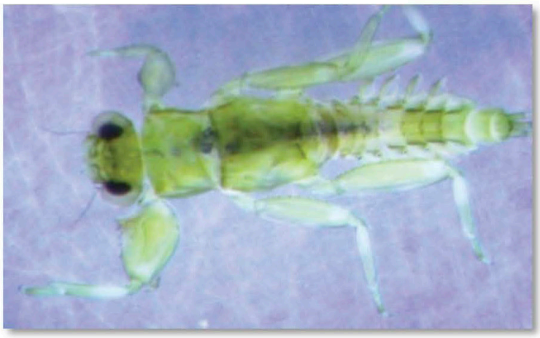
(Heptageniidae)



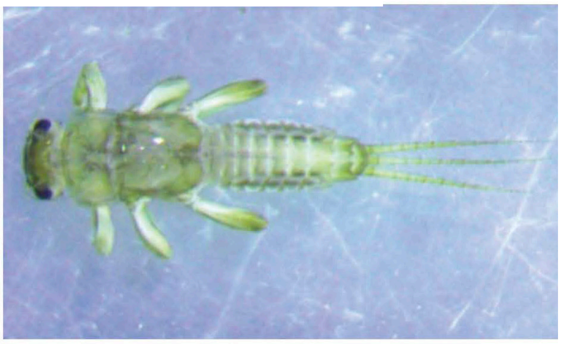
(Heptageniidae)

(Ephemerellidae)

(Ephemerellidae)



(Ephemerellidae)

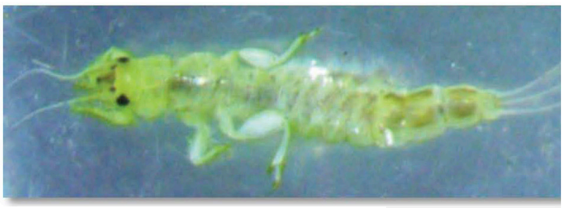


(Ephemerellidae)

(Ephemerellidae)

(Ephemerellidae)

(Siphlonuriidae)



(Ephemeridae)

PLECOPTERA (Stone Flies)

(Nemouridae)

(Nemouridae)

(Nemouridae)

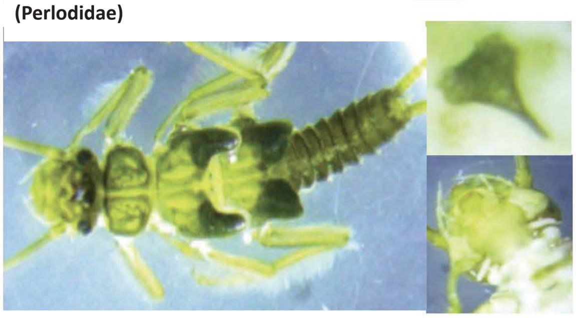
(Perlidae)

(Perlidae)

(Perlidae)

(Perlodidae)

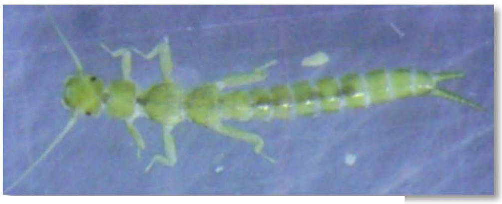
(Perlodidae)



(Perlodidae)

(Chloroperlidae)

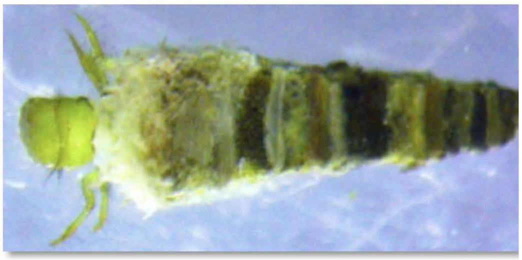
(Chloroperlidae)



(Chloroperlidae)

TRICHOPTERA (Caddisflies)

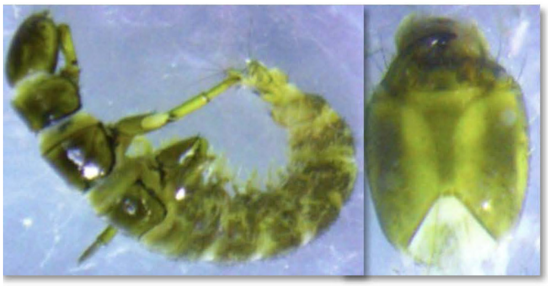
(Brachycentridae)



(Brachycentridae)

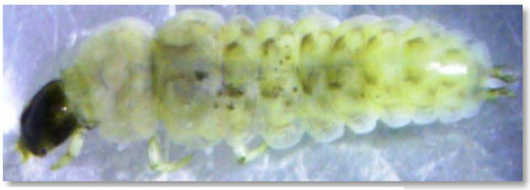
(Glossomatidae)

(Glossomatidae)



(Hydropsychidae)

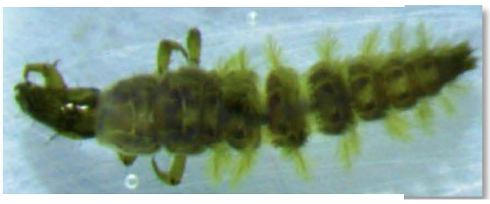
(Hydrobiosidae)



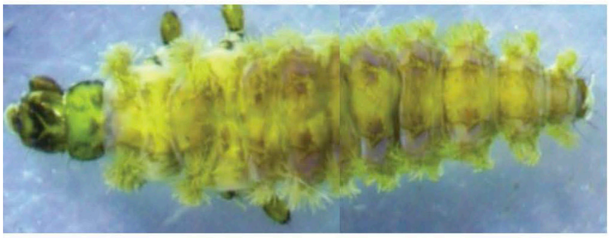
(Rhyacophilidae)

(Rhyacophilidae)

(Rhyacophilidae)



(Rhyacophilidae)

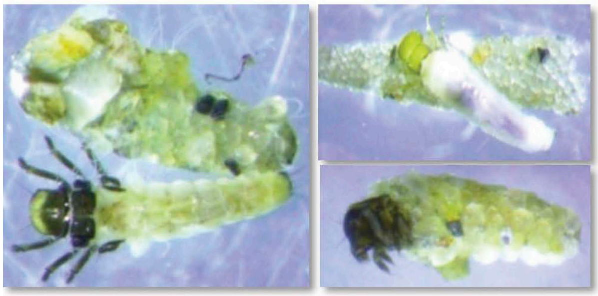


(Rhyacophilidae)

(Rhyacophilidae)

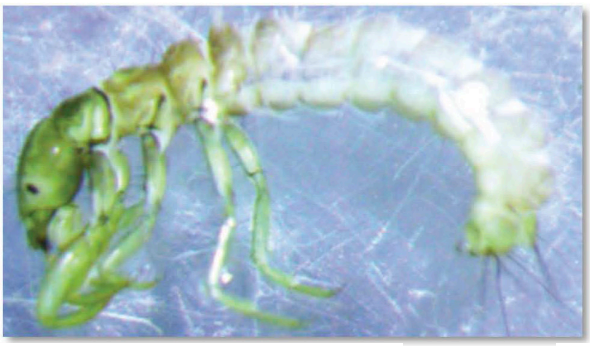
(Polycentropodidae)

(Polycentropodidae)

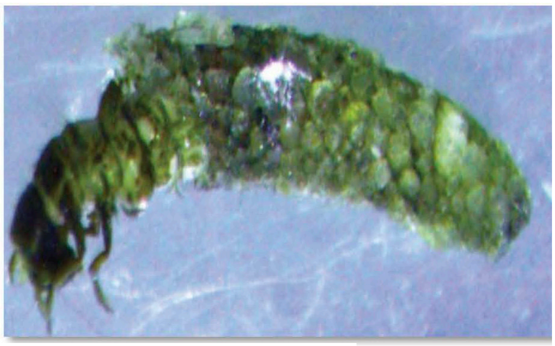


(Lepidostomidae)

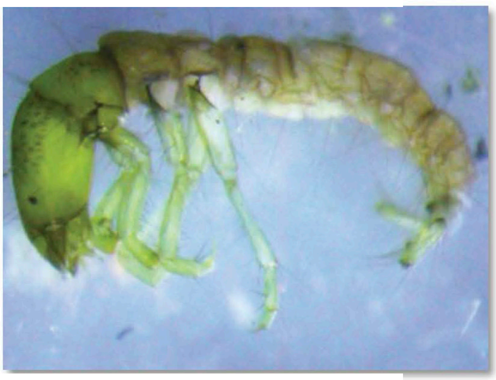
(Lepidostomidae)



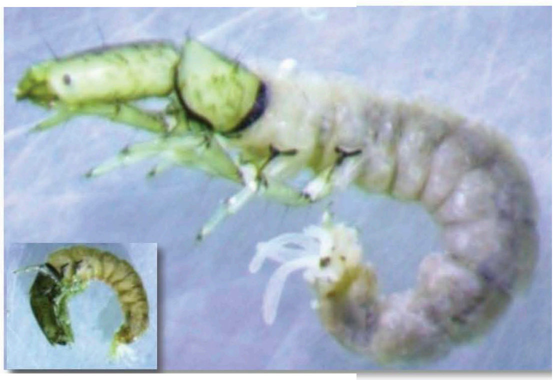
(Leptoceridae)



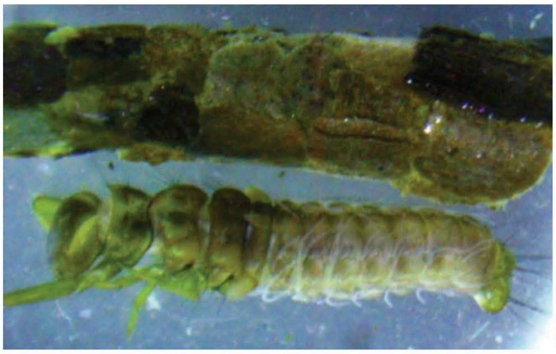
(Limnephilidae)



(Philopotamidae)



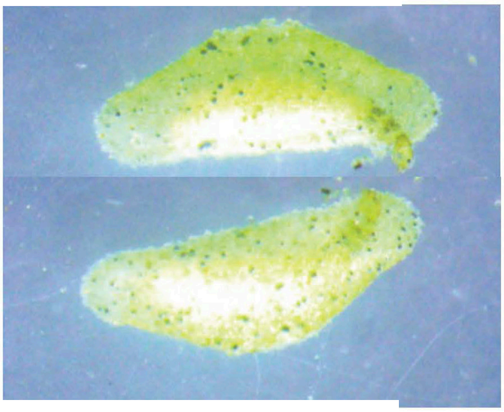
(Stenopsychidae)



(Phryganidae)

(Odontoceridae)

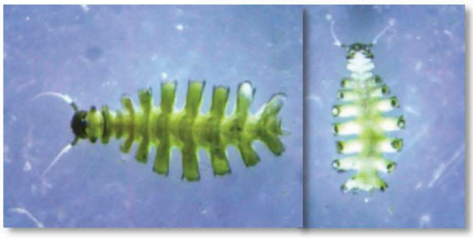
(Psychomyiidae)



(Hydroptilidae)

DRIPTERA

(Limoniidae)

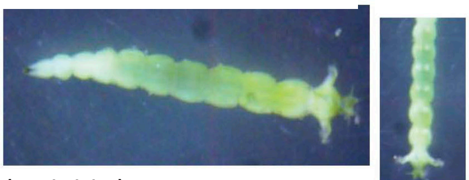


(Deuterophlebiidae)

(Simuliidae)

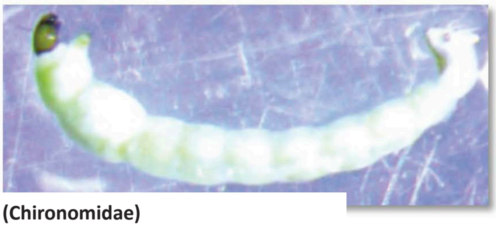
(Athericidae)

(Limoniidae)

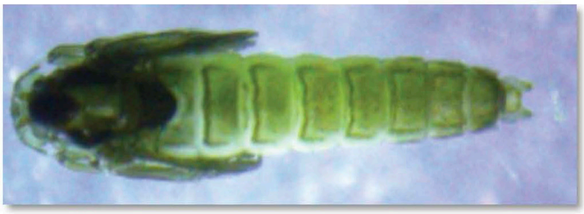


(Emphididae)

(Tipulidae)

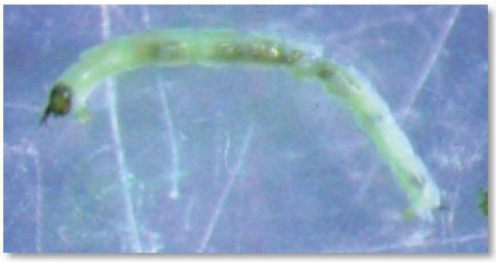


(Chironomidae)



(Chironomidae)

(Chironomidae)



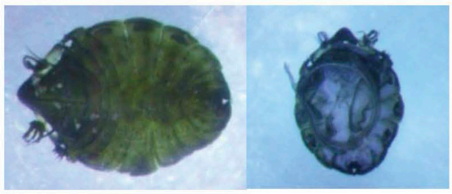
(Chironomidae)

(Pediciidae)

(Pediciidae)

(Ceratopogonidae)

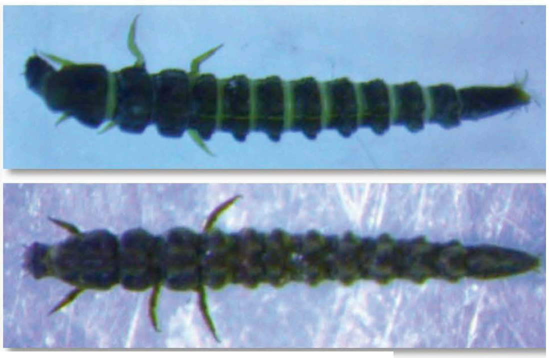
(Dolichopodidae)



(Deuterophlebiidae)

OLEOPTERA

(Elmidae)



(Elmidae)

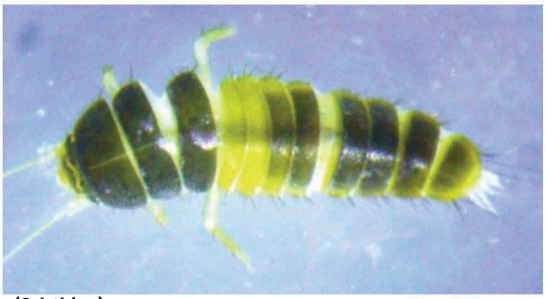
(Elmidae)

(Elmidae)

(Elmidae)

(Lampyridae)

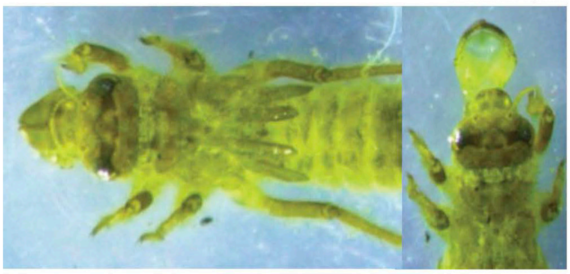
(Staphylinidae)



(Scirtidae)

(Dytiscidae)

ODONATA (Dragon Flies)



(Cordulegastridae)

(Aeshnidae)

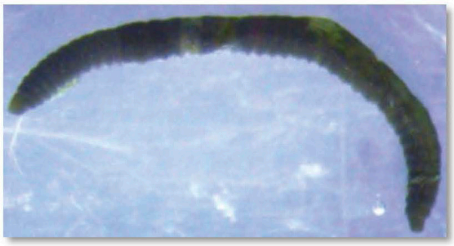
(Epiophlebiidae)

(Epiophlebiidae)

(Gomphidae)

(Gomphidae)

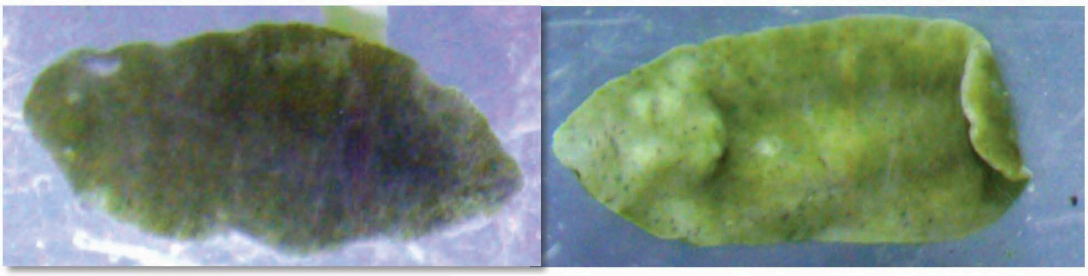
HAPLOTAXIDAE (Aquatic Earthworms)



(Lumbriculidae)

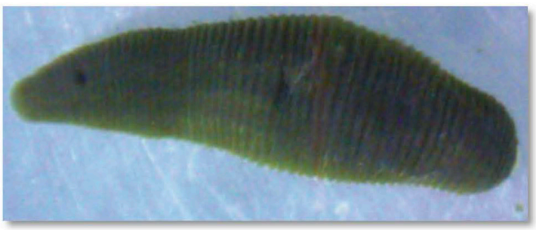
(Lumbriculidae)

TRICLADIDA (Planarian)

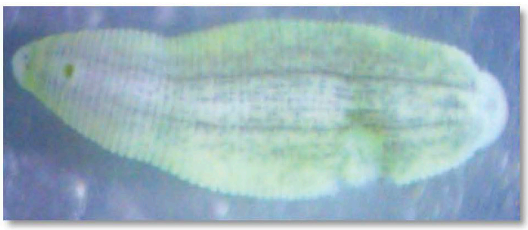


(Planaridae)

RHYNCHOBEDELLIDA

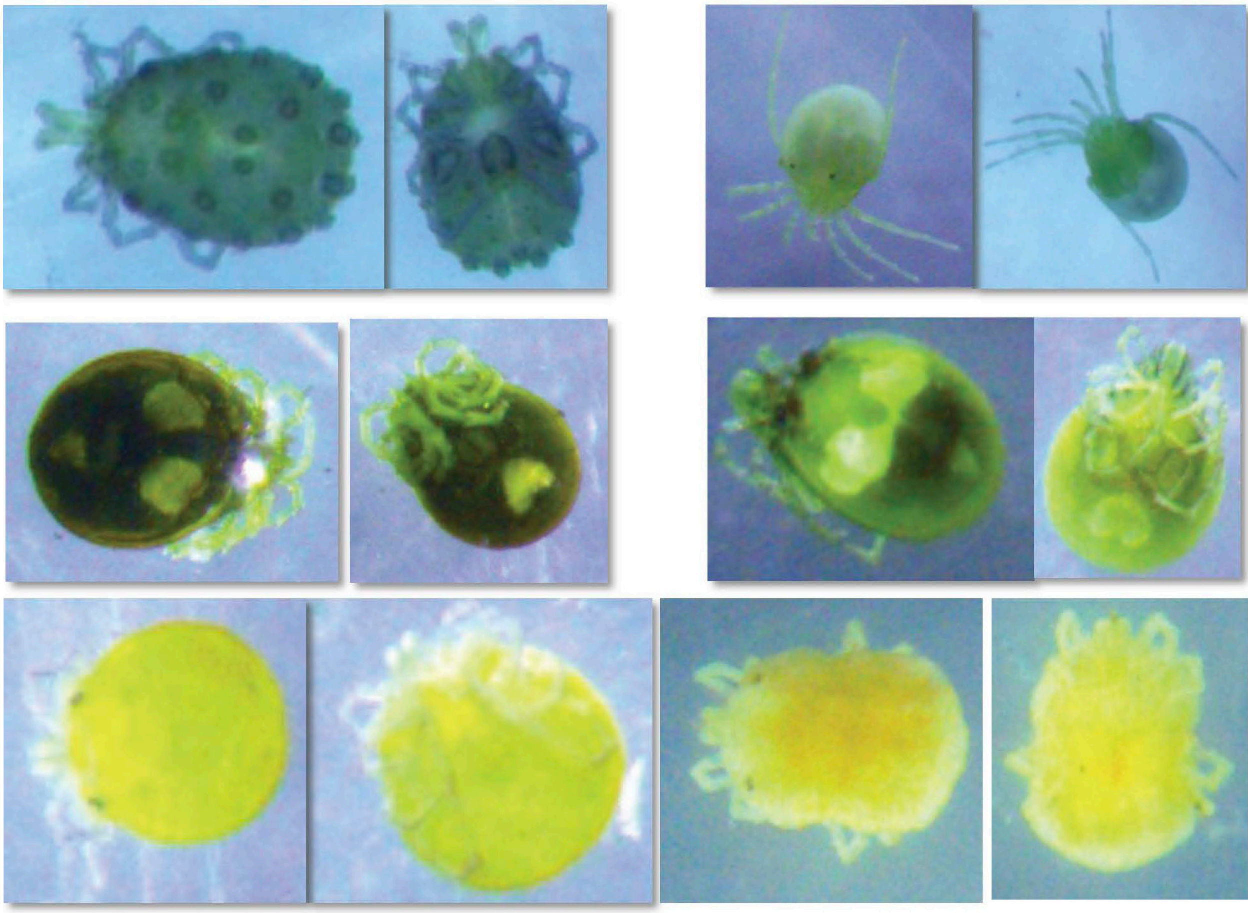


(Glossiphonidae)

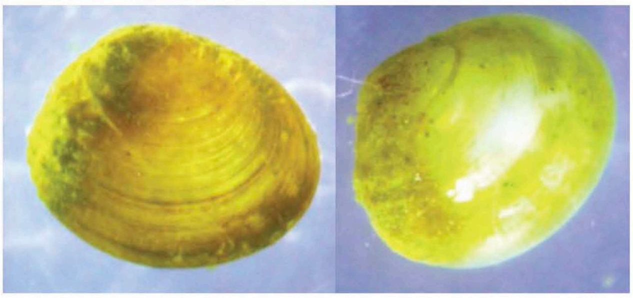


(Glossiphonidae)

HYDRACARINA (Aquatic Mite)

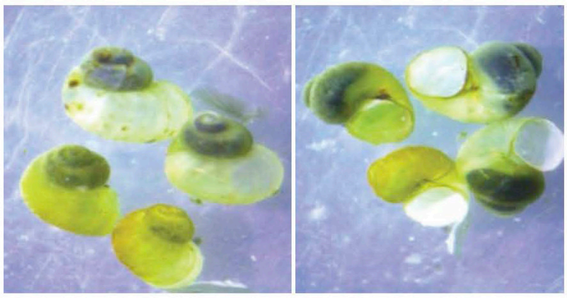


VENEROIDA



(Sphaeriidae)

CAENOGASTROPODA



(Amnicolidae)

OSTRACODA (Seed / Mussel Shrimp)

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