Aquatic Biodiversity Assessment
- A Pilot Study in Bumthang, Bhutan

Citation:
Wangchuk, J & Eby, A. L (2013). Aquatic Biodiversity Assessment –A pilot study in Bumthang, Bhutan. Royal Government of Bhutan, UWICE Press, Bumthang.

Layout and Design: Norbu Wangdi & Tshering Wangdi
Cover and layout redesigned: Jigme Wangchuk & Chenten Dawa

ISBN: 978-99936-678-3-4

Aquatic Biodiversity Assessment

- A pilot study in Bumthang, Bhutan

Ugyen Wangchuck Institute for Conservation and Environment

Water tower of Chamkharchhu

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Executive Summary

The pilot aquatic biodiversity survey was initiated as a part of collaborative research between Ugyen Wangchuck Institute for Conservation and Environment (UWICE) and the University of Montana (UM). The study area falls under the Bumthang Territorial Division and Thrimshingla National Park. The assessment was carried out during the pre-monsoon (14th to 27th May 2012) at different sites covering the four *Gewogs*. Six sampling reach were assessed in collaboration with the Thrimshingla National Park and Bumthang Territorial Division. At each site, we sampled aquatic invertebrates with a kick-net proportional to habitat types. After dividing the sample into morphotypes, field pictures were taken of each morphotype, and representative individuals were retained for identification in the laboratory. Fish sampling involved a variety of techniques including cast nets, kick nets and dewatering of stream section.

This assessment covers more in-depth analysis of freshwater macroinvertebrates, than fish, as only one exotic species, brown trout (*Salmo trutta*) was encountered. We documented 43 families from nine different orders. Further 76 morphotypes were identified using the dissecting scope at the UWICE. We observed the highest diversity of morphotypes in Trichoptera with 32 morphotypes followed by 23 Ephemeroptera, 19 Diptera, 13 Plecoptera, three each Coleoptera and Odonata, one each Hydracarina, Oligochaeta and Tricladida respectively. Entire sampling site was dominated by the order Ephemeroptera. Over 100 specimens of different morphotypes were collected and preserved at UWICE laboratory in the reference collection.

Through our collection, the most diverse assemblage of macroinvertebrates was typically found in the cascade and riffle habitats. Area associated with human-disturbance had less diversity of macroinvertebrates. Ephemeroptera was collected in the highest densities across all sampling areas while the lowest was Hydracarina. This report only begins to document the aquatic biodiversity of Bumthang because of many limiting factors; including human resource experts particularly for identification of different taxa, time, inadequate sampling effort and area. Thus, we suggest expanding this type of effort to extrapolate across a broader region with admissible sampling sites through experts collaboration from the region.

Acknowledgement

The need to assess the aquatic biodiversity was identified as very crucial, which could only materialize in the recent collaborations between the Ugyen Wangchuck Institute for Conservation and Environment (UWICE) and the University of Montana (UM). This collaboration has helped start this process. Without the research collaboration between UM and UWICE, this small piece of work would have been not possible. The authors would like to thank the management for having strengthened the strong collaborative research in various fields. Without the inspirational advise and guidance from the Director, Research Head and Education, and Head of the Departments at UWICE, this work wouldnot be possible.

Despite the limit of this small piece of work, there are many, who involved physically, intellectually, and mentally to achieve these results. Invaluable thank goes to Ms. Nima Dolma (Intern), Mr. Ugyen Tenzin (Research Assistant), Mr. Sonam Penjor (Driver) from UWICE for their hard work rendered during the field work. We would like to extend our heartiest thanks to Chief Forestry Officer of Divisional Forest Office, Bumthang and Chief Forestry Officer of Tshrimshingla National Park for contributing their unconditional support during the fieldwork in their respective area. We would like to Mr. Nima Gyeltshen, Mr. Thapa Rai, Mr. Sangay Penjor of Bumthang Territorial Division and Mr. Pema and Mr. R.B Rai of Thrimshingla National Park for their support and hard work with us in the field.

It is not possible to identify the macroinvertebrates with our naked eyes. We are grateful to the National Environment Commission (NEC) allowing us to use their dissecting scope for a very long duration for identification of specimens. We would like to thank NEC for encouraging, being supportive and providing dichotomous identification keys whenever we were in need.

Chapter 1: Introduction and background

Aquatic macroinvertebrates and vertebrates are very important organisms that inhabit the entire range of aquatic ecosystem. Different communities of macroinvertebrates and vertebrates occur in different types of lotic and lentic water ecosystem, such as streams, rivers, lake, wetlands, and thermal springs (hot springs). This initial survey focused only on lotic systems including streams and small rivers.

1.1 Importance of aquatic macroinvertebrates and vertebrates

Rivers provide food, income, transportation, power, and jobs for millions of people in the world. Rivers, streams, and wetlands sustain ecosystem services and population throughout the world despite the range of impacts due to increasing population, ecosystem modification, pollution, and higher demand for water use. The value of freshwater ecosystems not only provide direct services such as fish for food and water purification for drinking but also provide indirect ecosystem services such as flood control, nutrient cycling, and water filtration for healthy ecosystem (Allen et al. 2011). Aquatic macroinvertebrates play an important role in maintaining healthy ecosystem. They feed on the algae and other organic matter which help to control nutrients and therefore influences the water quality. They are a key component of the food web by providing a wide range of food sources for predators including larval and juvenile life stages of every fish species, small adult fish as well as terrestrial animals like birds and bats (Wallace & Webster, 1996). Macroinvertebrates are also useful indicators of water quality and the overall health of aquatic ecosystems. Because different species have different capabilities for living in a particular physical and chemical environmental conditions (e.g., temperature, salinity, turbidity, metal concentrations, nutrient levels), one can measure the different species inhabiting aquatic ecosystems to assess the health of water bodies. Typically, healthy aquatic ecosystems have both a greater diversity and higher densities of aquatic organisms (Hussain & Pandit, 2012).

The physical and chemical conditions of the aquatic system are affected by the intensity of pollution, dissolved oxygen and temperature. Some macroinvertebrates can tolerate a wide range of conditions while other groups can only tolerate a narrow range. Many aquatic insects play an important role in the aquatic and riparian food web. They are good indicators of water quality because they live in the environment that is cold and well-oxygenated. Insects within the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT) all play a vital role in ecological function because they provide an important food source for fish and other aquatic animals including fish, amphibians, birds, and bats. The emergence of EPT implies the existence of a healthy environment indicating the good water quality as they are relatively intolerant of pollution.

1.2 Status of freshwater biodiversity in the world

Global freshwater diversity continues to be relatively high compared to the terrestrial and marine ecosystem (Allen et al. 2010).

Freshwater systems support various living organism including the mammals, insects, plants, and fungi. Almost a quarter of the global vertebrate diversity is supported by freshwater system. (Allen et al. 2010). Fishes are the most diverse group of vertebrates in the world. There are 31,300 known species of fishes in the world. This includes over 13,000 freshwater fish species (Levegue et al. 2008). Aquatic invertebrate diversity is also considerably high and still scientists continue to describe the species. According to the world checklist database maintained by the Freshwater Animal Diversity Assessment (FADA), there are about 3,141 named species of freshwater Ephemeroptera in the world (Barber et al. 2010). As of 2009, there are 2,000 described species of Plecoptera (Skeel, 2009). According to the Fochetti and Figueroa (2008), there are more than 3,400 described species in the world, while in Asia alone, constitute an about 1,527 described Plecoptera species endemic to the small pockets of areas (Yang, Li & Zhu, 2004). As per the Howard (2012) there are some of 14,000 described caddisflies (Trichoptera) species worldwide.

1.3 Status of freshwater biodiversity in Bhutan

Previous work by Petr (1999) has record of 41 species of fish and 11 species has been added by the work of Bhattarrai and Thinley (2005) which make up the list to 52 species. For many years the list remained almost consistent between 1980 to 2005 as reported by Dubey (1978), FAO (1987), Petr (1999), Gyeltshen (2001) and Tenzin (2006). Bhutan had introduced eight species in the ponds and rivers (Petr & Swar, 2002). Brown trout (*Salmo trutta*) was first introduced in 1930 (Petr, 1999) and are generally abundant in most of the rivers. It is expected that population of native fishes have gone down because of the invasive behavior of brown trout. Two native species have likely been negatively impacted are the snow trout (*Schizothorax progastus*) and Himalayan trout (*Barilus sp.*) (Petr, 1999; Gyeltshen, 2001), reported from almost all the river system of western and central Bhutan. Fish species are still being documented, and the status of most fish species in the country is unknown. Bhutan likely has over 100 indigenous fish species but only 52 species are reported to have been documented as per the existing literature. Generally, the eastern Himalayan region has about 500 fish species (Abell et al. 2008), thus the number of fish species in Bhutan is grossly an underestimate of the actual diversity. In addition, the distribution, population, density of fish in the streams and rivers of Bhutan are unknown (Tenzin, 2006).

The diversity assessment of macroinvertebrates in Toebrongchhu stream, a tributary of Punatsangchhu has 20 species of macroinvertebrates belonging to 13 different orders (Wangyal et al. 2011). More detailed sampling and identification has occurred for two orders; Trichoptera and Odonata in the last few decades. A preliminary list of caddisflies that was identified from a three week trip in the country listed 166 species (Malicky, Karma & Moog, 2008). Several of these species; *Rhyacophila milanippe, R. mnemosyne, R. pyrrha, Agapetus oiopion, A. wolfarmi, Glossoma lar, Chimara mars, C. megara, C. ionone, C. oreithyia, C. pasiphae, C. pontos, C. semiramis, C. talos, C. vesta, Kisaura maia, K. teisiphone, Ecnomus hyas, Hydropsyche gkarmai,* *Hydromanicus makareus, Neurocyta drukpa, Apatania ottomoogi, Paraphlegopteryx astridae, Ocetis dvichakha, Setodes er, S. fragilis,* and *S. sychaeus* are described and endemic to Bhutan (Malicky et al. 2008). Bhutan has over 69 species of odonates recorded in the country, first contributed by Fraser (1936), followed by Lieftinck (1977), Tsuda (1991), Mitra (2002; 2006; 2008), Mitra and Thinley (2005), Brockhaus and Hartmann (2009) and Mitra et al. (2012). Hotspots within the eastern Himalayan region have been estimated to support about 38.2% of the continental South East Asia diversity within Odonata (Mitra et al. 2011), and approximately 960 species and subspecies of Odonata are reported from the region (Mitra, 2000). This exemplifies the potential unknown diversity likely in Bhutan.

1.4 Study objectives were:

- Develop list of of macroinvertebrates within the jurisdiction of the Bumthang *Dzongkhag* as a pilot-based study, covering four *Gewogs*
- Impart the skills to the forest officials working in the Territorial Division and National Park on aquatic sampling and identification of macroinvertebrates
- Collect the voucher specimen for building a reference collection

Chapter 2: Study area, methodology and data analysis

Bumthang is located in the North-Central part of Bhutan. The *Dzongkhag* has four *Gewogs,* namely Chhoekhor, Chhumey, Tang and Ura covering a total area of 2,708.46 km^2 (National Statistical Bureau, 2010) with an altitude ranging from 2400-6000 m. Bumthang has 1,462 households and a total population of 16,116 out of which 11,913 live in the rural areas. It is projected that the population may rise to 1,142 person in 2009 (PHCB, 2005). Today Bumthang is expected to have the population of 17,829 person. The *Dzongkhag* has a road network of 193.1 km including 124 km national highway stretching from Yotongla to Thrimshingla.

2.1 Study area

The study area was selected in collaboration with the Bhumthang Forest Division and Thrimshingla National Park. The study site is predominantly covered by blue pine *(Pinus wallichiana)* forest across the entire study site except in Shingkharchhu had the fir forest. The undergrowth and riparian vegetation are mostly the *Rosa* sp. *Elaeagnus pervifolia*, *Hippophae rhamnoides* and *Microphila* sp.

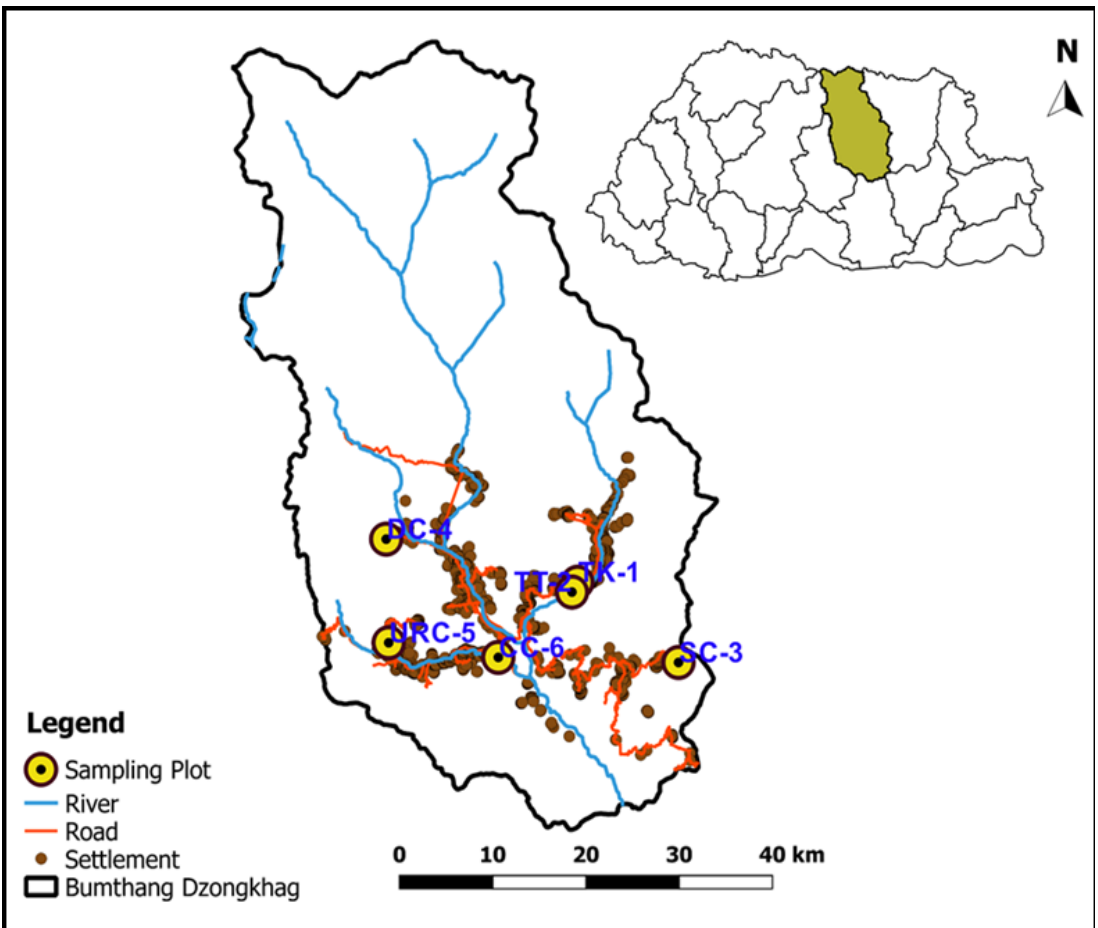


Figure 1. Map showing the study area and sampling sites

2.2 Methodology

Streams flowing into the river were measured and assessed at five different locations. The streams were selected at different elevation, forest, and land use types. This is because aquatic organisms prefer varieties of habitats. For instance some fishes live in higher gradient reaches of river and stream network whereas other species are found in lower gradient, in the wide alluvial valleys (Baley and Li, 1992). The sampling plot was laid out in the stream reach as described in the sampling protocol (Appendix I). The sampling reach was categorized into different habitats types that included pools, riffles, runs, cascades, and woody debris.

Macroinvertebrates were collected by disturbing bottom sediments (e.g., gravel, cobble, pebble, stone, and wood) and organism in a net are held downstream. Organisms were dislodged by disturbing the substratum materials and captured as they got swept into the net by the water current. These samples were rinsed and placed in a plastic bucket adapted from Merritt and Cummins (1996) and assessed. The field data was collected as presented in the sampling data sheet (Appendix II). Field pictures were taken to ensure the same morphotypes associated with the same names in the field to avoid a mistake in the subsequent sampling sites. Taking pictures of each morphotype in the field helps ensure consistent calls and maintain correct data as we proceeded further with sampling units in different sites. Representative samples of each morphotype (from each sampling site) were retained for identification in the laboratory and preserved in a 70% ethanol solution. Individuals (except for reference samples) associated with each morphotype were examined, counted, and released back to the streams.

In the laboratory, the collected samples were thoroughly assessed by using the dissecting scopes. Specimens were identified to the lowest taxonomic level possible, typically family, but occasionally genus. To do this, we used multiple existing identification keys from Hindu Kush Himalayan region: i) key to Trichoptera prepared by Graf, Malicky, and Schmidt-Kloiber (2006); ii) key to Coleoptera prepared by Huber, Graf and Schmidt-Kloiber (2006); iii) key to Diptera prepared by Janecek (2006); iv) keys to Plecoptera prepared by Graf, Sivec, and Schmidt-Kloiber (2006); v) key to the larval stages of common Odonata of Hindu Kush Himalaya, with short notes on habitats and ecology by Nesemann et al. (2011); vi) Aquatic Invertebrates of Alberta, North America (key to Coleoptera, Plecoptera, Ephemeroptera, Trichoptera Tricladida, Hydracarina, Diptera, Arenae, Odonata) by Clifford (1991); and vii) Aquatic Invertebrate Families of Mongolia by Bouchard (2012).

To sample the potential fish species present, we used a variety of gear-types, including cast nets, seine nets, and stream diversion. A variety of sampling gear is necessary, as different fish species are found in different habitats and areas of the stream (e.g., cast nets are very effective for fish swimming in the water column, but not for fish that are typically found hiding under rocks).

For any fish that were captured, we performed an external examination of fish, took reference photos, and measured the total length (tip of head to the end of tail). However, we only encountered brown trout in our study area. We collected physical and chemical measurements to understand water characteristics and water quality for these sites (Appendix II). Water quality data can provide a useful baseline as impacts to the watershed changes, inform us about the habitat requirements of the species that we sample at the same time and link macroinvertebrate assemblages with different water quality types to further validate using them as indicators in this region. Specifically, we measured temperature, pH, conductivity, and turbidity with a multiparameter tester PCStestr 35. We took multiple (at least 3) measurements at each site before sampling macroinvertebrates and fish, but the date and time of day measurements were taken varied, which may affect the data quality and consistency. So these informaton can be useful to be considered when we analyze and compare the sites.

2.3 Data Analysis

Data were analyzed using the Microsoft Excel and map was generated in QGIS 2.18.4. A quantitative description of taxa groups in different sampling site were presented in the graph, tables, and figures. The pictorial images are also presented so as to guide the beginner in identifying different taxa groups.

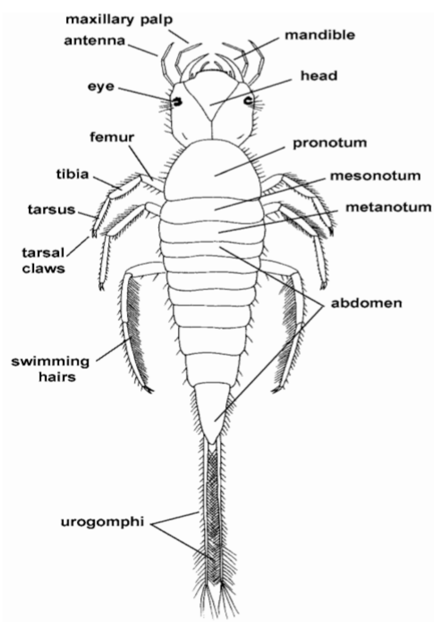
Chapter 3: Results; morphological characteristics of major taxonomic groups of aquatic macroinvertebrates and findings from different sites

3.1 Coleoptera

Aquatic beetles are found in almost all aquatic systems but have rich diversity in the lentic habitat like wetland and ponds. Some species are aquatic throughout their metamorphic stages from larvae to adult while in some cases the larva is developed in aquatic systems and emerge into terrestrial when they become adult. The aquatic beetle larva has a sclerotized head and three pairs of segmented thoracic legs (pronotum, mesonotum, and metanotum) and abdomen but has no wing pads.

Coleoptera has very similar features to aquatic insects but are distinguished by the shape of the body almost conical from the pronotum to the end of the abdomen. The legs have 4 segments; femur, tibia, tarsus, and tarsal claws usually covered by the hairs that help in swimming.

Dorsal view of Scirtidae



Source: Bouchard (2012). Guide to Aquatic Invertebrate Families of Mongolia.

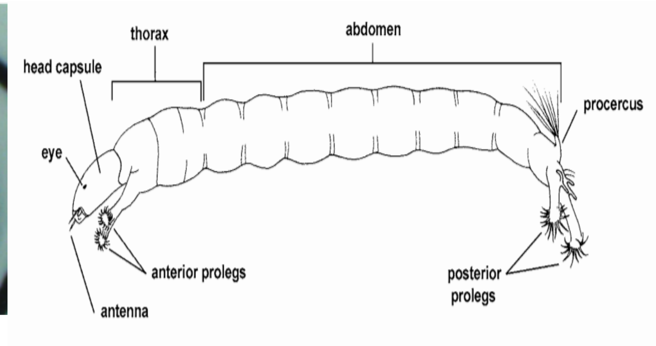
Figure 2. Morphological view of Coleoptera

3.2 Diptera

Diptera is commonly referred to as "true flies". They are the most diverse taxa in many freshwater habitats. Some are highly tolerant of warmer, less oxygenated habitat, more nutrient-rich habitats and can dominate the biomass in more polluted water bodies. Most species of Diptera are worm-like and have a head capsule that is sometimes reduced compared with their body. The identifying characteristics of Diptera is that they have no thoracic segmented legs or wing-pads during the larval stage.

A diagnostic characteristic of Diptera is that they have pro-legs at both the anterior and posterior part of the insects.

Lateral view of Chironomidae



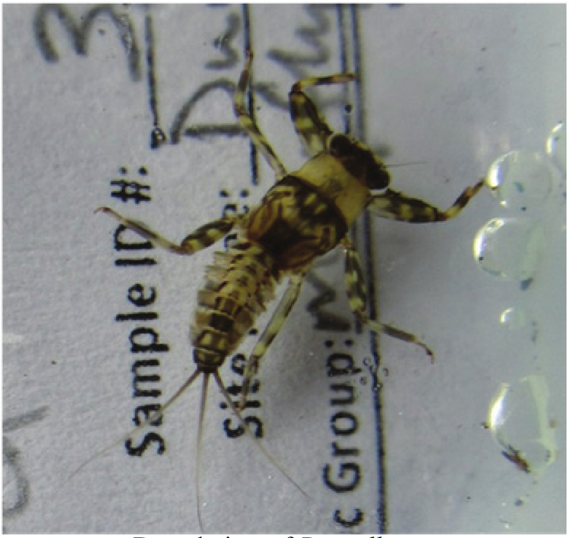
Source: Bouchard (2012) Guide to Aquatic Invertebrate Families of Mongolia.

Figure 3. Morphological view of Diptera

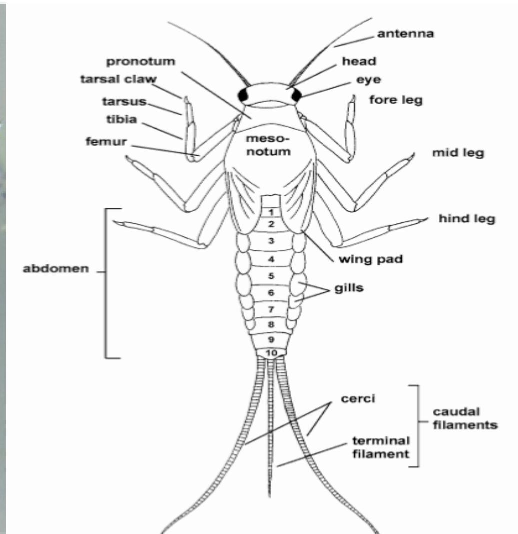
3.3 Ephemeroptera

Mayflies occur in different habitat types in the streams and rivers. Their diversity varies across stream order and altitude. Their eggs are laid at the water surface while a few exceptional species of *Baetis* crawl under the water and lay the eggs on the substrate (Edmunds & Waltz, 1996). Embryonic development takes a few weeks and in few cases, eggs may remain dormant over 11 months. The metamorphosis of mayflies includes a number of instars and the larval life varies from three to six months. Species within the families of Baetidae, Caenidae and Tricorythidae are reported to take a short period of 10-14 days while *Hexagenia limbata* may take as long as two years for development because they occur in very cold habitats. Mayflies are typically collectors or scrappers that feed on varieties of aquatic plants, algae, and detritus but a few species are carnivores, feeding on animal materials.

Mayflies generally have a head, thorax, and abdomen. The head has the eyes positioned laterally or dorsally and antennae are raised from the anterior or ventral part of the eyes. Wings originate from the thorax. In addition, each thorax bears a pair of legs (prothorax, mesothorax, metathorax). Almost all Ephemeroptera have abdomens with 10 segments, some of which bear gills. The gills can be lateral, ventral or dorsal that mostly occurs from one to seven abdominal segments (Edmunds & Waltz, 1996).



Dorsal view of *Drunella* sp.



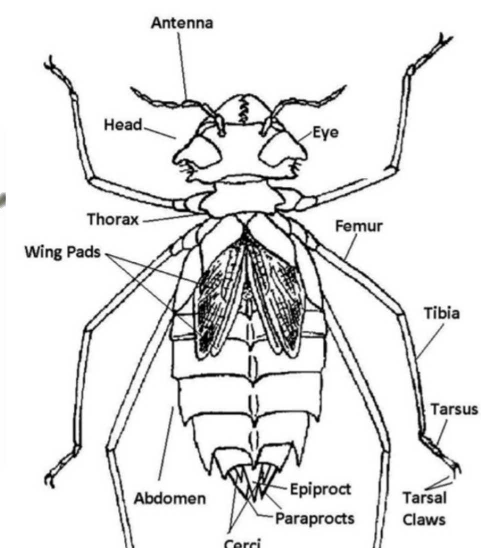
Source: Bouchard (2012). Guide to Aquatic Invertebrate Families of Mongolia.

Figure 4. Morphological view of Ephemeroptera

3.4 Odonata

Odonata has a head, three segments of thorax, and abdomens. Odonate heads have unique structures called labium folded and turned backward. Their abdomens are composed of a prothorax that is moveable in adults and the mesothorax and metathorax that are fused to the synthorax. The legs are in the position to permit walking. The larval abdomens of Odonata are usually shorter and stouter than the adult.

Dorsal view of *Epiophlebia laidlawi*



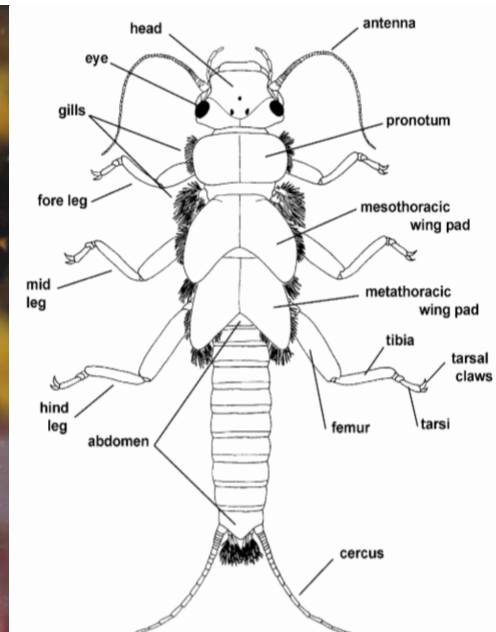
Source: Nesemann, Shah & D. Shah (2011).

Figure 5. Morphological view of Odonata

3.5 Plecoptera

Plecoptera is very sensitive to water quality and are associated with clean, well-oxygenated flowing streams and rivers. The habitats of the stoneflies are cobbles, debris, gravels and leaf packs. Stoneflies can be either shedders or predators. The nymphs in the aquatic system have a distinct head, thorax, and abdomen. Heads contain long sensory antennae, compound eyes, and mouth parts. The thorax is composed of prothorax, mesothorax and metathorax, each bearing a pair of legs. Legs have five segments; coxa, trochanter, femur, tibia, and tarsus. The dorsal surface of the meso and metathoracic segments bear wing pads in the mature nymphs. The abdomen is composed of 10 segments and at the end of the last segment, there are two long cerci.

Dorsal view of Perlidae



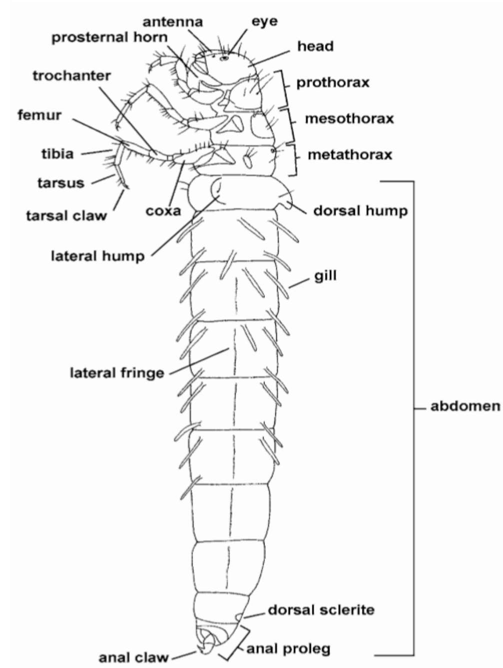
Source: Bouchard (2012). Guide to Aquatic Invertebrate Families of Mongolia.

Figure 6. Morphological view of Plecoptera

3.6 Trichoptera

Trichoptera is very close to the caterpillar like in appearance. The larvae of the trichoptera are usually observed, divided dorsally in Y shape and can be identified by their short antennae and sclerotized head. The larvae have hard plate like structure on top of the first second and third thoracic segments. But in some larva have the plates absent either on the mesothorex and metathorax. The three pairs of legs originating from the thorax have different lengths; some have short forelegs and long hind legs and others will have almost same length. The end of abdomen bears pairs of prolegs bearing the hooks/claws. The most interesting diagnostic character of the caddisfly larvae have the ability to spin the silk for retreat, net for collecting the food, for anchoring to the substrate and making the cocoon for development of pupa. Almost all the caddis flies live in the case with the exception of Rhyacophilidae (free living caddisflies).

Lateral view of Hydropsychidae



Source: Bouchard (2012). Guide to Aquatic Invertebrate Families of Mongolia.

Figure 7. Morphological view of Trichoptera

3.7 Sampling sites

Sampling sites are selected representative from each *Gewog*; Chhoekor, Chhumey, Tang and Ura. Sampling sites are abbreviated with sites code (Table 1) which are used and presentated in respect to the site-specific description in the following.

Table 1. Study sites in different Gewogs

Gewog name	Site name	Site code	Easting	Northing	Altitude (m)
Tang	Khekharte	TK-1	27.5707083	90.86244	2709
Tang	Gengaeergangchhu	TT-2	27.5613778	90.85666	2621
Ura	Shingkhar	SC-3	27.4965306	90.97360	3491
Chhumey	Uruk	URC-5	27.5102639	90.65873	2903
Chhoekhor	Dhur	DC-4	27.6081111	90.65393	2795
Chhumey	Chummey	CC-6	27.4983472	90.77798	2631

3.7.1 Khekhartechhu

Khekhartechhu is locally referring to white water. The sampling site is located just above the Tang Beat forest office. The small permanent spring-fed stream is a 1st order stream, flows through the V-shaped valley and a channel was observed almost sinuate. The catchment is covered by blue pine forest and altitude measured 2709 masl (meter above sea level). The nearest village from the sampling site is Jamjong under Tang *Gewog*. Only macroinvertebrates were assessed as the stream support no fishes.

Plate 1. Sampling site TK-1

Five samples were collected from the sampling reach. Riffle habitat dominated the stream reach thus, entire samples were collected from the riffle habitats. About 580 individual macroinvertebrates were examined. Individuals in the order Ephemeroptera dominated our samples, followed by Diptera Trichoptera, Plecoptera, Oligochaeta, Odonata, and Tricladida (Figure 8).

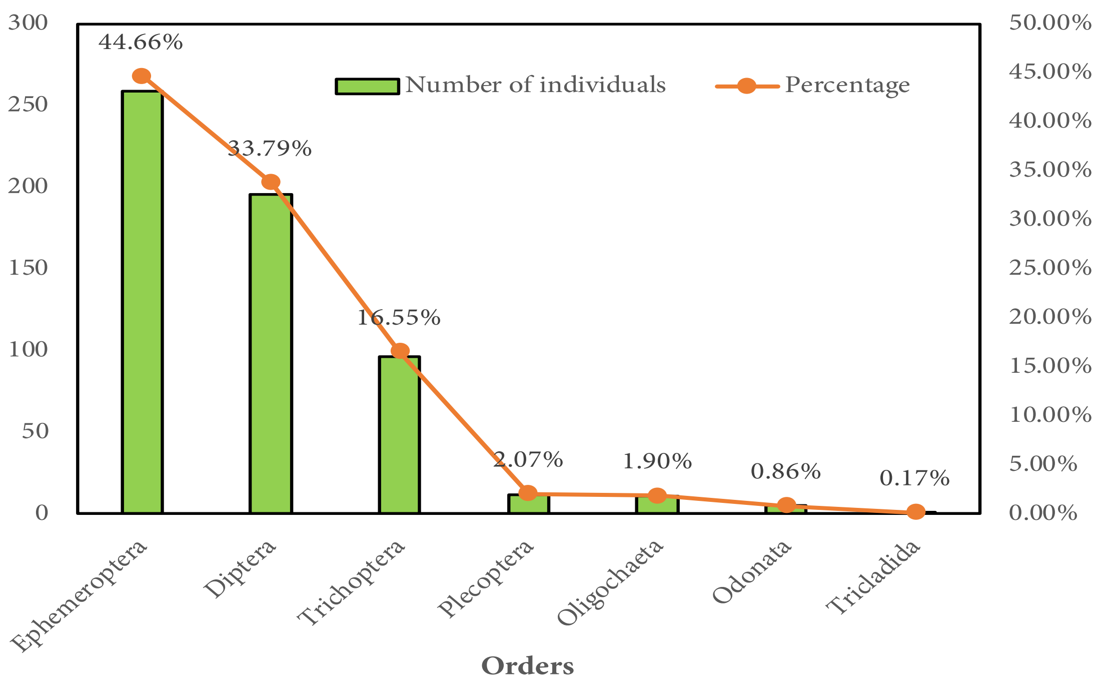


Figure 8. Number of individuals in different orders and percent composition (TK-1)

Table 2. Number of families and morphotypes in different orders (TK-1)

Order	Number of families	Number of morphotypes
Diptera	5	7
Ephemeroptera	5	7
Odonata	3	3
Oligochaeta	1	1
Plecoptera	2	3
Trichoptera	5	6
Coleoptera	1	1
Tricladida	1	1
Total	**23**	**29**

Amongst eight orders and 23 families, we detected the greatest diversity of morphotypes in Ephemeroptera and Diptera followed by Trichoptera, while only a single morphotype was observed in case of Coleoptera, Oligochaeta and Tricladida (Figure 9).

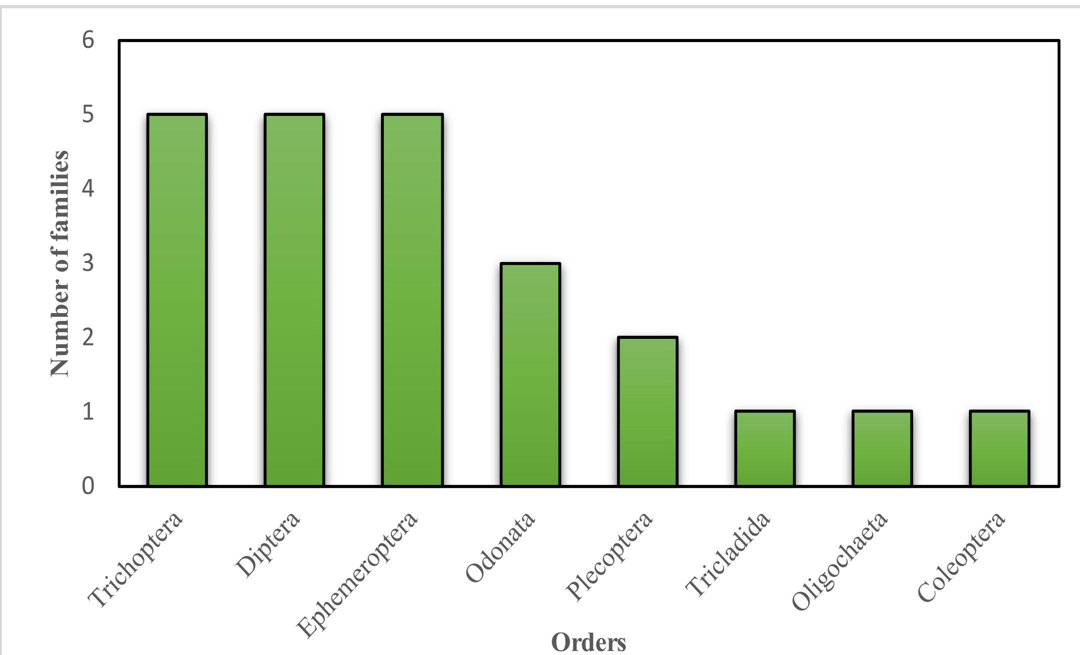


Figure 9. Number of families under different orders (TK-1)

3.7.2 Gengaeergangchhu

Gengaeergangchu is away from the Beat office but opposite to the site TK-1. The catchment of the stream is covered by the conifer forest. This permanent spring-fed stream is a tributary of Tangchhu which joins to the Chamkharchhu. The stream flows through a canyon and forms a sinuate channel. Both macroinvertebrate and fish were assessed at the sampling site and for fish assessment, a part of Tangchhu was also assessed. The site was situated at an altitude of 2621 masl.

Plate 2. Sampling site TT-2

Three samples were assessed from two different habitats, cascades and riffles. A total of 462 individuals were collected. Most of the morphotypes belongs to Ephemeroptera, followed by Diptera, Trichoptera, Plecoptera, Oligochaeta, Tabullaria, and Coleoptera respectively (Figure 10).

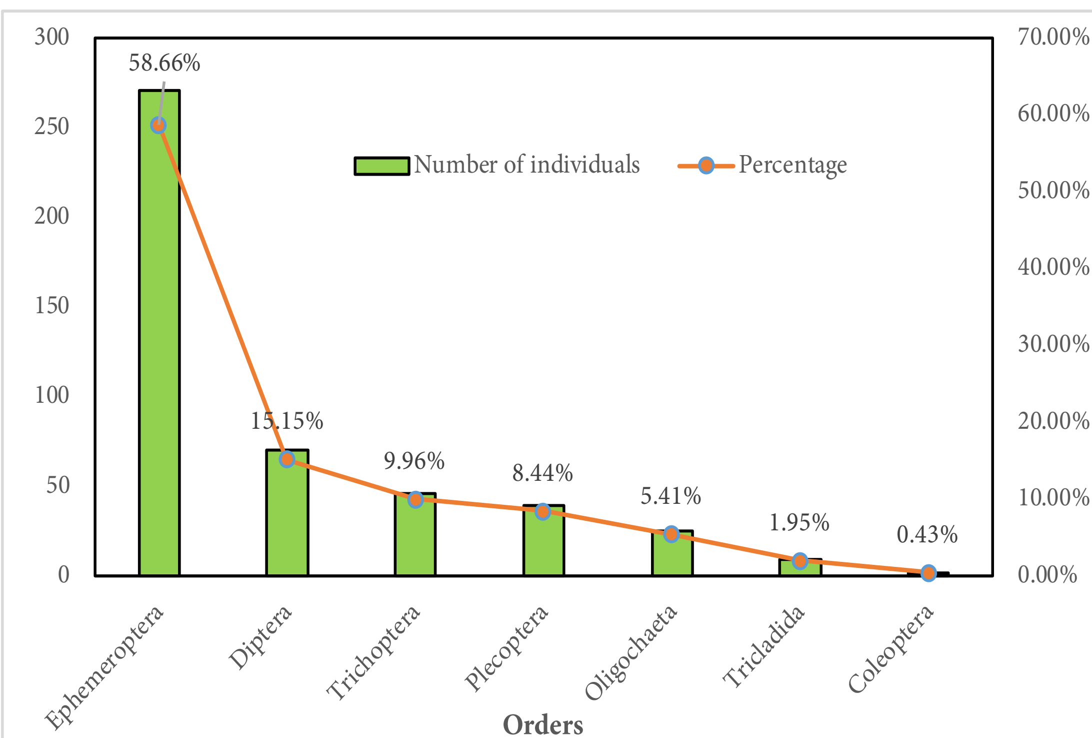


Figure 10. Number of individuals under different orders and percent composition (TT-2)

At this site, we detected 44 morphotypes from 20 families and seven orders. The order Ephemeroptera and Trichoptera had the highest diversity compared to other orders, while we detected only one morphotype under Coleoptera, Tricladida, and Oligochaeta.

Table 3. Number of families and morphotypes in different orders (TT-2)

Order	Number of families	Number of morphotypes
Coleoptera	1	1
Diptera	5	5
Ephemeroptera	3	12
Oligochaeta	1	1
Plecoptera	4	8
Trichoptera	5	16
Tricladida	1	1
Total	**20**	**44**

We used the cast net (14 mm mesh size) for sampling the fish. Seine and dip nets were ineffective due to the stream morphology and depth. Within a distance of 800 to 1000 m, approximately 50 cast nets were thrown, and 43 brown trout individuals were captured. The total length of fish ranged from 230 mm (largest) to 40 mm (smallest). The average length of fish was 152 mm. We captured brown trout in fast flowing water and did not encounter any other fish species.

3.7.3 Shingkharchhu

Plate 3. Sampling site SC-3

Shingkharchu is located slightly more than two kilometers away from Shingkhar village. The catchment of the stream is covered by mixed conifer forest dominated by fir and rhododendron. The site falls within the community forest of Shingkhar community under Thrimshingla National Park where trees are harvested for construction and firewood. This site is accessible by a forest road. This small permanent stream flows through the valley and the form of stream is slightly braided. The stream is also partly snow-fed in winter as it is located at an altitude of 3491 masl. The sampling site at Shingkhar had one of the higher number of macroinvertebrates sampled. A total of 1248 individuals were enumerated from 5 replicates. Once again, we found that dominant individuals were under the order Ephemeroptera, followed by Trichoptera, Plecoptera, and Tricladida. Only one individual Hydracarina (aquatic mite) was encountered from the stream (Figure 11).

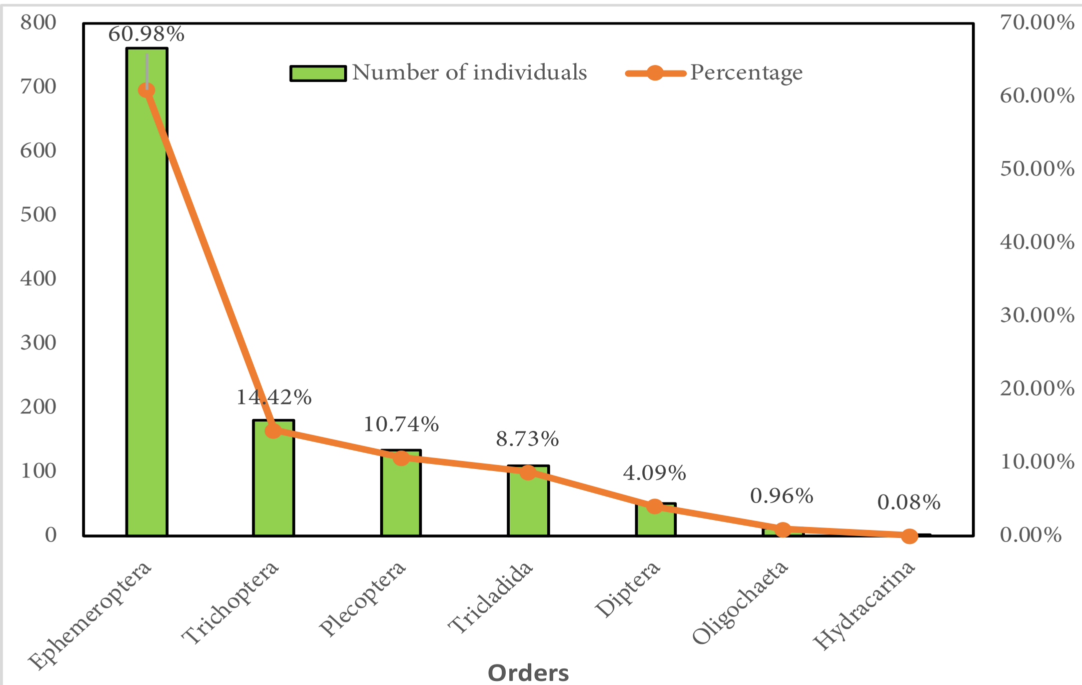


Figure 11. Number of individual under different orders and percent composition (SC-3)

In addition to having one of the highest number of individuals, the largest number of families and morphotypes were in the order Trichoptera, whilst only one morphotype was detected in each of the orders Hyracerina, Tricladida and Oligochaeta (Table 4).

Table 4. Number of families and morphotypes in different orders (SC-3)

Order	Number of families	Number of morphotypes
Diptera	6	9
Ephemeroptera	5	8
Hydracarina	1	1
Oligochaeta	1	1
Plecoptera	4	8
Trichoptera	9	16
Tricladida	1	1
Total	**27**	**44**

Macroinvertebrate inhabit in different habitats like riffle, pool, run and cascades. Almost all of the order prefers to occupy the best habitat where they can avail adequate food and oxygen. The figure 12 shows, amongst the different habitats, macroinvertebrates prefer cascade habitat. Next to cascade they prefer to live in riffle and run. However very few macroinvertebrates were found in pool, as the pool possess very less oxygen.

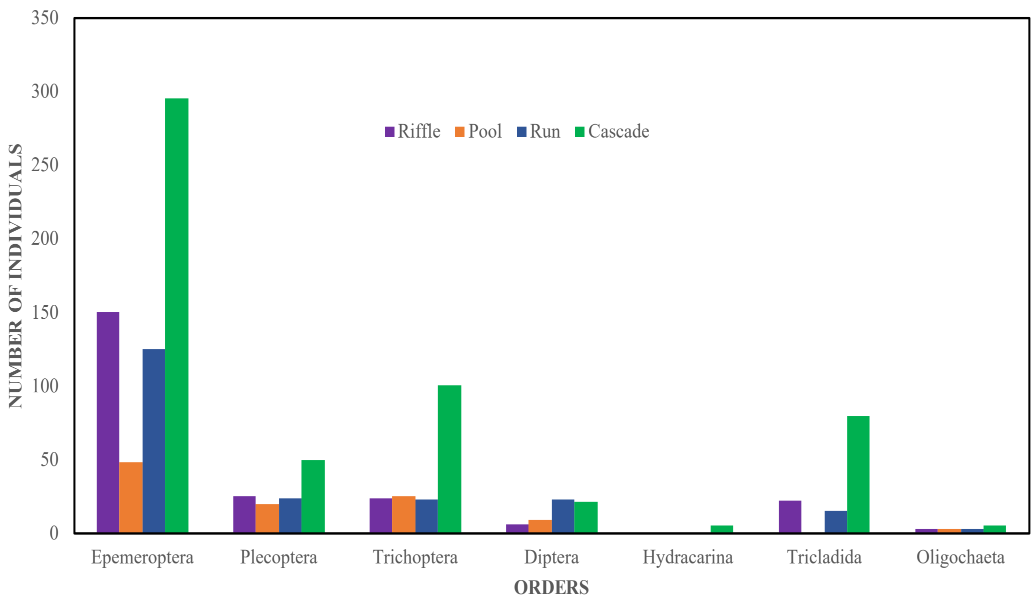


Figure 12. Number of individuals in different habitats under different orders (SC-3)

3.7.4 Dhurchhu

Plate 4. Sampling site DC-4

Dhurchu is a tributary of Chamkharchhu, and could be the 3rd order stream. The catchment of the tributary is mostly covered by the mixed conifer forest. The forest is harvested and has the depot near the sampling site. The permanent stream flows through the trough valley and is also glacier fed stream. The nearest village to the stream is Dhur. The channel form just below the sampling site was meandering. The altitude of the site was 2692 m. We sampled both macroinvertebrates and fish at the site.

A total of 521 individual macroinvertebrates from eight different orders were enumerated. Increasing sampling effort (six replicates) seem to come across, increasing richness of species (Table 5). The highest morphotypes measured may have attributed to sampling effort, other than characteristic of the river system (size, large rocks and bedrock substrate, high flow, or fish present) or the timing of sampling. For example during the sampling time period, the stream was at a relatively high flow. In addition, given the ease and number of brown trout collected in cast nets, it is apparent that the stream supports lots of brown trout indicating the richness of the site.

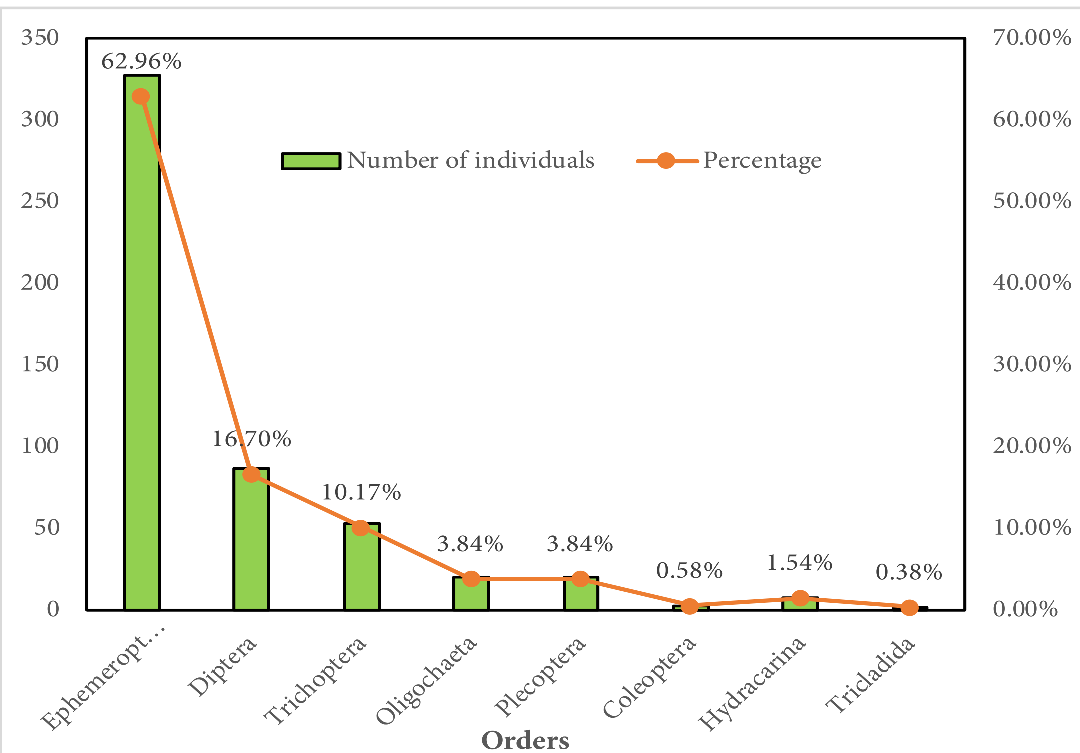


Figure 13. Number of individual under different orders and percent composition (DC-4)

Table 5. Number of families and morphotypes in different orders (DC-4)

Order	Number of families	Number of morphotypes
Coleoptera	2	2
Diptera	8	12
Ephemeroptera	3	13
Hydracarina	1	1
Oligochaeta	1	1
Plecoptera	3	5
Trichoptera	8	11
Tricladida	1	1
Total	**27**	**46**

We collected and enumerated 46 morphotypes from 27 families, from eight different orders. The order Ephemeroptera had the highest number of morphotypes followed by Diptera and Trichoptera. The minor orders like aquatic earthworm, planarian, and aquatic mite were represented by a single morphotype (Figure 14).

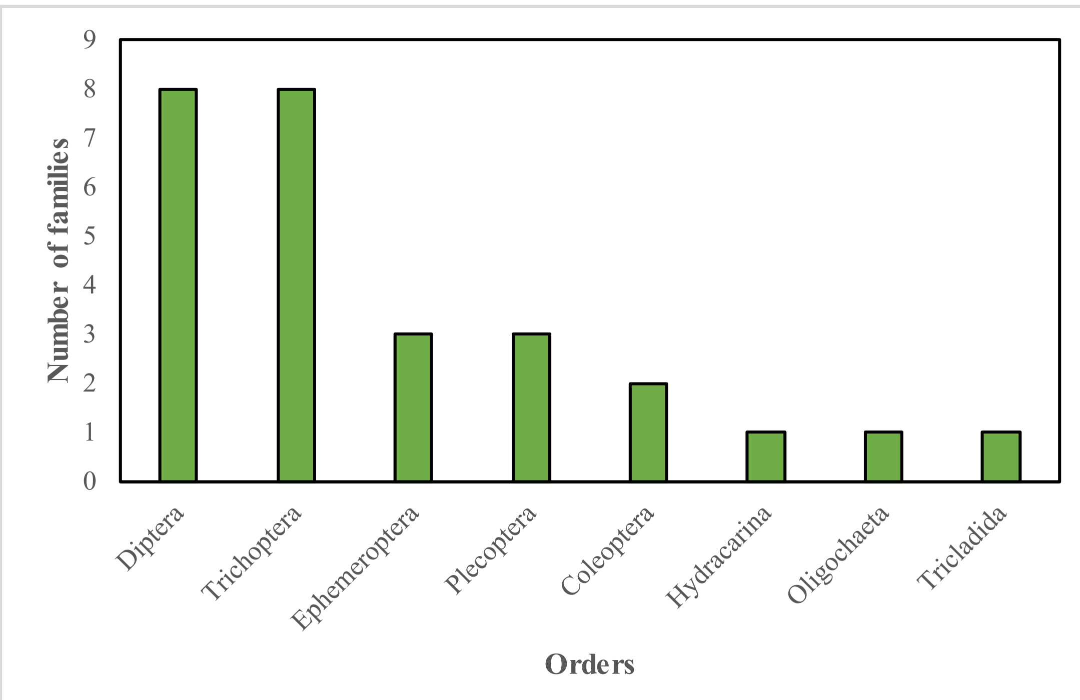


Figure 14. Number of families under different orders (DC-4)

To capture fish, we cast netted and initiated a stream diversion. The cast netter work a reach of approximately 500m, performed over 100 casts in fast flowing and shallow water, and caught 57 brown trout. Their total length ranged from 271 mm to 95 mm. The average length of the fish was measured 205 mm.

3.7.5 Urukchhu

Plate 5. Sampling site URC-5

Urukchhu is one of the tributary of Chhumeychhu, which then joins to the river, Chamkharchhu. It is located below the road towards Tharpaling approximately a kilometer away from the highway. The nearest village is Uruk and the valley is along the downstream, utilized for cultivation and pasture. The catchment of the stream is covered by mixed conifer forest and catchment is utilized for extraction of timber and grazing land for cattle.

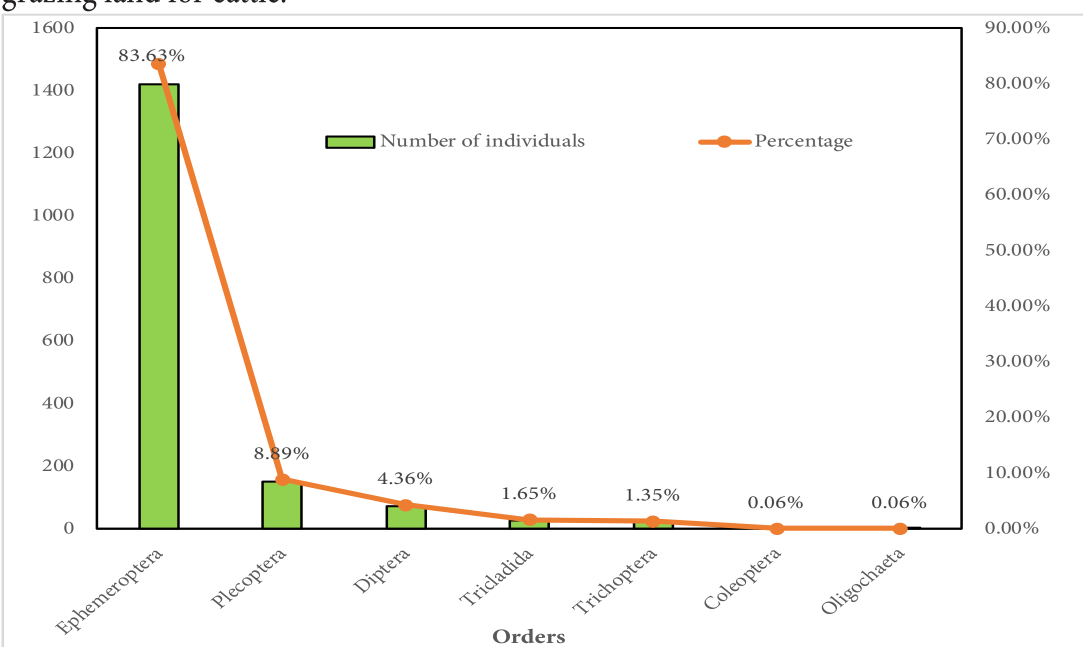


Figure 15. Number of individuals in different orders and percent composition (URC-5)

The permanent spring fed stream flows through to flood plains and the stream channel was not only wide but also meandering with braiding form. We performed macroinvertebrate sampling at this site. Urukchhu has one of the highest population densities comparing to other sampling sites. Amongst the seven orders of macroinvertebrates, Ephemeroptera had the highest abundance of individuals while Coleoptera and Oligochaeta had the lowest (Figure 15). Haptageniidae dominated over other families where we enumerated over 1000 individuals from the site.

Table 6. Number of families and morphotypes in different orders (URC-5)

Order	Number of families	Number of morphotypes
Coleoptera	1	1
Diptera	3	4
Ephemeroptera	3	7
Odonata	1	1
Oligochaeta	1	1
Plecoptera	3	3
Trichoptera	8	10
Tricladida	1	1
Total	**21**	**28**

At URC-5 sampling site, there were 28 morphotypes, across 21 families, within seven different orders. Unlike other sampling sites, Trichoptera had the highest diversity of families, followed by Ephemeroptera. Orders such as Tricladida, Oligochaeta and Coleopteran were represented by one morphotype (Figure 16).

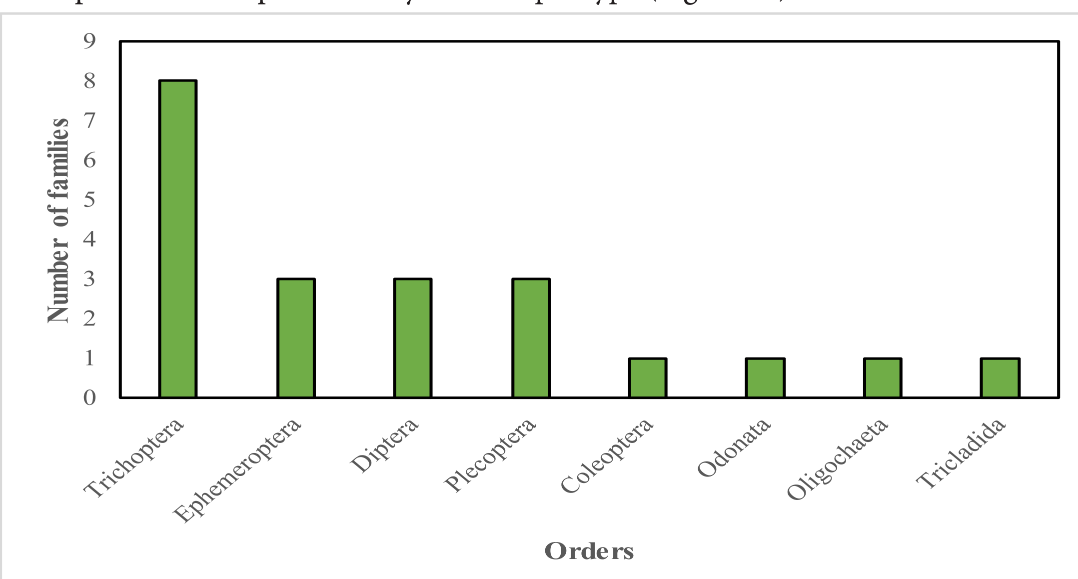


Figure 16. Number of morphotypes in different orders (URC-5)

3.7.6 Chhumeychhu

Plate 6. Sampling site CC-6

Chummechu is a tributary of Chamkharchuu and could be a 3rd or 4th order stream. The catchment of the stream is covered by conifer forests. The valley is utilized for cropland and pasture for cattle. The nearest village from the sampling site is Nangar, under Chummey gewog. The stream is spring fed and flows through the valley and a sampling reach of the river is naturally constrained. A micro-hydropower plant is upstream of the sampling site, but very little water is impounded associated with this plant. In this site, only fish assessment was carried out. The altitude of the site was 2640 m. Over 71 cast nets were thrown in fast flowing and shallow habitats across a 750 m section. In addition, we kick netted over 50 m in the same stream section. We did not catch a fish in the kick net, but 47 brown trout were caught in the cast nets. The largest fish measured 285 mm while the smallest measured 145 mm and average length of fish was recorded 183 mm. No native fish was encountered during our assessment though the habitat at the site seem to be favorable for snow trout. Since brown trout is non native and invasive in most of the river system in the country, we have not presented detail analysis in this report.

Plate 7. Brown trout captured in the cast net at CC-6

3.8 Synopsis of results

This study recorded 96 morphotypes and 43 families from 9 orders. Trichoptera has the highest diversity of families and morphotypes, then Ephemeroptera was next (Figure 17; Table 7). Orders such as Hydracarina, Oligochaeta and Tricladida were typically represented by one morphotype. It is very likely more orders and families are present in the study area. Even for these sites, this is likely an underestimate. Taking voucher samples from every site for microscopic evaluation is critical to ensuring a more comprehensive diversity survey. In addition, more complete sampling would increase our understanding of the aquatic diversity in the study area.

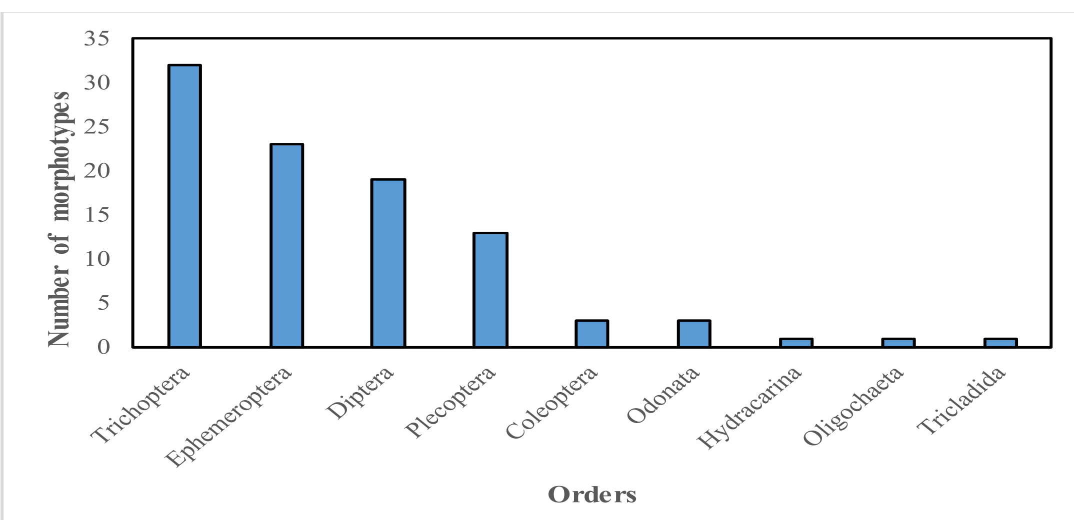


Figure 17. Morphotype richness under different orders

Table 7. Total number of families and morphotypes under different orders

Order	Number of families	Number of morphotypes
Coleoptera	3	3
Diptera	10	19
Ephemeroptera	6	23
Hydracarina	1	1
Odonata	3	3
Oligochaeta	1	1
Plecoptera	5	13
Trichoptera	13	32
Tricladida	1	1
Total	**43**	**96**

3.8.1 Diversity of macroinvertebrates at different sampling sites

Five samples were taken in proportion to habitat types to represent the diversity that is present, except at sites TK-1 (3 samples) and URC-5 (4 samples). The number of orders, families, and morphotypes detected was fairly similar across all sites. Fewer families and morphotypes were identified at TK-1 and URC-5 (figure 18) than at other sites. These sites had fewer replicates but are also impacted by human activities.

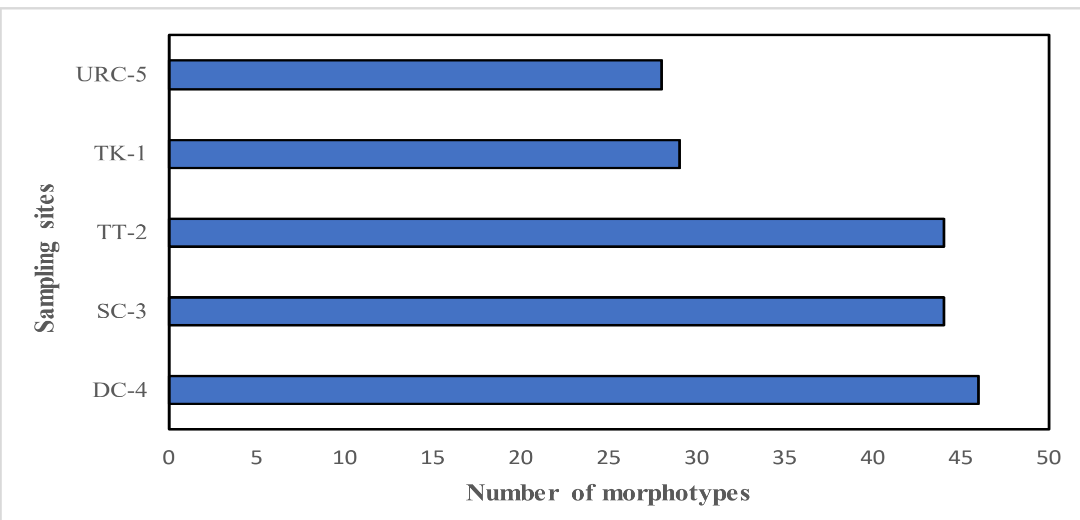


Figure 18. Number of morphotypes in different sampling sites

Given the area is undisturbed, diversity and density of macroinvertebrates depend on the type of substrates in addition to habitat type (pool, riffle, run). In terms of both diversity and density of species, fewer macroinvertebrates were collected in samples from the sandy substrate with slow water velocity. We found much higher diversity and abundance of macroinvertebrates in the run, riffle and cascade habitats that are characterized by higher water velocity and larger substrate (such as gravel, cobble, and stone substrate).

The highest diversity was found at site DC-4 while the lowest was found at URC-5 (Figure 19). Stream sampling sites that had a higher diversity of habitat types are also associated with a higher diversity of macroinvertebrates. At site TK-1, entire samples were measured from riffle but at TT-2, SC-3, and DC-4 had habitat such as riffle, pool, run, and cascade. Thus for a complete diversity survey, representative sampling covering diffrent habitat types within multiple stream sections (likely with different habitat represented because of differences in stream gradient) is necessary to accout the biodiversity.

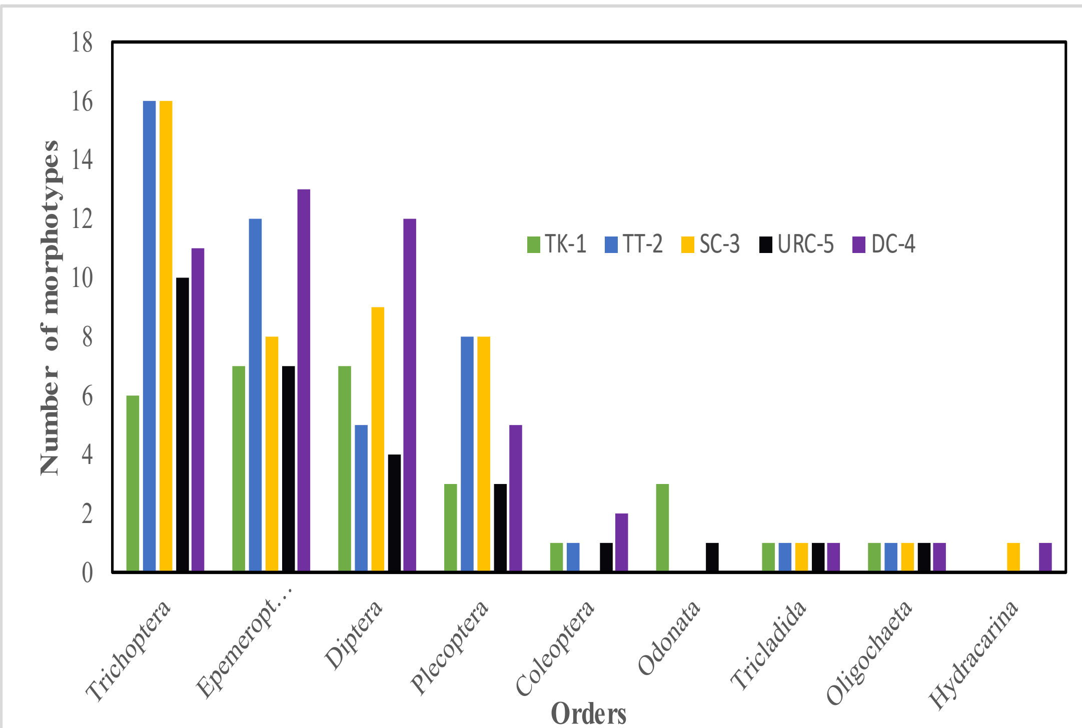


Figure 19. Morphotype richness under various orders at diffrent sites

Chapter 4: Recommendation and conclusion

4.1 Recommendation

- Voucher collection from the field and preservation in the laboratory for identification is an indispensable job as it requires extra care and patience for the quality result. In order to correctly identify, voucher specimens of each species should be collected in separate bottles. This is because most of the live color of the voucher in our collection had changed in the ethanol/preservatives, which makes the identification difficult.

- In this study, samples were only collected from a few locations and it does not represent the whole Bumthang area. Moreover, the samples were collected during pre-monsoon which lacks the diversity of information on other seasons such as post monsoon. To obtain a comprehensive measure of aquatic biodiversity, we recommend sampling both in post and pre-monsoon seasons within a year and broadening the coverage to all habitat types across a broader to obtain a better representative sample for Bumthang.

- Standardize the number of replicate samples from all sites in order to ease the analysis and compare the similarities/differences of diversity, the higher sampling effort, greater diversity is expected. So, samples are critical to getting a reliable measure of diversity. In addition, species diversity is positively related to habitat diversity across most systems, so ensuring coverage of all habitats is important in studying the biodiversity.

- Even though Schizothorax rechardsonii has been reported in this area in the past, but we did not encounter any fish from that group. Throughout the entire sampling effort, we only captured Brown Trout (*Salmo trutta*). We suggest diversifying the extensive and intensive survey in order to confirm the absence or presence of native fish in this drainage, as it is assumed that brown trout have taken over this area.

4.2 Conclusion

This report is one of the stepping stone towards exploring the freshwater biodiversity particularly the invertebrates. As sampling continues, broadly across time (season) and space (more ecoregions, watersheds, and habitats), more species will be discovered. Increasing sampling effort and study area across the country, hundreds of additional species will be encountered. To fully describe and map the aquatic invertebrate biodiversity, more effort is necessary (time, resources, experts) to complete an assessment across all different seasons, across all types of freshwater habitats (including ponds, lakes, and wetlands) and across the entire Bumthang Dzongkhag. This study demonstrated the feasibility of this type of work and piloted a useful protocol in order to troubleshoot problems and make improvements.

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Annexure I. Sampling protocol for diversity of stream macroinvertebrates

Macroinvertebrates have varied life spans (two weeks to 4 to 5 years) and aquatic insects go through metamorphosis of different times during the year. In North America, early autumn is the most important time to collect because population are most stable (fewer adults are emerging), insects are larger and more readily identifiable, and it is after the potential stressful summer months (low flow and high temperature). If the study can afford two collection times, spring is the second most useful time to collect, after snowmelt and before leaves are fully out. In the Himalayan region, these two sampling periods correspond to pre-monsoon and post-monsoon. The seasonal effect of the monsoon climate on the abundance of the benthic macroinvertebrates in mountain stream in Nepal found no change in abundance pre-monsoon versus post-monsoon season (Brewin et.al 2000). Similarly, Korte et al. (2010) found different species but few differences in assessment metrics pre-monsoon versus post-monsoon for the Hindu Kush-Himalayan region. Thus, any time during the dry season appears appropriate and acceptable.

Macroinvertebrates inhabit all habitat types in streams and rivers, including riffles, runs, pools and cascades. The most diverse assemblage are often in rifles, where cobbles are predominant but with dome gravels and boulders. While riffles typically support the most diverse community, some species prefer other habitats, such as clumps of leaves, plants and woody debris, silty bottoms, and large bounders. Thus, to examine the entire macroinvertebrate diversity, you would want to sample all available habitats. Depending on the question of interest (biodiversity, monitoring over time, or impact assessment) you may choose to take replicate samples in riffles only where you may expect to detect differences in community composition overtime or you may choose to sample all habitats representative of the area. This decision of where to sample (single habitat such as riffles or all habitat types) should be made by the study designer and standardized across all data collectors for each study.

Picking your site

1. There should be no major tributaries discharging to the stream in the study area and the site should be at least 100m from a road crossing to minimize impact of the crossing on the stream habitat conditions. Also, sampling should be avoided during or shortly after floods or drying of the stream reach.
2. Measure a study area reach 10 x the width of the stream, up to 100m in length.

3. Determine the proportion of habitat (pools, riffles, runs, cascades) in the reach and record on the data sheet. Ideally, the sampling area should not be disturbed until sampling begins.
4. Write down the types of sample used and mesh size of the sampler. Typically standardized samplers are used as a Hess sampler, D-net, kick-net, or surber sampler. Any net (at least 25cm by 25cm) with a 500μm mesh is very functional.
5. Sample multiple habitat in a way that is reflective of the proportion of microhabitats present within the sampling reach. All habitat types that compose at least 5-10% of the habitat should be sampled.

6. Collect samples systematically from all available habitats by kicking the substrates or jabbing the D-frame net. Often 20 samples are taken across major habitat types in the reach proportion to their occurrence in the reach.
7. After sampling, the coverage of habitat types and percentages should be reviewed for completeness.

Sampling riffles and runs

1. Collect your samples at the upstream end, just below a pool as this is the area that is typically most productive.
2. Approach the riffle from downstream and place the net at the downstream edge you wish to sample. Place the net perpendicular to flow and lay the bottom of the net firmly on the stream bed (if using kick net, tilt it back at downstream angle but not so water flows over the top and anchor the bottom to prevent invertebrates from escaping).
3. Pick up rocks and debris (over 5mm in diameter) from the same area, hold them in front of net and gently rub the organism from the rock into the net, then place these "cleaned" items outside the sampling area.
4. After rocks and debris have been rubbed step inside the sampling area, start at the upstream end and working towards the net disturb the sediment by kicking. Do this until the stream bottom is thoroughly disturbed digging down as far as possible. Typically, 5-10cm for fine sediments, 10-15cm for intermediate sized substrate, and 15-20cm for larger substrate. Remove the net with forward scooping motion and place the content into a wide-mouthed dish pan.
5. Be sure the entire sample makes it into the pan.

6. Depending on the number of invertebrate in the sample, you may be able to subsample. It is important to have a sample consisting of >100 individuals, so only very large samples should be subsampled.
7. To subsample, put the sample in dish pan or on a sieve (mesh frame). Divide it into four even sections and put 1 or 2 sections into bucket or pans for picking and identification. Remember, the goal is to count >100 individuals per sample. Indicate how the sample was split on the data sheet (e.g., ½ or ¼ so that you can adjust the densities and sampling effort as u process the data). If you do not have >100 individuals in the sample, then do not continue to subsample the remaining replicates.
8. Examine the body morphology of the invertebrates separate them into easily recognizable groups using forceps or pipettes. Ice cube trays or egg cartons work well for separating invertebrate into groups. Most individuals can be identified to class or order using simple invertebrate keys.
9. Identify organisms to the finest taxonomic level possible, then count and record the numbers present. For each morphotype, photograph up to 3 individuals for the records, being sure to link the picture with the date, site, and sample #. Return the animals to the stream.
10. Examine the body morphology of the invertebrates separate them into easily recognizable groups using forceps or pipettes. Ice cube trays or egg cartons work well for separating invertebrate into groups. Most individuals can be identified to class or order using simple invertebrate keys.

9. Identify organisms to the finest taxonomic level possible, then count and record the numbers present. For each morphotype, photograph up to 3 individuals for the records, being sure to link the picture with the date, site, and sample #. Return the animals to the stream.

If additional habitat types are sampled, take a similar approach using a net and coming into the site from the downstream direction. Sample the habitat as described below and then complete steps (e) and (i) above.

Sampling pools

a. Areas can be sampled similar to riffles by dislodging fine sediments or if the sediment is very soft, the net can be bumped along the surface to dislodge sediment and organisms which are then swept into the net.

Sampling leaves and debris

a. Woody debris: wash the samples into the net or pick up wood and pick off larger animals then spray into net.
b. Roots: initial sweep with net, then vigorously shake into net.
c. Leaves: Wash and wipe leaves into net try to avoid large amount of leaves in net.
d. Plants: all parts of the macrophytes (i.e., roots, stems, and leaves) are removed and shaken into the net so that the animals float into the net. The plant must be rinsed and thoroughly examined as many of the organism on these plants cling to the plants.

Sampling boulders and bed rocks

a Certainly lifting is not possible, brush or scrape the surface of the rock and sweep the animals into the net.

Completion of the sampling

Once the invertebrate sampling has been completed the habitat types can be reviewed and additional in streams habitat measures taken. Data sheets, sample labels and notes regarding pictures must be completed properly. After sampling has been completed at a site, all nets, pans, and equipment should be rinsed thoroughly, examined and free of organism or debris. Equipment should also be either dipped in alcohol or dried for several hours between to avoid transfer of organisms.

Note

Once organism are identified streamside they may be recorded, photographed, and released. Even though identification to family is also part of the diversity story, Korte et al. (2010) found that identification to the family level was useful in the Hind-Kush Himalayan assessment metrics. To identify macroinvertebrates to species, sorted samples will have to be preserved and returned to the laboratory for identification under a dissecting scope. Samples that are going to the lab should be placed in a jar, preserved in a 70% ethanol solution and labeled with the date, site, collector, and sample number. If a complete biodiversity assessment is important, it may be necessary to preserve some entire samples and sort them under a dissecting scope in the lab as small species may only be detected at the microscope level (such as midge larvae, and microcaddis)

Annexure II. Data sheet

Pre-Field site location information Site location information (GIS, maps, google earth)		Page 1
River name:	Investigator:	Instituion:
Date: Start time: End time: Sampling site code:		
District and nearest village:	Altitude (m):	Catchment area(km2):
Latitude (decimal degrees):	Longitude (decimal degrees):	

Catchment land use (round to nearest 10%):

[ ] deciduous native forest [ ] coniferous native forest [ ] mixed native forest [ ] silviculture (harvest and plantation) [ ] partial cutting [ ] clear cutting [ ] Rhododendron	[ ] non-native forest [ ] bamboo [ ] open grass and bushes [ ] alpine meadow [ ] naturally unvegetated [ ] open standing water [ ] wetland/marsh	[ ] pasture [ ] crop land [ ] villages [ ] urban sites (residential) [ ] urban sites (industrial) [ ] other *These should sum to 100%*

Hydrological characteristics for watershed:

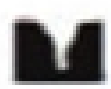
Classification [ ] periodically dry in ___ summer __ winter [ ] episodic (non-predictable?) [ ] permanent	Water source [ ] spring fed [ ] snow fed [ ] glacier fed [ ]rain fed (monsoon) Estimates of discharge?

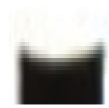
lakes/ponds/reservoirs upstream?

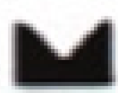
lakes/ponds/reservoirs downstream?

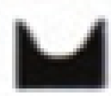
Gradient of river (~ 10km)?

Valley Shape

Canyon 

Wide 

Floodplains V- shaped 

Trough 

Other?

Channel Form

Meandering 

Constrained (natural) 

Braided 

Constrained (artificial)

Sinuate 

Sampling site information Page 2

Site name:

Investigator:

Date:

Start time:

End time:

Sample code for site:

Hydro-morphological impact at site

Dams at sampling site
_____ number
_____ height of dam (cm)

Other transverse structures (e.g., diversion barriers, bridges)
_____ number
_____ height of structures (cm)

Proportion of stream bottom (%)

Concrete/Plastering ___________
Stones________________________
Wood/Lumber_________________
Trees_________________________
Other materials________________
No bank stabilization____ ________
(natural state)
Sum of 100%

Proportion of bank (%)
Left Right
Concrete/Plastering
Stones
Wood Trees
Other materials
No bank stabilization

(natural state)
Sum of 100%

Water Uses
______ fisheries
____recreation/tourism
______ hydropower
______ drinking water
______ industrial
______ cattle watering
______ irrigation (crops)
______ other (specify)

Channelization:
_____ Yes ____ No

Straightening:
_____ Yes ____ No

Connected to floodplain:
_____ Yes ____ No

Water Abstraction:

_____ Yes ____ No

Residual flow?

Purpose of water abstraction:
____ Irrigation
____ Drinking water
____ Other

Picture #:

Signs of pollution at sampling site

Point source pollution: _
____ Yes ____ No

Non-point source pollution:
_____ Yes ____ No

Sewage Overflows:
_____ Yes ____ No

Mining:
_____ Yes ____ No

Toxic substances:
_____ Yes ____ No

Nutrient enrichment (lots algae):
_____ Yes ____ No

Visual water turbidity:
_______ Clear _______ Slightly Turbid ______ Turbid ________ Stained

General Ranking of presence of the following from visual estimation 0 = absent, 1 = rare (<5%), 2 = common (5-30%), 3 = abundant (30-70%), 4 = dominant (>70%)

Filamentous algae	0	1	2	3	4
Slime	0	1	2	3	4
Macrophytes	0	1	2	3	4
Algae	0	1	2	3	4
Moss	0	1	2	3	4
General Comments:					

Sampling site characteristics and information collected Page 3

Site name:

Investigator:

Date:

Start time:

End time:

Sample code for site:

Site type (River, stream, lake, wetland)

Stream order:

Reach length:

Gradient:

Aspect:

Shading (% canopy cover)
______ _______ ______

Reach width (m):
____ ____ ____

Reach thalweg depth (cm):
_________ _________ _________

Width of riparian vegetation (m):

# of debris dams:

# of large wood over 10 cm (diameter 3m long):

Densities shrubs/trees in riparian circle
Sparse Intermediate Dense

Visual propotion of reach habitat types
_________% Riffle _________% Run
_________% Pool _________% Cascades

Notes on riparian sites:

Notes on sites:

Types of substrate sampling (<6μm=silt/mud ; 6μm-2mm=sand ; 2mm-6cm=gravel; 6cm-20cm is stones; 20cm-40cm cobble, >40cm is boulder)
% coverage: _____ silt/mud, _____ sand, _____ gravel, _____ pebbles/stones, _____ cobble, _____ boulder

Embeddedness?

Water Chemistry?
pH: ______, ______, _____ Turbidity: ______, _______, _____ Conductivity _____, _______, ______

Temperature: ______, _______, ________ Dissolved oxygen: ______, _______, _______ mg/l

TDS: __________, ________, ________ Dissolved oxygen: ______, _______, _______ % saturation

Notes for water chemistry (sunny/cloudy; recent rain; time of sample collection)

Site sketch with location of samples

Sampling site macroinvertebrate information

Page 4

River basin:		Investigator:
Site name:		Sample code for site:
Date:	Start time:	End time:
Site type (Stream, river, wetland, pond)		Habitat type present:
Site description:		
Equipment type:	Area sampled (each replicate:	Mesh size:

For each replicate note what habitat the sample was from and if it was from split (if so how many times)

	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Sample ID # (picture)	Notes
Habitat							
Split (1/2, 1/4)							
Major groups							

Sampling site fish information

Page 5

River basin:	Investigator:
Site name:	Sample code for site:

Date:	Start time:	End time:

Habitat type present:

Site description:

Equipment type:	Sampling area/efford:	Mesh size:

**Total length, fork length, standard length*

ID	Species	Length (mm)*	Picture #	Tissue sample	Gear	habitats	Notes

Voucher label form

Sampling Code:	Sampling Code:	Sampling Code:	Sampling Code:	Sampling Code:
Sampling Site:	Sampling Site:	Sampling Site:	Sampling Site:	Sampling Site:
Latitude:	Latitude:	Latitude:	Latitude:	Latitude:
Longitude:	Longitude:	Longitude:	Longitude:	Longitude:
Altitude:	Altitude:	Altitude:	Altitude:	Altitude:
Date:	Date:	Date:	Date:	Date:
Taxa name:	Taxa name:	Taxa name:	Taxa name:	Taxa name:
Collector:	Collector:	Collector:	Collector:	Collector:
Sampling Code:	Sampling Code:	Sampling Code:	Sampling Code:	Sampling Code:
Sampling Site:	Sampling Site:	Sampling Site:	Sampling Site:	Sampling Site:
Latitude:	Latitude:	Latitude:	Latitude:	Latitude:
Longitude:	Longitude:	Longitude:	Longitude:	Longitude:
Altitude:	Altitude:	Altitude:	Altitude:	Altitude:
Date:	Date:	Date:	Date:	Date:
Taxa name:	Taxa name:	Taxa name:	Taxa name:	Taxa name:
Collector:	Collector:	Collector:	Collector:	Collector:
Sampling Code:	Sampling Code:	Sampling Code:	Sampling Code:	Sampling Code:
Sampling Site:	Sampling Site:	Sampling Site:	Sampling Site:	Sampling Site:
Latitude:	Latitude:	Latitude:	Latitude:	Latitude:
Longitude:	Longitude:	Longitude:	Longitude:	Longitude:
Altitude:	Altitude:	Altitude:	Altitude:	Altitude:
Date:	Date:	Date:	Date:	Date:
Taxa name:	Taxa name:	Taxa name:	Taxa name:	Taxa name:
Collector:	Collector:	Collector:	Collector:	Collector:
Sampling Code:	Sampling Code:	Sampling Code:	Sampling Code:	Sampling Code:
Sampling Site:	Sampling Site:	Sampling Site:	Sampling Site:	Sampling Site:
Latitude:	Latitude:	Latitude:	Latitude:	Latitude:
Longitude:	Longitude:	Longitude:	Longitude:	Longitude:
Altitude:	Altitude:	Altitude:	Altitude:	Altitude:
Date:	Date:	Date:	Date:	Date:
Taxa name:	Taxa name:	Taxa name:	Taxa name:	Taxa name:
Collector:	Collector:	Collector:	Collector:	Collector:
Sampling Code:	Sampling Code:	Sampling Code:	Sampling Code:	Sampling Code:
Sampling Site:	Sampling Site:	Sampling Site:	Sampling Site:	Sampling Site:
Latitude:	Latitude:	Latitude:	Latitude:	Latitude:
Longitude:	Longitude:	Longitude:	Longitude:	Longitude:
Altitude:	Altitude:	Altitude:	Altitude:	Altitude:
Date:	Date:	Date:	Date:	Date:
Taxa name:	Taxa name:	Taxa name:	Taxa name:	Taxa name:
Collector:	Collector:	Collector:	Collector:	Collector:

Field picture background

Sampling date: Sample ID #:
Sampling Code: Site name:
Rep #: Taxonomic Group:

Sampling date: Sample ID #:
Sampling Code: Site name:
Rep #: Taxonomic Group:

Sampling date: Sample ID #:
Sampling Code: Site name:
Rep #: Taxonomic Group:

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Sampling Code: Site name:
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Sampling date: Sample ID #:
Sampling Code: Site name:
Rep #: Taxonomic Group:

Sampling date: Sample ID #:
Sampling Code: Site name:
Rep #: Taxonomic Group:

Checklist of taxa from different sampling sites

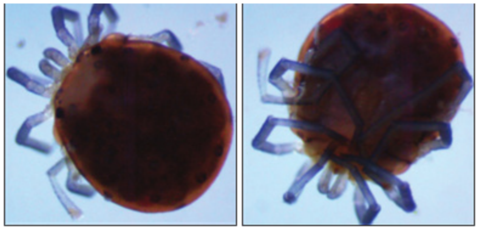
Order	Family	Genus	TK-1	TT-2	SC-3	DC-4	UR-5
Coleoptera	Scirtidae		-	*	-	*	*
	Lampyridae		-	-	-	*	-
	Hydraenidae		*	-	-	-	-
Diptera	Athericidae	Athericidae 1	*	-	*	*	*
		Atherix sp.	*	*	-	*	-
	Blephariceridae	*Horaia* sp.	-	*	-	-	-
	Ceratopogonidae	Heleinae	-	-	-	*	-
	Chironomidae	*Chironomus* sp.	-	-	-	*	*
		Chironomidae 1	*	-	-	*	-
		Chironomidae 2	*	-	*	-	-
		Chironomidae 3	-	-	-	*	-
		Tanypodinae	-	-	-	*	-
	Deuterophlebiidae	*Deuterophlebia* sp.	-	*	*	*	-
	Dolichopodidae		-	-	-	*	-
	Limoniidae	*Antocha* sp.1	-	-	*	*	*
		Antocha sp.2	-	*	-	-	-
		Dicranota sp.	-	-	*	-	-
		Eloeophila sp.	*	-	-	-	-
	Psychodidae	*Pericoma* sp.	-	-	*	-	-
	Simuliidae	Simuliidae 1	-	-	*	*	*
		Stegoptera sp.	*	-	*	-	-
	Tipulidae	*Tipula* sp.	*	*	*	*	-
Ephemeroptera	Baetidae	*Baetis alpinus*	-	-	*	-	*
		Baetis sp.1	-	*	*	*	*
		Baetis sp.2	*	*	-	*	-
		Baetis sp.3	*	-	-	*	-
		Baetis sp.4	-	*	-	*	-
		Baetis sp.5	-	-	*	*	-
	Ephemerellidae	*Cincticostella* sp.1	-	*	-	-	-
		Cincticostella sp.2	-	-	-	*	-
		Caudatella sp.	-	*	-	-	-
		Serratella sp.	-	*	-	-	*
		Drunella sp.1	*	*	-	*	*
		Drurella sp.2	-	-	*	*	-
		Drunella sp.3	*	-	-	*	-
	Ephemeridae	*Ephemera* sp.	*	-	-	-	-

Order	Family	Genus	TK-1	TT-2	SC-3	DC-4	UR-5
	Heptageniidae	*Rhithrogena* sp.	-	*	*	-	-
		Cinygmula sp.	-	*	-	-	*
		Epeorus sp. 1	-	-	-	*	*
		Epeorus sp. 2	-	*	-	*	-
		Heptagenia sp.	-	*	-	-	*
		Pseudiron sp.	*	*	-	*	-
		Heptageniidae	-	-	*	*	-
	Leptophlebiidae	*Paraleptophlebia* sp.	*	-	*	-	-
	Siphlonuridae	*Parameletus* sp.	-	-	*	-	-
Hydracarina	Sperchonidae		-	-	*	*	-
Placoptera	Chloroperlidae	Chloroperlidae 1	-	-	*	*	*
		Chloroperlidae 2	-	*	-	*	-
		Suwallia sp.	-	-	*	-	-
	Leuctridae	*Despaxia* sp.	*	-	*	-	-
	Nemouridae	Nemouridae1	-	-	*	-	-
		Amphinemoura sp.	-	*	*	*	-
		Nemoura sp.	*	*	*	*	*
		Shipsa sp.	*	-	*	-	-
		Sphaeronemoura sp.	-	*	-	-	-
	Perlidae	*Claassenia* sp.	-	*	-	*	*
		Perlinae	-	*	*	-	-
		Paragnetina sp.	-	*	-	-	-
	Perlodidae		-	*	-	-	-
Trichoptera	Brachycentridae	*Brachycentrus* sp.1	*		*	*	*
		Brachycentrus sp.1	-	-	*	*	-
		Micrasema sp.	-	-	-	*	-
		Amiocentrus sp.	-	-	*	-	-
	Glossosomatidae	*Agapetus* sp.	-	*	*	*	-
		Anagpetus sp.1	-	-	*	*	-
		Anagpetus sp.2	-	*	-	-	-
		Glossosoma sp.	-	*	*	-	*
	Hydropsychidae	*Hydropsyche* sp.	-	*	*	*	*
		Parasyche sp.	*	*	-	-	-
		Potamiya sp.	-	*	-	-	-
		Arctopsyche sp.	-	-	-	-	*
		Diplectroma sp.	-	*	-	-	-
	Helicopsychidae	*Helicopsyche* sp.	-	-	*	-	-

Order	Family	Genus	TK-1	TT-2	SC-3	DC-4	UR-5
	Lepidostomatidae	*Lepidostoma* sp.	*	-	*	*	*
	Limnephilidae	*Philocasca* sp.	*	-	-	-	-
	Philopotamidae	*Chimarra* sp.	-	-	*	*	-
		Dolophilodes sp.	-	-	*	-	-
	Phryganeidae	*Phryganea* sp.	-	-	*	-	*
		Phryganeidae 1	-	-	-	*	*
	Polycentropodidae	*Polycentropus* sp.	-	-	-	-	*
	Psychomyiidae	*Psychomyia* sp.	-	*	*	-	-
	Rhyacophilidae	Rhyacophilidae 1	*	*	*	-	*
		Rhyacophilidae 2	*	*	-	-	-
		Rhyacophilidae 3	-	-	*	-	-
		Rhyacophila sp.1	-	*	-	-	-
		Rhyacophila sp.2	-	-	*	-	-
		Rhyacophila sp.3	-	*	-	-	-
		Rhyacophila sp.4	-	*	-	-	-
		Himalopsyche sp.1	-	*	-	*	-
		Himalopsyche sp.2	-	*	-	-	-
	Stenopsychidae		-	*	-	*	*
Odonata	Corduliidae	*Somatochlora* sp.	*	-	-	-	-
	Gomphidae	*Gomphus* sp.	*	-	-	-	*
	Epiophlebiidae	*Epiophlebia laidlawi*	*	-	-	-	-
Tricladida	Planariidae	*Planaria* sp.	*	-	*	*	*
Oligochaeta	Lumbriculidae	*Lumbriculus* sp.	*	-	*	*	*

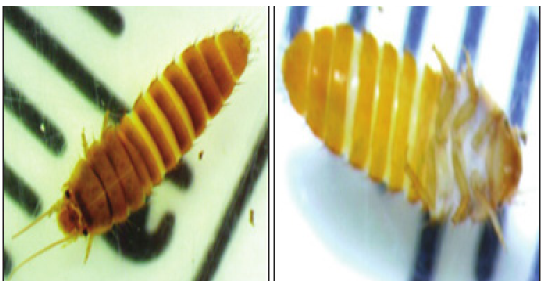
Pictorial images of freshwater biodiversity

ARANEAE (Water mite) Hydracarina

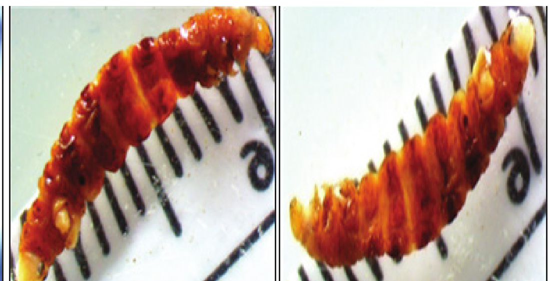


Sperchonidae

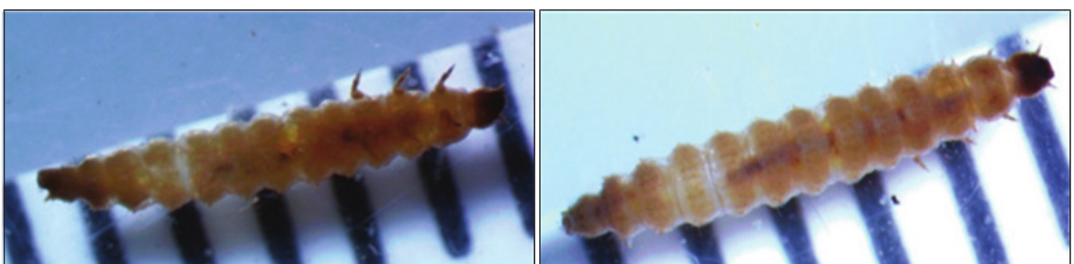
COLEOPTERA



Scirtidae

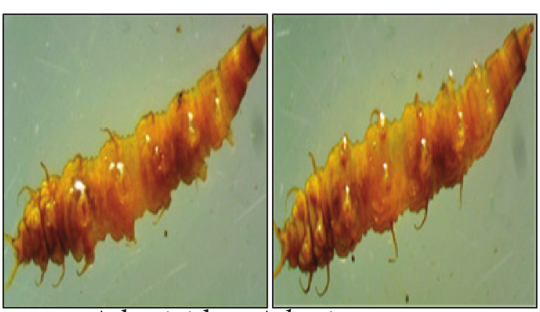


Lampyridae



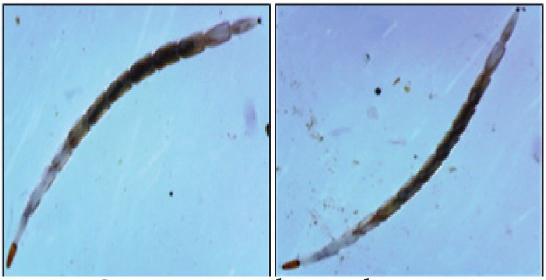
Hydraenidae

DIPTERA

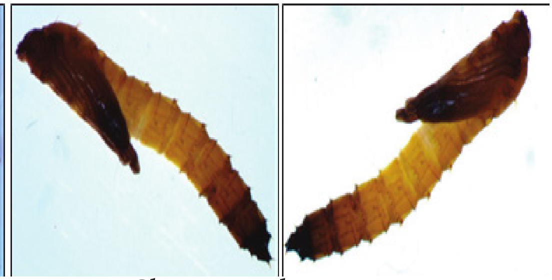


Athericidae: *Atherix* sp.

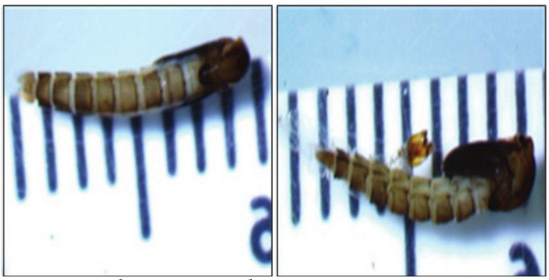
Blephariceridae: *Horaia* sp.



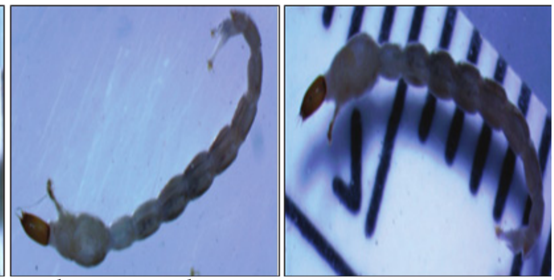
Ceratopogonidae, Heleinae



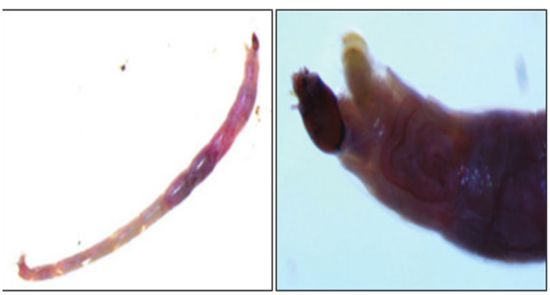
Chironomidae1



Chironomidae 2

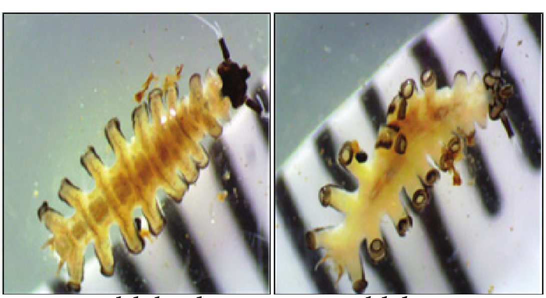


Chironomidae 3

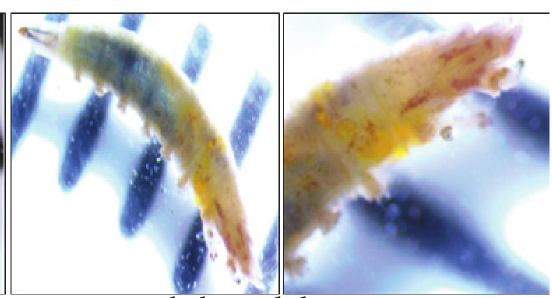


Chironomidae: *Chironomus* sp.

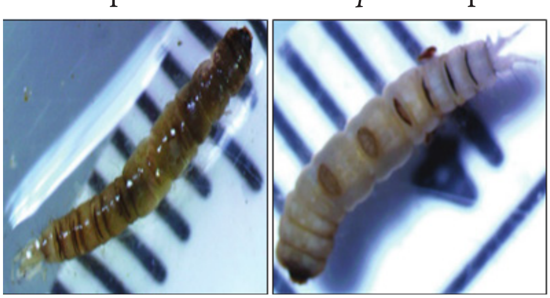
Athericidae 1



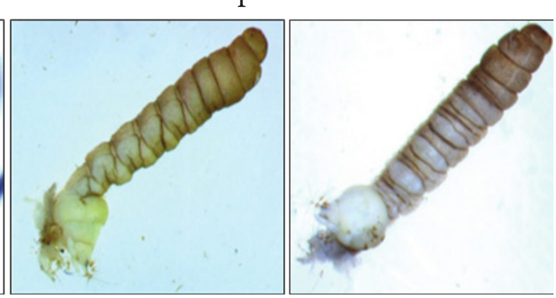
Deuterophlebiidae: *Deuterophlebia* sp.



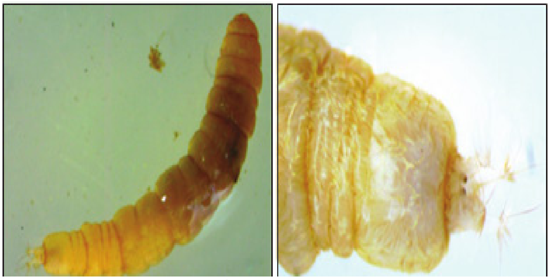
Dolichopodidae



Limoniidae: *Antocha* sp.

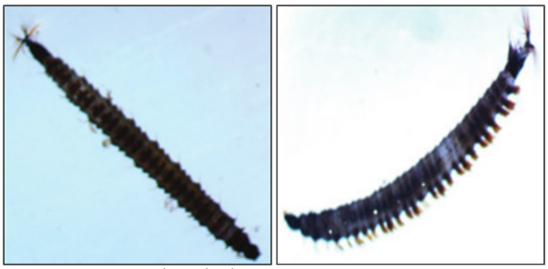


Limoniidae: *Antocha* sp.

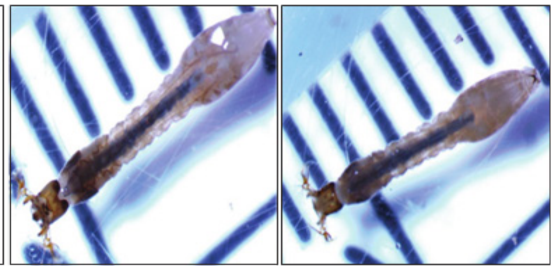


Limoniidae: *Dicranota* sp.

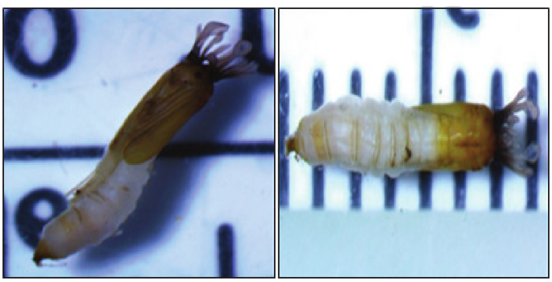
Limoniidae: *Eploeophila* sp.



Psychodidae: *Pericoma* sp.



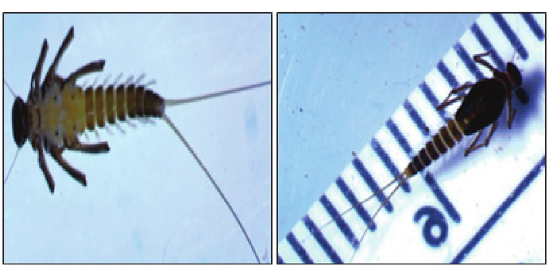
Simuliidae: *Stegoptera* sp.



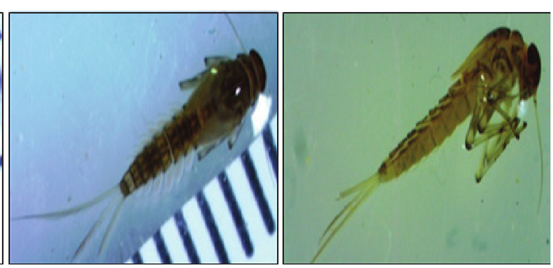
Simuliidae (Pupa)

Tipulidae: *Tipula* sp.

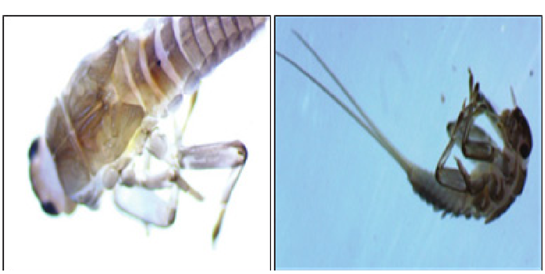
EPHEMEROPTERA



Baetidae: *Baetis* sp.1



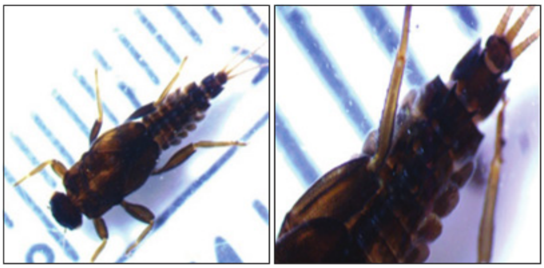
Baetidae: *Baetis* sp.2



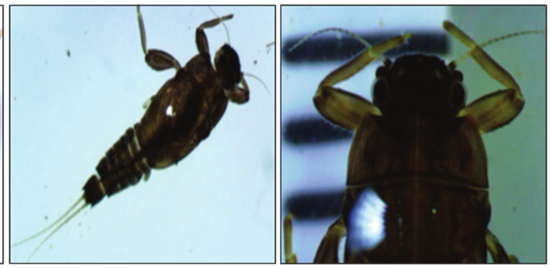
Baetidae: *Baetis* sp.3

Baetidae: *Baetis* sp.4

Baetidae: *Baetis* sp.5



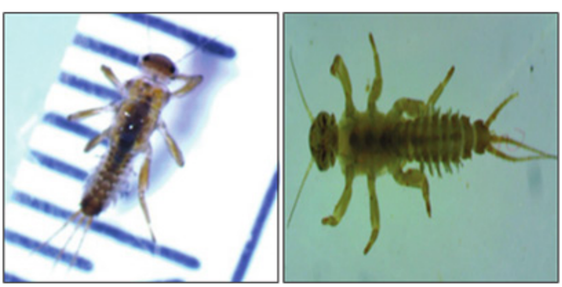
Ephemerellidae: *Cincticostella* sp.1



Ephemerellidae: *Cincticostella* sp.2

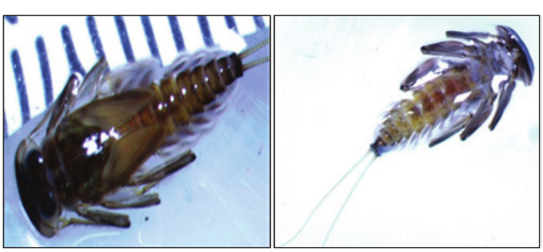
Ephemerellidae: *Drunella* sp.1

Ephemerellidae: *Drunella* sp.2

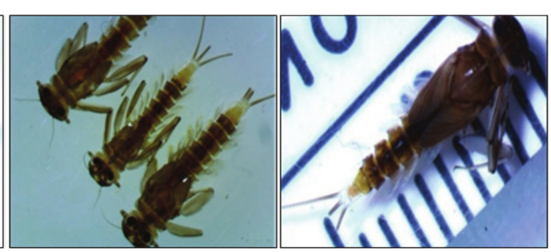


Ephemerellidae: *Serratella* sp.

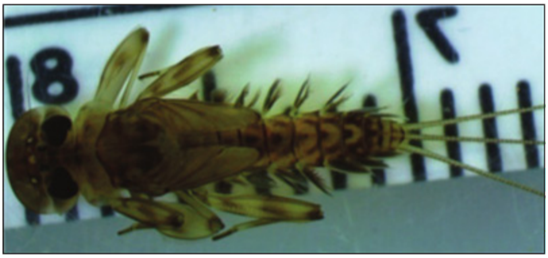
Ephemeridae: *Ephemera* sp.



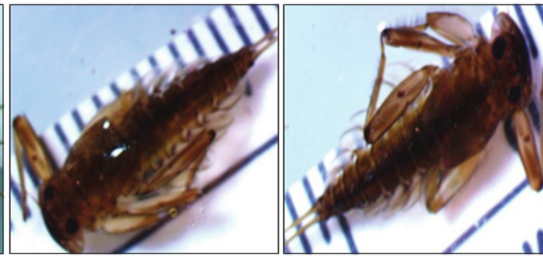
Heptageniidae 1



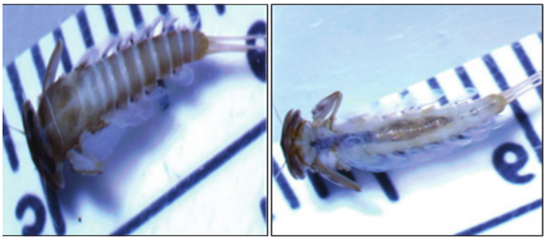
Heptageniidae: *Cinygmula* sp.



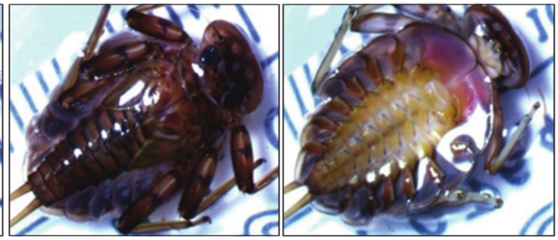
Heptageniidae: *Cinygmula* sp.



Heptageniidae: *Epeorus* sp.

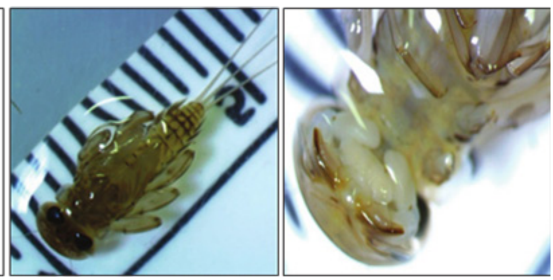


Heptageniidae: *Rhithrogena* sp.

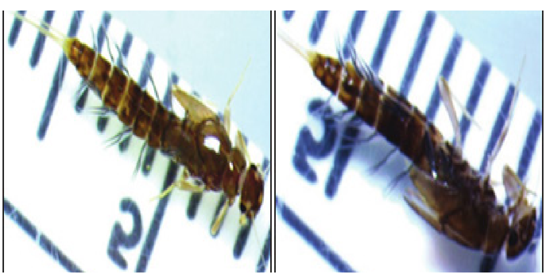


Heptageniidae: *Epeorus* sp.

Heptageniidae: *Pseudiron* sp.



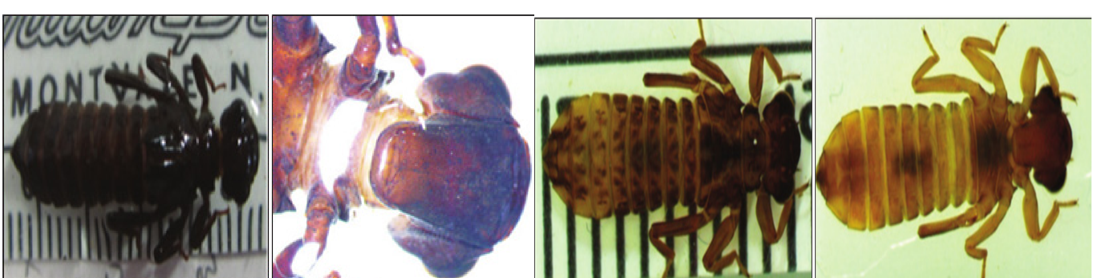
Heptageniidae 2



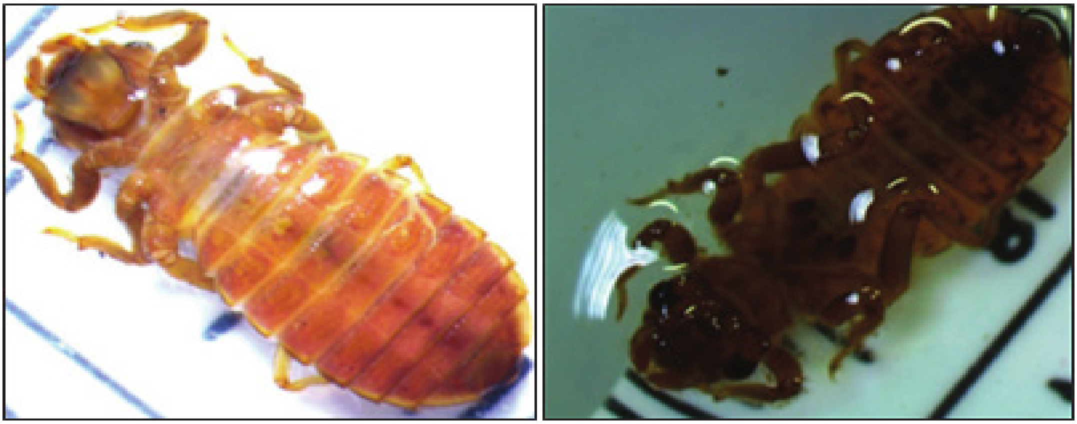
Leptophlebiidae: *Paraleptophlebia* sp.

Siphlonuridae: *Parameletus* sp.

ODONATA



Epiophlebiidae: *Epiophlebia laidlawi*

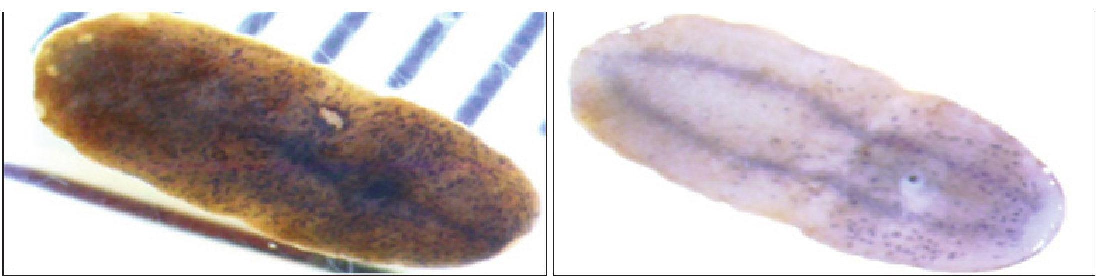


Gomphidae: *Gomphus* sp.

OLIGOCHAETA (Subclass)

Lumbriculiidae: *Lumbriculus* sp.

TRICLADIDA (Planarians)



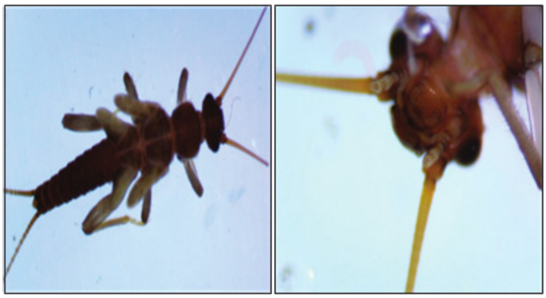
Planariidae: *Planaria* sp.

PLECOPTERA

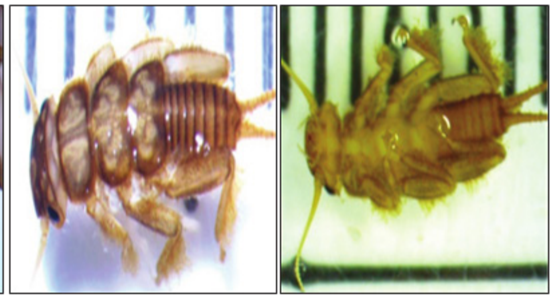
Chloroperlidae 1

Chloroperlidae 2

Perlidae: *Claassenia* sp.



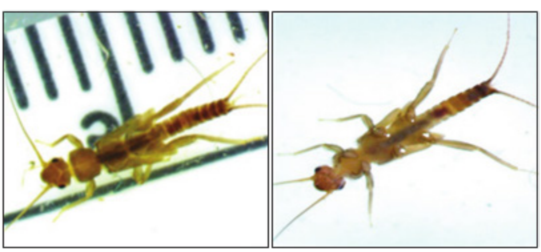
Perlodidae



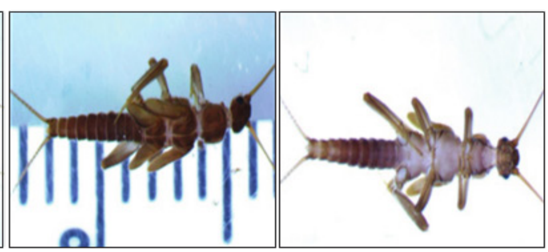
Perlidae: Perlinae

Perlidae: *Paragnetina* sp.

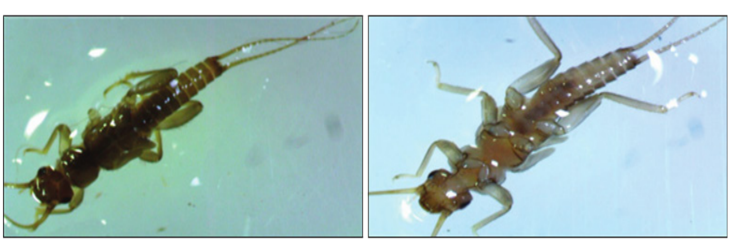
Leuctridae: *Despaxia* sp.



Nemouridae: *Amphinemoura* sp.

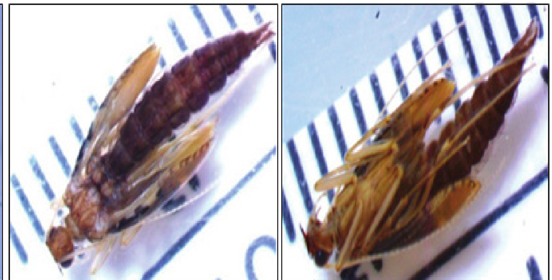


Nemouridae: *Nemoura* sp.



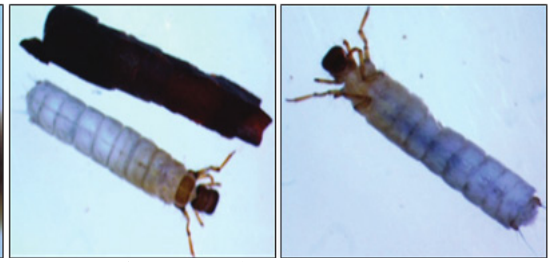
Nemouridae: *Shipsa* sp.

Brachycentridae: *Miscrasema* sp.

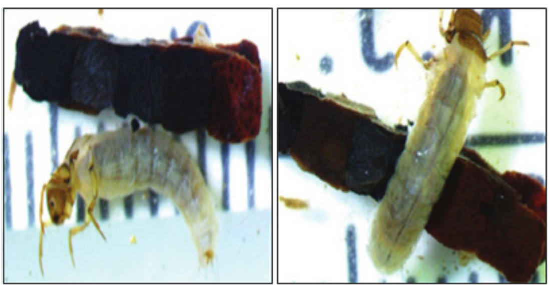


Nymph

Brachycentridae: *Brachycentrus* sp.



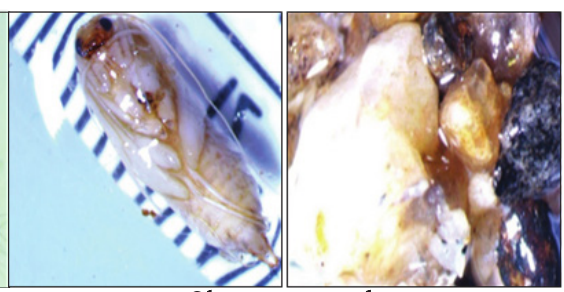
Brachycentridae: *Amiocentrus* sp.



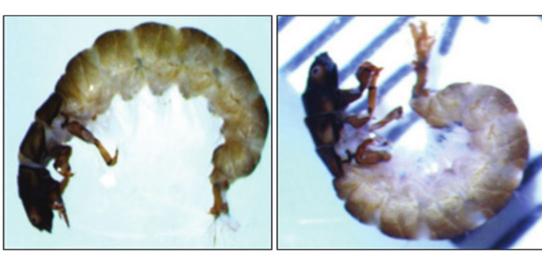
Brachycentridae: *Brachycentrus* sp.

Glossosomatidae: *Agapetus* sp.

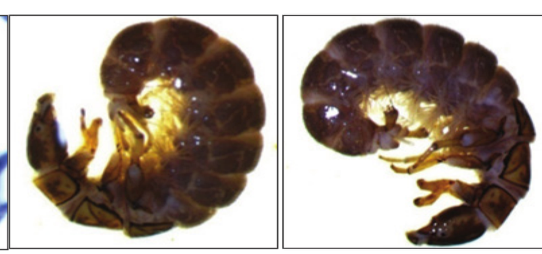
Glossosomatidae: *Anagapetus* sp.



Glossosomatidae

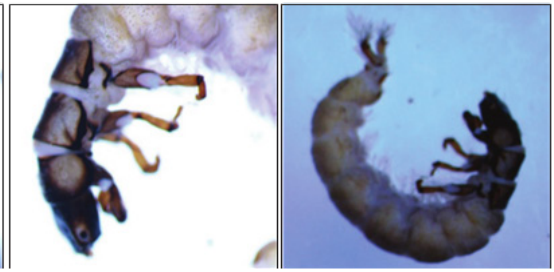


Hydropsychidae: *Diplectroma* sp.

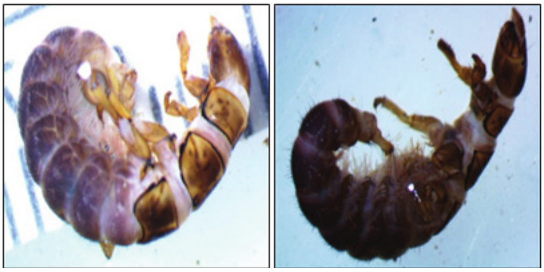


Hydropsychidae: *Arctopsyche* sp.

Hydropsychidae: *Hydropsyche* sp.

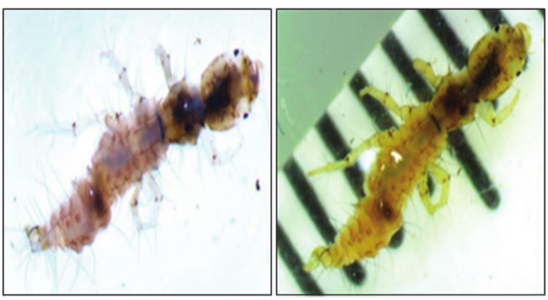


Hydropsychidae: *Potamiya* sp.

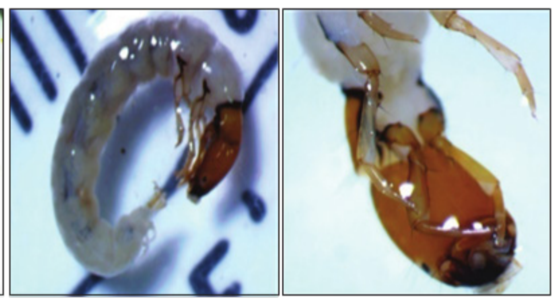


Hydropsychidae: *Parapsyche* sp.

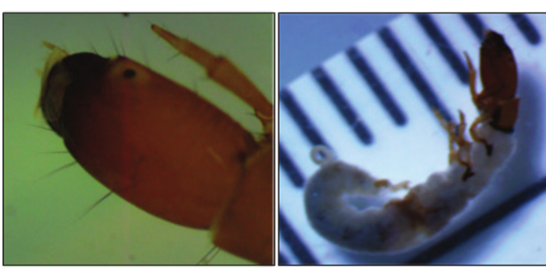
Lepisdostomatidae: *Lepidostoma* sp.



Polycentropodidae

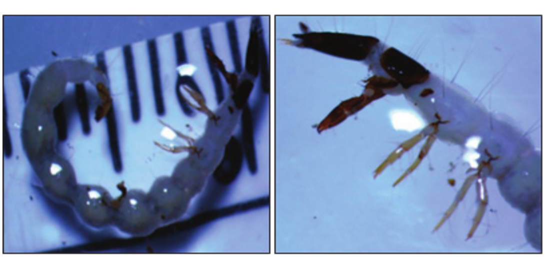


Philopotamidae: *Dolophilodes* sp.



Philopotamidae: *Chimarra* sp.

Philopotamidae 1

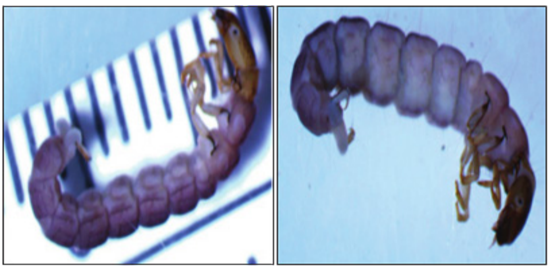


Philopotamidae 2

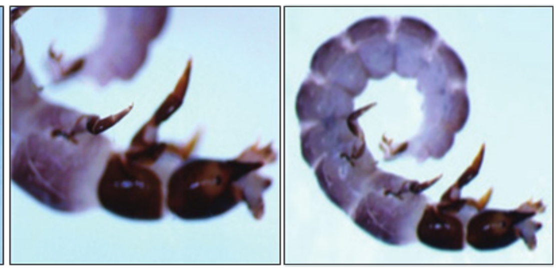
Philopotamidae 3

Phryganeidae

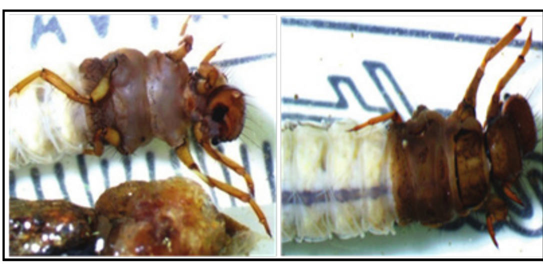
Phryganeidae: *Phryganea* sp.



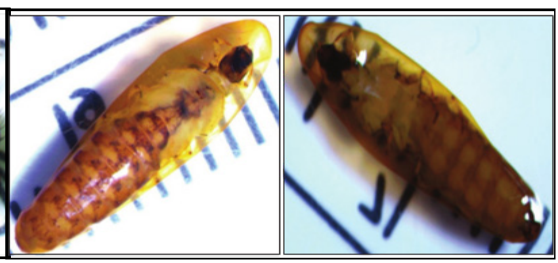
Polycentropodidae: *Polycentropus* sp.



Psychomyiidae: *Psychomyia* sp.



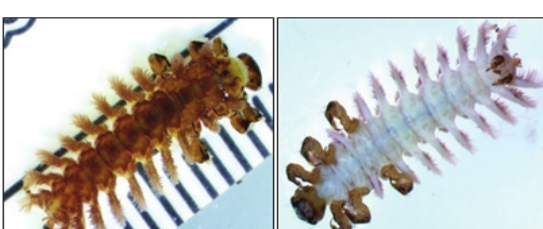
Limnephilidae



Rhyacophilidae (pupa)

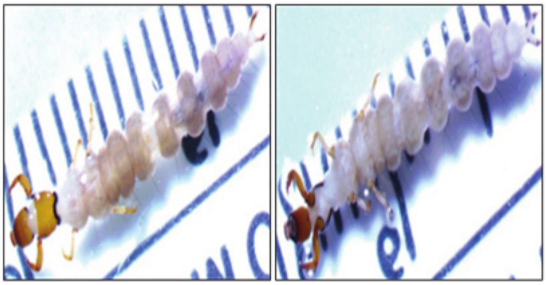
Rhyacophilidae 1

Rhyacophilidae 2



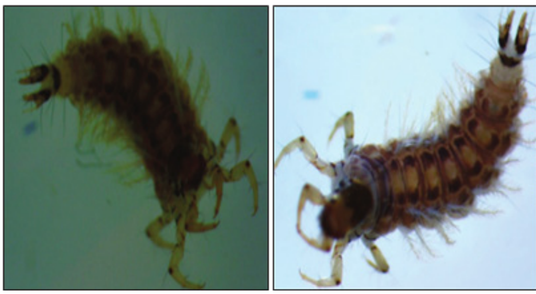
Rhyacophilidae: *Himalopsyche* sp.1

Rhyacophilidae: *Himalopsyche* sp.2

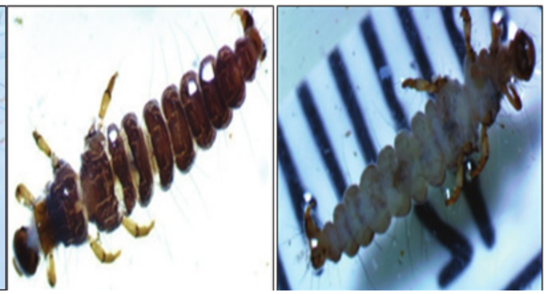


Rhyacophilidae: *Rhyacophila* sp.1

Rhyacophilidae: *Rhyacophila* sp.2

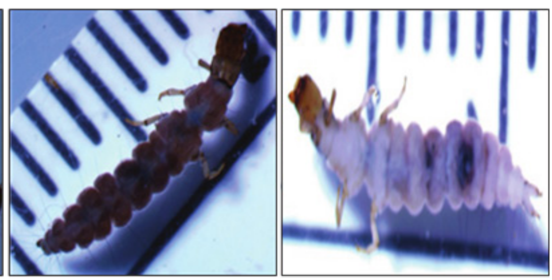


Rhyacophilidae: *Rhyacophila* sp.3



Rhyacophilidae: *Rhyacophila* sp.4

Stenopsychidae



Rhyacophilidae 3

Salmo trutra

A pristine external environment can help nurture innner peace.

Join our efforts in helping conserve Bhutan's natural environment.

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