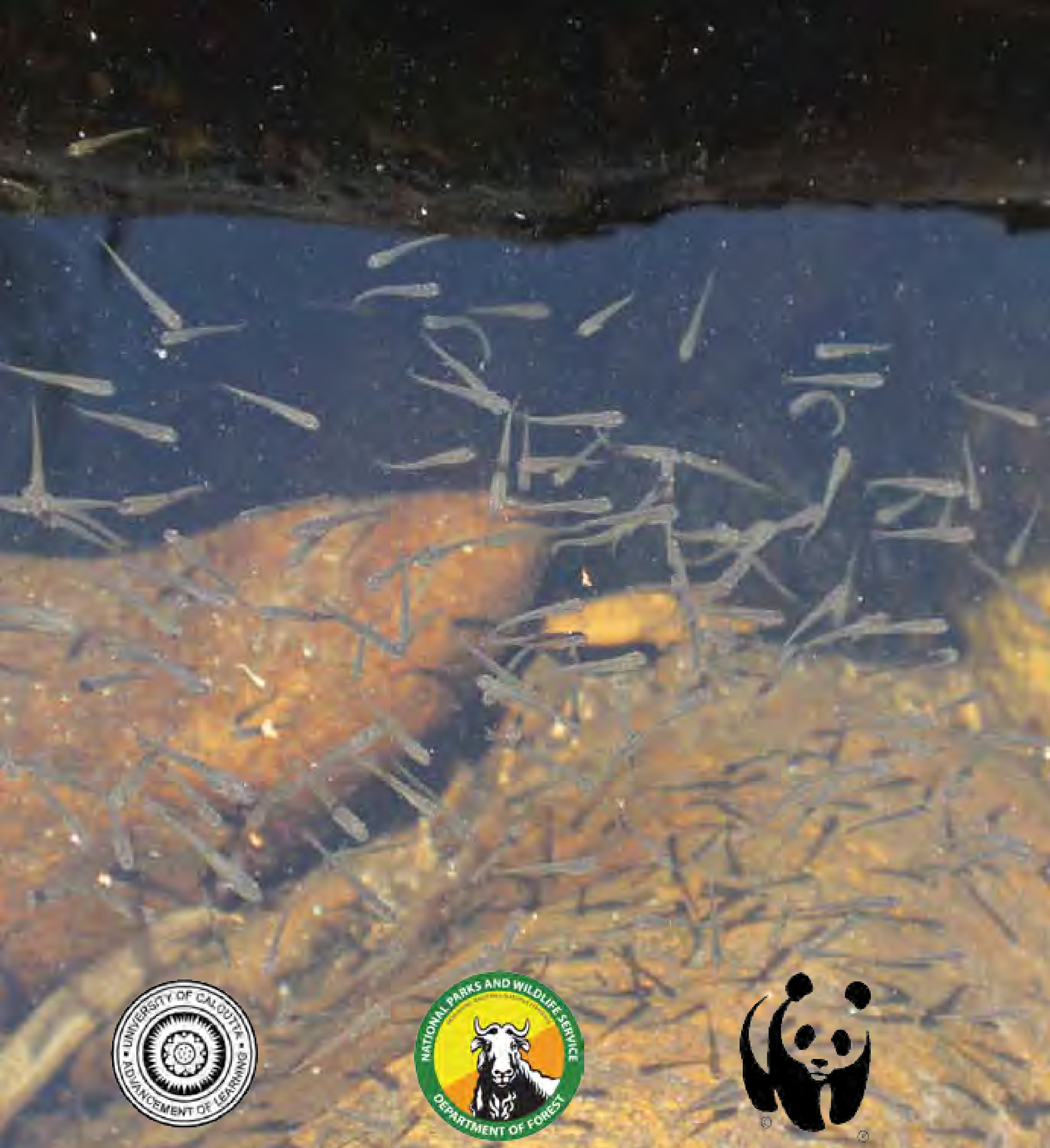

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Preliminary report on Freshwater biodiversity in Wangchuck Centennial Park, Bumthang

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Preliminary study on the freshwater biodiversity in Wangchuck Centennial Park, Bumthang

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A C K N O W L E D G E M E N T

This report is a joint effort of Wangchuck Centennial Park, University of Calcutta and WWF Bhutan. The report attempts to document the first freshwater macro-invetrabrates in the country.

For the successful completion of this research, we would like to thank that Royal Government of Bhutan for allowing us to conduct this research in the Wangchuck Centennial Park and moreover for supporting the research team in the field.

Help rendered by National Biodiversity Centre, Serbithang in facilitating the material transfer protocol for obtaining government approval to take samples to Calcutta University for further identification is highly appreciated.

Lastly, we would like to thank all other individuals and organizations including the local communities who directly or indirectly participated in the research.

Tashi Delek

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FOREWORD

Bhutan is endowed with rich freshwater resources fed by glaciers, snow and rain. The rivers, streams, lakes and wetlands are crucial components of freshwater ecosystems for biodiversity and sustainable economic growth, both locally and regionally.

Freshwater ecosystems regulate micro-climates and has immense livelihood, cultural and spiritual significance for local communities across the country. Plants and animals found in and around these water bodies are often endemic and highly adapted to their locations. However, as biodiversity conservation in Bhutan is challenged by increasing threats of extinction, it is quite certain that some species might go undocumented.

Over the years, substantial scientific knowledge has been generated on terrestrial ecosystems and appropriate legislations are in place for their protection. Nonetheless, information on freshwater biodiversity is still very limited. Even protected area management plans say little about freshwater biodiversity conservation.

Therefore, to develop a better understanding of the aquatic biodiversity, a systematic study of the macro-invertebrate and fish communities of freshwater bodies was carried out at Wangchuck Centennial Park (WCP). This study should cater to establish a firsthand scientific notion on the state of aquatic biodiversity in aquatic ecosystems at WCP, and further appreciate its role and value in the ecosystem.

This report will uncover the rich aquatic biodiversity in the country and spark off enthusiasm in others to conduct similar studies thereby furthering contribution to global biodiversity conservation. More importantly, it will help draw appropriate management interventions to protect our freshwater biodiversity for future to adapt to emerging threats of climate change and water pollution.

Kinzang Namgay
Country Representative
WWF Bhutan

EXECUTIVE SUMMARY

The Wangchuck Centennial Park (WCP) is the largest protected area in Bhutan and it constitutes the critical ecosystem connectivity of the mountainous northern portion of the country. WCP's streams and rivers form the headwaters that feed four major rivers in Bhutan, supporting both secluded habitats for numerous species of flora and fauna and the country's major economic development through hydropower. Although, substantial scientific knowledge has been generated on terrestrial ecosystems, information on biodiversity is virtually absent.

A pilot survey of the frehwater biodiversity of WCP was carried out with special reference to macro-invertebrates and fish fauna to understand the diversity and also to appreciate their role and value in the ecosystem. This pilot study was conducted in the four rivers and their tributaries in WCP viz. Nikka Chu, Mangde Chu, Chamkhar Chu and Kuri Chu.

The study consisted of 18 sampling sites from where 1107 insect specimens belonging to nine orders were encountered. Most of them are aquatic larval forms along with accidental occurrence of some semi-aquatic adults. Their preferable habitat and ecological functioning has been described and comparison of family distribution and total abundance of different insect orders were mad along with the correlation matrix among abundance of different taxonomic groups and physico-chemical parameters. Samples collected indicated the dominance of insects over the other macro-invertebrate groups (e.g. *Molluscs, Annelids* etc.). The ichthyofaunal diversity of the park was limited to a exotic species (*Salmo trutta trutta*) and very few native species. The overall ecosystem health was found to be sustainable as evident by the rich and diverse macro-invertebrate community mainly the aquatic insects, since they form the most important links in the aquatic food chain by maintaining a balanced nutrient recycling within the aquatic system.

The analysis of the various physico-chemical parameters indicates more or less healthy water quality within the park. The correlation evaluation pooled out showed the parameters viz. Dissolved Oxygen, TDS and temperature to be the most influencing factors in determining aquatic faunal diversity of the park. However, long-term regular monitoring of the diversity and the environmental parameter will ensure to maintain the pristine ecosystem habitat within the park and can be idealized for future monitoring programmes in other protected areas of Bhutan.

1. INTRODUCTION

Freshwater ecosystems represent a major group of habitats around the world. All freshwater bodies are dynamic systems, not only are their organisms affected by the physico-chemical conditions, but also the plant and animals interact and may influence both the habitat and one another. They have a major influence on the physical and chemical conditions, while intra and inter-specific relationships among plants and animals may be of critical importance to both water quality and the structure of communities. Freshwater bodies are one of our most vital natural resources. Not only are they essential to sustain life itself, but they also play a crucial role in our economic development and social well-being. It is important that our water bodies are protected from pollution and managed as a sustainable resource for all of the activities that depend on them. The freshwater ecosystem includes rivers, streams, lakes, ponds and wetlands. Streams are dynamic aquatic ecosystems that connect all types of aquatic ecosystems.

The biodiversity has remained as one of the central themes of ecology since many years. The global campaign to help biodiversity began with the Earth Summit in Rio in 1992. This was followed up by various strategies and targets developed at international and national level, finally filtering down to a local level from around 2004 onwards and the development of Local Biodiversity Action Plans. It is at local level where conservation has perhaps greatest impact on us – because it involves where we live and the biodiversity on our doorstep. However after the Rio's Earth Summit, it has become the main theme for not only ecologists, but the whole biological community, environmentalists, planners and administrators. Owing to the Convention on Biological Diversity, each nation has the solemn and sincere responsibility to record the species of plants and animals occurring in their respective countries to assess the biodiversity properly and evolve suitable management strategies for conserving the biodiversity. At present, freshwater biodiversity is of outstanding global importance and as such is the over-riding conservation priority during the International Decade for Action – 'Water for Life' – 2005 to 2015.

Aquatic biodiversity can be defined as the variety of life and the ecosystems that make up the freshwater, tidal, and marine regions of the world and their interactions. Freshwater aquatic biodiversity encompasses freshwater ecosystems, including lakes, ponds, reservoirs, rivers and streams, groundwater, and wetlands. The banks of streams or riparian areas are also important being associated with freshwater systems in terms of energy flow and affecting the associated aquatic faunal diversity in concern to water quality. Aquatic ecosystems provide a home to many species including phytoplankton, zooplankton, aquatic plants, insects, fish, birds, mammals, and others. They are organized at many levels, from the smallest building blocks of life to complete ecosystems, encompassing communities, populations, species, and genetic levels. Aquatic biodiversity has enormous economic and aesthetic value and is largely responsible for maintaining and supporting overall environmental health.

Humans have long depended on aquatic resources for food,medicines, and materials as well as for recreational and commercial purposes such as fishing and tourism. Aquatic organisms also rely upon the great diversity of aquatic habitats and resources for food,materials, and breeding grounds. Therefore study of aquatic biodiversity becomes imperative in order to maintain aquatic health leading to a sustainable environment in future. As some species begin to disappear and new ones arrive, it has become more important than ever to document biodiversity and understand the various factors that shape and affect aquatic biodiversity.

Fresh water makes up only 0.01% of the World's water and approximately 0.8% of the Earth's surface, yet this tiny fraction of global water supports at least 100,000 species out of approximately 1.8 million – almost 6% of all described species. Inland waters and freshwater biodiversity constitute a valuable natural resource, in economic, cultural, aesthetic, scientific and educational terms. Their conservation and management are critical to the interests of all humans, nations and governments. Describing patterns of and controls on distribution of organisms is central to the study of ecology and historically has been the focus of much limnological study. Freshwater habitats contain representatives of many of the groups of organisms on Earth.

Correct identification of freshwater organisms is essential to understanding their ecology. Biodiversity of invertebrates and fishes can be used to indicate chronic pollution problems. Taxonomic identification of invertebrates and fishes is required for techniques that use species diversity as an indicator of pollution. Freshwater biodiversity provides a broad variety of valuable goods and services for human societies – some of which are irreplaceable (Covich et al., 2004). The value of this biodiversity has several components: its direct contribution to economic productivity (e.g. fisheries); its 'insurance' value in light of unexpected events; its value as a storehouse of genetic information; and its value in supporting the provision of ecosystem services. Conservation of biodiversity is complicated by the landscape position of rivers and wetlands as 'receivers' of land-use effluents, and the problems posed by endemism and thus non-substitutability. In addition, in many parts of the world, fresh water is subject to severe competition among multiple human stakeholders. Protection of freshwater biodiversity is perhaps the ultimate conservation challenge because it is influenced by the upstream drainage network, the surrounding land, the riparian zone, and – in the case of migrating aquatic fauna – downstream reaches.

Knowledge of the total diversity of fresh waters is woefully incomplete – particularly among invertebrates and microbes, and especially in tropical latitudes that support most of the world's species. Even vertebrates are incompletely known, including well-studied taxa such as fishes. Adequate data on the diversity of most invertebrate groups in tropical fresh waters do not exist, but high levels of local endemism and species richness seem typical of several major groups, including decapod crustaceans, molluscs and aquatic insects such as caddisflies and mayflies. Maintenance of biodiversity is a critical test of whether water use or ecosystem modifications are sustainable, and this assumption underlies all use of freshwater organisms as biomonitors or indicators of habitat condition (Rosenberg and Resh, 1993; Karr and Chu, 1999). Preservation of all elements of freshwater biodiversity would guarantee that water use for humans is sustainable, and the magnitude of the threat to and loss of biodiversity is probably a reliable indicator of the extent to which current practices are unsustainable.

The IUCN (2010) study on freshwater biodiversity in the Eastern Himalaya claimed that the region supports significant numbers of species dependent upon freshwater habitats (Allen et al., 2010). The assessment showed that, out of 1,073 species, 77 species (7.2%) are globally threatened, and none are considered to have become extinct. However, from Bhutan, only 50 species of dragonflies have been reported and the country has been considered a severely data deficient area with respect to freshwater biodiversity (Allen et al., 2010). The assessment report also considered portions of the Brahmaputra River drainage located in Arunachal Pradesh, Meghalaya, and northern Bengal, together with parts of Assam and the Himalayan foothills between Nepal and Bihar, to exhibit the most diverse fish fauna, which indicates that Bhutan might also be similarly rich since it is located in the same geographic area. Imphal River and its tributaries draining from the surrounding hills and the central plain of Manipur, as well as the adjoining areas in Myanmar, have been identified as habitat for the majority of threatened fish species in the region (Allen et al., 2010). Other species found in the Himalayan region include 180 freshwater molluscs and 367 species of Odonata (Allen et al., 2010), with no information on other taxa. The IUCN (2010) assessment also identified several threats to freshwater biodiversity in the eastern Himalayan region including dam construction, wetland drainage, pollution, deforestation, etc., which are very much prevalent in Bhutan.

The Biodiversity Action Plan of Bhutan (2009) states that there are 50 freshwater fish species, including eight introduced species (MoAF, 2009). Himalayan trout (*Barilius* spp.) and Mahseer (T*or tor*) are the main indigenous fish species listed as totally protected species in the Forest and Nature Conservation Act, 1995 (MoAF, 2009). However, there is no information available on other freshwater species in Bhutan, such as aquatic plants and macro-invertebrates, which are an integral part of the aquatic food web. As the state of aquatic biodiversity becomes increasingly of concern and importance, it is equally important to find ways to monitor and measure biodiversity. Measuring aquatic biodiversity can be accomplished in many ways. Everything from fish to bird to insect to plant species can be in one way or another counted and therefore measured and evaluated. The presence-absence and abundance of species give scientists an idea of the state of aquatic biodiversity within a particular aquatic ecosystem. If the same type of information is collected from the same ecosystem every year, the biodiversity of that area can be monitored. This is how change in biodiversity over time is detected. Till date, no systematic studies of the macro-invertebrate and fish communities of the freshwater bodies have been conducted in Wangchuck

Centennial Park This initiated the need for a comprehensive survey of the aquatic biodiversity of WCP with special reference to macro-invertebrates and fish fauna and to appreciate their role and value in the ecosystem. The present study will cater to the need to protect habitats that conserve species diversity and control degradation of water quality in order to manage, identify, map and protect biodiversity hotspots for the concerned ecosystem and taxa and also toward the benefit of sustainable use of resources.

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2. MATERIALS AND METHODS

2.1 Study area

The study was conducted in Wangchuck Centennial Park (Figures 1), located in the north-central part of Bhutan. It was formally gazetted as a National Park on June 10, 2008. The 4914 sq km park is now the largest protected area in the country. It connects with Bumdeling Wildlife Sanctuary to the east and Jigme Dorji National Park to the west, forming a contiguous protected area covering the entire northern frontier of the country. As such, it plays an important role in conserving, protecting, and maintaining the integrity of specific ecosystems, species, as well as cultural, historical, and religious sites. The park includes three ecological zones with six different habitat types, namely: cool broadleaved forests, mixed conifer forests (hemlock and spruce), fir forests, juniper forests, alpine meadows and scrub, and alpine scree.

The assignment was aimed at pilot survey of the aquatic macro-invertebrates and fish available within WCP. The investigation included collection and taking stock of information and data on habitats, collection and documentation of the aquatic biodiversity (macro-invertebrates and fish), measuring hydrological parameters, preparing fact sheets and future recommendations.

Figure 1. Map of protected areas in Bhutan shwoing Wangchuck Centennial Park

2.2 Habitats and Physiography

The water ways in the study area comprised freshwater habitats. The main habitats studied were rivers and associated streams and lakes. Variation of altitudinal gradient was well marked between specific sites (1267-3744 meters). The rivers' stretch studied have longitudinal profiles characterized by steep slopes with some fast flowing rocky reaches to slow flowing sandy and/or muddy floors along with associated streams. Most of the stretches were characterized by faster flow, low temperature, higher oxygen concentrations with rocky or gravel to sandy beds (Plates 1, 2 and 3).

2.3 Sites Sampled

This pilot survey was undertaken in April-May 2012 at various sites to mount sample collection of aquatic macro-invertebrate and ichthyofauna as well as water samples from rivers, streams and lakes in and around WCP area. The sampling sites covered parts of the districts of Bumthang, Lhuntse, Wangduephodrang and Trongsa falling within and buffer area of the park. A total number of 18 sampling sites were selected. Most of the sites are selected at the lower parts of the park since the accessibility into higher areas is difficult and also the diversity at higher altitude is assumed to be comparatively low.The map of WCP and it buffer zone where sample sites are located is show in Figure 2. The main river channels as well as associated streams and lakes were sampled. The details of sampling sites covered are tabulated in Appendix A.

Figure 2. Map showing sampling sites in Wangchuck Centennial Park

2.4 Sampling Procedure

2.4.1 Micro-invertebrates collection methods

Insects

They form the major group among aquatic macro-invertebrates. There is an amazing diversity of aquatic insects, but these organisms often go unnoticed because many are secretive and spend most of there life well hidden. Insects from riffle areas were collected from leaf packs caught among the rocks and also by gently scrubbing organisms from rocks.

Annelids

Aquatic oligocheates formed the major collection among this group. They are usually found clinging to plants or under rocks. Freshwater species live in swamps, ponds, streams, and rivers where they are live on wood, rocks, vegetation, or on other animals. They were collected mainly by spotting attached to under-surface of rocks and by hand picking and putting into vials. Specimens were preserved in 70% ethanol.

Molluscs

Molluscs are sufficiently large and conspicuous. Hand net or water net fitted with a wooden handle is useful to drag over the aquatic vegetation. Leaves and branches of the plant were carefully searched out and snails are picked up with hand or forceps. Specimens are then transferred to specimen tubes. Bivalves can be collected by dredge or by hand picking in case of bigger specimens from the bottom of small ponds or slow moving streams. Collection was made by scooping the bottom mud and then putting into ordinary sieve and washing with water. After washing the small bivalves are picked up from the sieve. Specimens were also preserved in 70% ethanol. Samples were collected directly by hand picking or by using dip-net.

Hand collecting: Many aquatic insects were collected with only a pair of forceps and a vial of alcohol. It was done by picking up substrates such as rocks, logs, sticks, leaves, or even human trash (e.g., tires, buckets) and picking insects directly from the substrate into a vial of 70% alcohol (Figure 3). When removed from the water, many insects crawl from the substrate so it is sometimes a good idea to place it in a white pan making them easy to identify. Slow moving insects or insect cases attached to substrates or a piece of wood were also collected.

Figure 3. Collecting samples using hand

Dip netting: The best way to qualitatively collect as many species of aquatic insect as possible from the water is by using a dip net shown in Figure 4. These usually have fine mesh (500 μm), a sturdy frame, and a long handle. A dip net was used by holding the net downstream and disturbing the substrate with hands or feet so that the insects are dislodged and washed downstream into the net. This is effective in areas where there is overhanging terrestrial vegetation or submerged tree roots.

Figure 4. Sample collection using dip-net

Preservation of specimens: Specimens were preserved in small vials on spot after collection (Figure 5). Specimens were preserved in 70% ethanol. If a large number of specimens are picked into a vial, it is best to change the alcohol after 24 to 48 h to prevent decomposition.

Figure 5. Sample preservation at site

2.4.2 Ichthyofauna (Fish fauna) collection methods

Mainly cast net was used for catching the fishes. The fish samples were collected mainly through experimental fishing. Fish samples collected were preserved on spot in 4% formaldehyde and transported to the laboratory for identification.

Cast nets: The cast-net method for sampling freshwater fish is a small-scale method of net fishing that can be conducted by one person (Figure 6). A cast net is made up of three parts: the upper section (net band), the middle section (a conical-shaped net mesh), and the lower section, which is weighted. The practitioner casts using both hands and the shoulder, throwing the net onto the surface of water in an area likely to have the targeted fish.

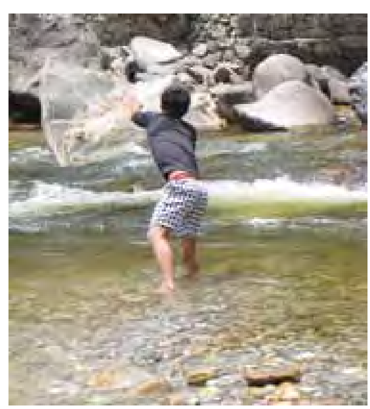

Figure 6. Using cast net to collect sample

With the weights dragging along the stream bottom, the net is slowly drawn back to the caster's hands, collecting the captured fish within the net. In most cast nets, the internal part of the lower section of the net (the section with rounded margins) forms a pouch-like structure in which the fish are caught.

Hooks: Another most effective way of catching fish is by hooks. Metal hooks with artificial baits attached to it are used to catch fish fauna from the rapids.

For collecting data from the sample sites, a standard format is used which is presented in Appendix B.

2.5 Physico-chemical parameters

Water parameters are the primary determinant of the quality of aquatic habitat and diversity of biotic assemblage thereof. Each of the parameters and their interactions describe the habitability of a template of a stream or lake. Candidate habitat variables are evaluated for the relevance to the biotic community, responsiveness to human impact, applicability to target landscape, and assessment reliability. Habitat indicators as water quality criteria can be used to evaluate the water quality standard and biotic integrity. In concert with measures of water parameters and evaluations of biotic assemblage pattern, habitat indicators assist in diagnosis of the habitat ranking status. Although variability is inherent in aquatic systems, there is an observed pattern of habitat conditions that are necessary to support aquatic communities. This report covers a narrative water quality standards and estimations in wide region of WCP to provide more specificity in regard to macro invertebrate and fish diversity status at an eco-regional scale.

2.5.1 Collection and preservation of water samples

Water samples from the sub-surface level were collected manually by lowering a closed glass bottle (200 ml) / polyethylene bottle under the water surface film, opening and closing it there by hands and taking it out. After collecting the water samples, the containers are labeled properly indicating the site specification, date and time of collection and station number. Tightly capped water sample containers then brought to the laboratory for further analysis of the various water quality parameters.

Figure 7. Water testing on site and sample collection

Standard chemical methods (APHA, 2005) were followed for analysis of Total Hardness (ppm), Total Alkalinity (ppm) and Total Acidity (ppm). Whereas, Iron (ppm), Organic Phosphate (ppm), Nitrite-nitrogen (ppm), Total Ammonical nitrogen (ppm) and Nitrate-nitrogen (ppm) was measured using standard calorimetric kit (N.I.C.E.). Water Temperature (°C), Transparency (cm), pH, Dissolved Oxygen (mgL-1), Total Dissolved Solid (ppm) and Percentage of salt (%) were measured using digital scientific instruments. The format used for collecting data on the water quality (physico-chemical) parameters is presented in Appendix C.

2.5.2 Water quality assessment based on macro-invertebrates

An assessment of the aquatic macro-invertebrates at the waterway can provide an indication of water quality since different macro-invertebrates have different tolerances to pollution. Highly sensitive macroinvertebrate can only live in water with high water quality. Tolerant and very tolerant macroinvertebrate can withstand lower water quality. A healthy waterway has a higher biodiversity of macroinvertebrates.The Freshwater macroinvertebrates are rated according to their sensitivity to pollution.

Pollution rating' numbers from 1 to 10 indicates how sensitive each bug is. See table 1.

There are four 'grades':
- Very sensitive – 10, 9
- Sensitive – 8, 7, 6
- Tolerant – 5, 4, 3
- Very tolerant – 2, 1

Very Sensitive (10,9) Require higher DO, neutral PH, Cold Water	**Sensitive(8,7,6)**	**Tolerant (5,4,3)** Can tolerate low O_2,lower/higher pH,warm H_2O	**Very tolerant (2,1)**
• Stonefly Nymph • Mayfly Nymph	• Caddishfly Larva	• Dragonfly Nymph, • Damselfly Nymph • Coleopteran larvae, • Whirling Beetle	• Annelid worm • Hemipteran bugs

Table 1: Categories of Stream Macro-invertebrates

3. OBSERVATION AND ANALYSIS

3.1 Types of freshwater ecosystems and major taxa

The major categories of aquatic ecosystem are lakes, rivers, streams and wetlands. They harbor high diversity of plants, animals and soil types. From biodiversity conservation value, rivers are most important as they sustain the breeding, nesting and nursery habitats for a wide array of faunal groups and most importantly the fishes.

3.1.1 Aquatic macro-invertebrates

Aquatic macro-invertebrates consist of insect larvae, insect nymphs, annelids, mollusks, crabs and prawns. Insects are the most dominant of aquatic macro-invertebrates. They are mostly benthic and live in or near bottom sediments. Others are partly benthic and pelagic. They are the major links between primary producers (algae and macrophytes) and higher organisms specially fish.

3.1.2 Arthropoda (Insects)

They are the most important macro-invertebrate components of aquatic biodiversity and central to ecosystem function. They are characterized by a chitinous exoskeleton and stiff jointed appendages (including legs, mouthparts and antennae). There are three subphyla common in freshwaters; the Chelicerata (class Arachnida-water mites and aquatic spiders), the Uniramia (insects and collembolan) and the Crustecea. Among them class Insecta are the most studied freshwater invertebrates. The majority of these species spend most of their immature lives in the water, and the adults emerge from the aquatic environment to mate and disperse. The immature forms of the aquatic insects are variously referred to as larvae, nymphs, or naiads.

In our present study, we have encountered 1107 insect specimens belonging to nine orders (Plate 4-15). Most of them are aquatic larval forms along with accidental occurrence of some semi-aquatic adults. Their preferable habitat and ecological functioning has been briefed in the table 2.

Order	Commin Name	Habitats	Functional feeding groups
Ephemeroptera	Mayflies	Lentic and lotic	Scrapers,collectors,few predators
Odonata	Dradonflies and Damselflies	Lentic and lotic	Predators
Plecoptera	Stoneflies	Lentic and lotic	Shredders,collectors,predators
Tricoptera	Caddishflies	Lentic and lotic	Predators,scrapers,collectors
Hemiptera	Bugs	Lentic and lotic	Predators
Lepidoptera	Aquatic caterpillars	Lentic and lotic	Shredders,scrapers
Coleoptera	Water beetles	Lentic and lotic	Predatorsscrapers,collectors,shredders
Diptera	Flies and midges	Lentic and lotic	Predators,scrapers,collectors,shredders

Table 2: Characteristics of Orders of Aquatic Insects (Dodds, 2002)

Comparison of distribution by insect order of nine different insect orders collected in 17 different sites of WCP has been depicted in Appendix D and Figure 8. Among 18 sites samples, no samples were recorded from site 18. The site is a high altitude lake at Sombji called Dortsho located at 4185 meters high. This is due to the lack of dissolved oxygen at the higher altitude since atmosphere is the main source of dissolved oxygen in most aquatic systems, and low atmospheric oxygen pressure at higher altitudes has the potential to affect the life in water. The correlation matrix among abundance of different taxonomic groups is presented in Appendix E.

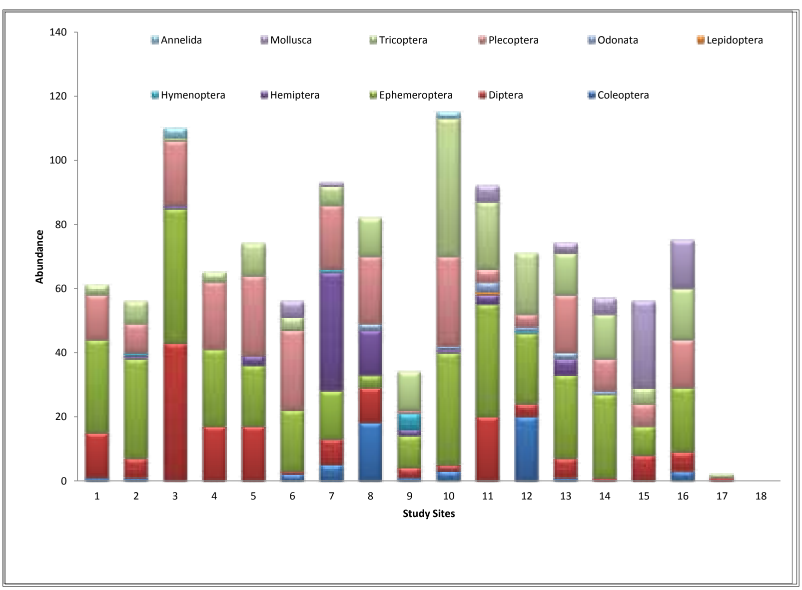

Figure 8. Comparison of total abundance of different taxonomic groups collected in 17 sites in WCP

Mayflies belonging to order Ephemeroptera are often abundant as larvae in streams (where they can provide an important food source for fish) and in the benthos of some lakes. Mayfly larvae are distinguished from other aquatic insects by long filaments on their posterior end (generally three) and presence of conspicuous gills on the first seven abdominal segments. The sub-imago stage is the only winged pre-adult stage known in insects. Mayflies are diverse in well oxygenated, unpolluted streams and are used as indicators of good ecosystem health. In the present study, Ephemeroptera was found to be the most abundant insect order comprising 33.06% of the total samples collected. They are the most dominant group in majority of the sites.

The **Plecopteran or stoneflies** larvae are important in streams as food for fish. Some species are predators of other invertebrates and others are important leaf shredders. They are associated primarily with pollution-free, cool, highly oxygenated running waters. Since they prefer clean habitats they are used as biotic indices for stream water quality. The larvae are generally distinguished from other aquatic larvae by the two long cerci (appendages) on their posterior end and their elongate, flattened bodies. In the current survey, they are found to be the second most dominant group (21.86% of total specimens collected) among aquatic insects.

Caddisflies belonging to order Tricoptera are best known for their cases, retreats, and nets that they construct using silk and materials from the environment. Some species are free living, highly mobile, and lack cases or nets. Most species occur in running waters. Along with the mayflies and stoneflies, a diverse caddisfly community indicates a clean stream or river. The free-living caddisfly are mostly predators, those that build cases are primarily herbivores on periphyton or eat leaves, and the species that spin nets are filter feeders. They formed the third major group (17.16%) among the aquatic insects collected in WCP. Their abundance showed a significant negative correlation with altitude as was evident by their high abundance in low altitude sampling sites (in Lhuntse and Nubi in Trongsa).

Flies and midges (Diptera) constitute a large group, to which about 40% of all aquatic insects belong (Hilsenhoff, 1991). In the current study, 15.18% of the total collected insects were found to be under dipteran group. This group is dominated by family Chironomidae which includes about one-third of all species of aquatic Diptera. The Diptera also includes many aquatic larvae with adults that are nuisance species for humans, including the mosquitoes, black flies, biting midges, horseflies and deer flies. Diptera are found in a wide variety of aquatic habitats and exhibit a tremendous array of adaptations to them.

The order **Heteroptera** (sub-order Hemiptera) includes the true bugs. About one-third of the aquatic species live on the water surface and two thirds in the water. The true bugs are distinguished by mouthparts modified to form sucking and piercing beak, a first pair of wing that are leathery at the base, a second pair entirely membranous and simple, and gradual development to adult stages. Most aquatic species are adapted for swimming and breathing through siphons. The groups includes the giant waterbugs (big enough to prey on small fish), the water boatman (often seen in shallow littoral zones), and the gerrids (water striders). Hemiptera constituted 6.05% of the total collected insects and they formed the most dominant insect group in Lhuntse (Site 7). In the present study, the distribution of hemipteran insects was found to bear a high positive correlation with the TDS. As sampling was conducted in late summer, mostly surface dwelling and semi-aquatic families were abundant.

The **Coleoptera** (beetle) species with aquatic larval and/or adult stages represent only about 3% of this mostly terrestrial order. However, they aquatic beetles are significant components of the biodiversity of both lentic and lotic habitats. The group includes the “riffle beetles” (Elmidae, with aquatic larvae and adults), the “water pennies” (Psephenidae larvae, attached to rocks), the Gyrinidae (whirligig beetles, with adults found on the surface), the predacious diving beetles (Dytiscidae), and the water scavenger beetles (Hydrophilidae). Many coleopterans are important predators, whereas others are adapted to scrape periphyton, graze aquatic plants, or utilize detritus. The pilot survey in WCP reveals lesser abundance of coleopteran insects (4.97%), they were mostly found in lower altitudes and warmer temperature zone as was evident by their negative correlation with altitude and positive correlation with water temperature.

The **Odonata** (dragonflies and damselflies) are voracious predators as aquatic larvae and terrestrial adults. The larvae can be distinguished by long hinged labium that has been modified to eject rapidly and seize moving prey. They are significant predators of mosquitoes and thus are viewed as beneficial insects. However, the abundance of odonates in the current survey was less (0.90% of the total sample collected) and most of their larvae were collected from Nubi (Site 11) with accidental occurrence of a matured dragonfly in Lhuntse (Site 10).

Truly aquatic **Lepidopteran** moth larvae are few. The larvae of some aquatic forms have numerous paired filamentous gills on the abdomen. Although majority of the species are found in ponds with dense macrophyte populations, others are abundant (e.g., Paragyractisi) can be abundant in streams and feed on periphyton. During the present survey, only one terrestrial lepidopteran larvae was obtained at Nubi. The site having dense overhanging vegetation, the occurrence of the larvae might be accounted as accidental. However, no aquatic larval forms were encountered during the whole sampling tenure.

Although 11 families of Hymenoptera include species that are considered, no aquatic forms have been obtained in the present survey. However, in Lhuntse, 5 examples of adult Hymenoptera have been collected which again might be considered as abnormal occurrence.

3.1.3 Mollusca

Freshwater mollusks include two classes, the Gastropoda (snails, slugs and limpets) and the Bivalvia (clams and mussels). These species are linked to many ecological processes through their role as filter feeders. In the present study distribution of shelled mollusks was found to be very scarce. Only one individual specimen has been collected from Lhuntse, which seems to belong in family Neritidae of the class Gastropoda. However several specimens of fresh water slugs (belonging to family Acochlidiidae of the class Gastropoda) have been collected during the whole study period (Plate 14).

3.1.4 Annelida

Annelids are segmented worms with a tubular body and a specialized digestive system with a terminal mouth and an anus. Their body cavities have thin transverse septa that delineate the segments. The freshwater annelids include the oligocheates, the leeches, and several other less diverse classes. They are used as indicator species of polluted waters and can be components of biotic indices to assess ecosystem health. Of the freshwater annelids, in our present survey only Oligochaet larvae were found in two study sites namely site 3 (in Bumthang) and site 10 (in Lhuntse) (Plate 15). Although leeches are the most predominant annelids in freshwater habitat, no leeches have been encountered during the sampling tenure.

During the whole study, altitudinal gradients have been encountered starting from 1267 m up to 4184 m. However, altitude plays lesser important role in determining the overall abundance of the major macro-invertebrates, see Figure 9.

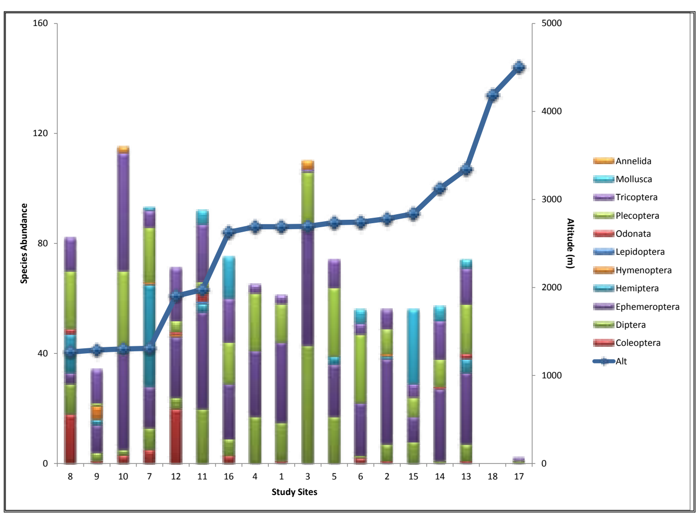

Figure 9. Comparative analysis of the correlation between total abundance of different taxonomic groups and altitudes

The diversity among the invertebrate groups is surely negatively related to the altitude. In high altitude sites fewer families of invertebrates have been reported (Figure 10). Although the result may not represent the real scenario and long term surveys in different seasons of a year might surely bring some light in the actual situation.

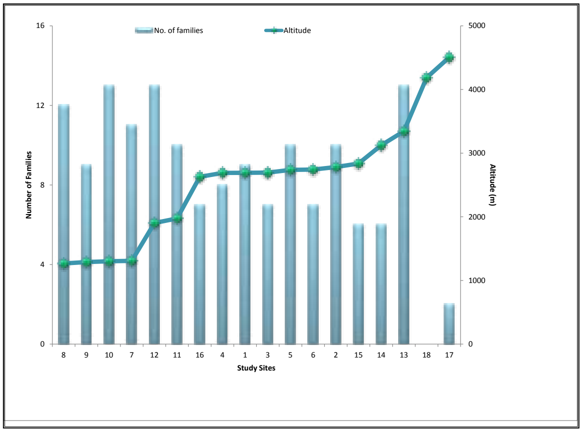

Figure 10. Comparative analysis of the correlation between number of families among different taxonomic groups and altitudes

3.1.5 Fish fauna

The fishes are considered the most important and diverse freshwater animals. Insights into fish biodiversity in the streams and wetlands of Wangchuck Centennial Park are near-absent. Fish biodiversity is likely to be very low, but there could be endemic cold-water species in the high altitude rivers, streams and wetlands. In the Indian Central Himalayas, it is considered exceptional to find any fish above an altitude of 2500 m (WWF-India, 2010). This is a function of temperature and oxygen tolerance, as well as scarcity of food sources. In contrast, three piscine species belonging to three orders and three families were found in the present study (Plate 16,17 and 18). *Salmo trutta trutta* Linnaeus, 1758 was the only representative species of Salmoniformes order and Salmonidae family, found in those study sites having more than 2500 m elevation (Sites 1, 2, 3, 4, 5, 6 and 15). This species is already recorded as an introduced species in the high altitude steams and rives of Eastern Himalayas. *Schizothorax richardsonii* Gray, 1832 (snow trout) belonging to order Cypriniformes and family Cyprinidae is a freshwater, demersal, potamodromous species distributed in the Eastern Himalayas. In the present study, this vulnerable (VU) species (according to IUCN Red List Status) *S. richardsonii* was found in the Sites 7, 8, 9 and 10 having lower altitudes (1200-1300 m). They mainly inhabit mountain streams and rivers, preferring to live among rocks. They generally have herbivore feeding habit and feed mainly on algae, aquatic plants and detritus. Lastly, *Oreoglanis insignis* Ng and Rainboth, 2001 (Siluriformes: Sisoridae) was another species found in the sites around 2000 m (Sites 11 and 12). This freshwater, benthopelagic species might be first time in report in Bhutan. The conservation status of *O. insignis* is not tilled evaluated according to IUCN Red List.

3.2 Physico-chemical parameters status

Since cold water holds more oxygen than warm water, a general trend of higher concentration was noted for Dissolved Oxygen. The trend went on as higher the altitude more is the DO_2 concentration (6.3-10.9 ppm) across the temperature-altitude template of study sites. Localized factor as bit disturbance would also impact the fluctuations in DO_2 results.

pH ranging from minor acidic (6.0) to high alkaline (9.9) nature of water quality was due to the natural contour of the particular site. Total Acidity showed an overall trend of low magnitude but with certain variation, ranging from 1.6-8.8 ppm. Total alkalinity was also found to be of low magnitude and certain variation was obvious due to absence/low availability of metallic ions ($Ca2^+$, $Mg2^+$) and salts, as evident from the results of TDS (5.49-84 ppm) and salts. Quite a varying result was observed for Ammoniacal nitrogen (0.1-0.65 ppm) and Nitrate nitrogen (0.1-1.2 ppm). Temperature, pH and substrate quality configured the high and low pattern for them. Inorganic phosphate was found in minor quantum, as expected, and not found to vary significantly. The physico-chemical parameters of WCP has is represented in Appendix F.

3.3 Threat analysis

The threat analysis was done mostly through direction observation at the site in consultation with the park staff and also with the local communities. The findings were cross checked with similar threats in the region to determine the magnitude or intensity of threats. Although, the aquatic ecosystem is relatively intact without much of threats, with the change in life style and more on going and planned developmental activities the threats are likely to increase. So of the current observed threats to aquatic ecosystem in WCP which would be similar in other parts of the countries are described below.

i***. Runoff from agricultural and urban areas***

People residing in and around WCP practice agriculture which is their main source of livelihood and to increase the productivity, some amount of chemical fertilizers, pesticides and weedicides are being used. These chemical substances get drained into the water and finally to the main river which is toxic for many aquatic species. Also, many urban areas (although small) do not have proper sewage treatment and all the waste gets drained into the water. Waste from the use of toiletry items is very harmful for the aquatic ecosystem.

ii. Development of Hydropower projects

In Bhutan hydropower is the major economic backbone for the country's development.

As a result, there are many small hydropower projects which intersect the rivers of WCP. They can cause significant and irreversible loss of many species changing the overall ecosystem for the native species. They can also prevent migration and dispersion of many species, mainly fishes. Although there are generally smaller dam located on a river that uses the river's natural flow to generate electricity. But with growing demands for hydropower, the number of dams is likely to be increased. The respective authorities must be careful about implementing new hydropower projects in WCP.

iii. Invasion of exotic species

Many rivers in Bhutan were introduced with Brown trout (*Salmo trutta trutta*) including the Chamkhar Chu in WCP. It was first introduced to Bhutan in 1930, and until the 1980s two trout hatcheries (in Haa and Wangchutaba) produced about 20000 trout fingerlings per annum. However, it was discontinued in 1983 on the assumption that it was feeding on and suppressing indigenous fish such as asla (Gyeltshen, 2002). Similarly, during the sampling the team failed to spot any native fish in Chamkhar Chu and it may have been due to the predation by the Brown trout. Local people also reported that it is hard to find local species although it appears that few are still available. The Global Invasive Species Database also claims that this fish has been introduced around the world for aquaculture and stocked for sport fisheries, but is blamed for reducing native fish populations, especially other Salmonids, through predation, displacement and food competition (Global Invasive Species Database, 2010).

4. CONCLUSION AND RECOMMENDATIONS

In food webs, aquatic macro-invertebrates capture, use and make available to other fresh water organisms nutrients that otherwise would be unavailable by processing the nutrients from coarse particulate organic matter (CPOM) and from fine particulate organic matter (FPOM) (Cummins and Merritt, 1996). In the present study, a total of 1178 individual of aquatic macro-invertebrate belonging to 3 major phyla and 12 orders have been collected and grouped under five major trophic categories viz. predators, scrapers, collectors, shredders and parasites.

CPOM occurs in freshwater ecosystems in the form of both living and dead vascular plants and larger animals. Shredders, predators and parasites fragment CPOM and release smaller organic particles as feeding scrapes and faeces; their own bodies assimilate nutrients from CPOM and become available to many predators. FPOM occurs in freshwater ecosystem as tiny particles of both living and dead plant and animal material or as flocculated dissolved organic matter (DOM). FPOM is abundant but cannot be consumed by many animals because it is too small for efficient consumption and cannot be used by plant because its nutrients are not in solution. Among the aquatic insects many collectors proficiently consume these materials (Wallace and Merritt, 1980; Merritt and Wallace, 1981) and then become prey for freshwater and nearby terrestrial predators.

Aquatic insects are thus important links in food webs to assure that nutrients are passed to other members of the community. Higher diversity and abundance of aquatic insects help these nutrients pass more efficiently to other animals thereby retaining the nutrients in the ecosystem longer, helping to assure the health of the overall ecosystem. The current study reveals more or less even distribution of the predators, scrapers, collectors and shredders throughout Wangchuk Centennial Park. However, the parasites partially replaced the shredders in few locations (Sites 2, 7, 9, 12) (Figure 11). Their overall distribution indicates the balanced nutrient utilization and cycling in the aquatic ecosystem of the park. To maintain this balanced and integrated environment conservation measures should be undertaken and carried forward in future.

Modern science recruits communities of freshwater macro-invertebrates as an alternative method to monitor the water bodies biologically (bioassessment or biomonitoring). The advantages of biomonitoring with insects and other macro-invertebrates can be realized only if the natural fauna of the region is known.

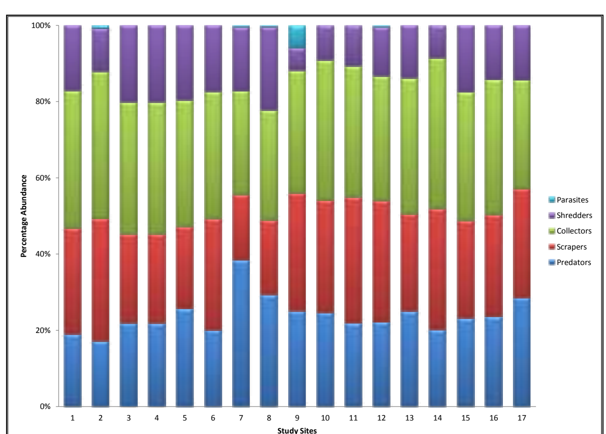

Figure 11. Precentage abundance of different Trophic groups

If the species diversity of freshwater macro-invertebrates is poorly known biomonitoring is possible only at relatively crude taxonomic levels such as order or families. But to make biomonitoring most effective, background knowledge of the invertebrate biodiversity at the genus and species level must be obtained from the wide variety of freshwater habitats of the region. In the present survey only 10% of the collected specimens were in adult forms thus making the identification process up to the species level very difficult.

In regions like WCP where species are less well known and documented, larvae were poor candidates to be recognized up to species level. For proper documentation, the entire life cycle needs to be studied in details and for that a long term study is recommended.

Due to environmental difficulties sampling could not be applied evenly in all the study sites. Again, the multiple sampling in individual sites will not only increase the chance of getting all the life forms of the resident species but also will minimize the sampling errors. Therefore a long term study covering every extreme season of the year is advised.

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Appendix A | Details of Sampling sites in WCP

Sites	Districts	Rivers/lakes	Habitat	Locations	Altitude (m)
1	Bumthang	Chamkar chu	River Channel	27.36'05.74"N 90.42'51.04"E	2642
2		Chamkar chu	Nasphel Stream	27.40'19.96"N 90.53'01.18"E	2770
3		Chamkar chu	Tang stream	27°36'17.66"N 90°41'54.4"E	2694
4		Chamkar chu	Dhur stream	27°36'22.6"N 90°41'54.4"E	2690
5		Chamkar chu	Menchugang stream	27°36'42.3"N 90°40'30.4"E	2737
6		Chamkar chu	Dhur stream	27°36'39"N 90°40'21.9"E	2742
7	Lhuntse	Kuri chu	Gangzur stream	27°41'42.55"N 91°9'52.66"E	1309
8		Kuri chu	Kuri chu (River)	27°42'52.71"N 91°09'7.07"E	1267
9		Kuri chu	Stream on a way to Ney	27°43'59.0"N 91°08'55.7"E	1289
10		Kuri chu	Kuri chu (Ney bridge)	27°43'59.43"N 91°07'54.38"E	1302
11	Trongsa	Mangde chu	Nubi stream	27°43'57.75"N 90°29'27.07"E	1975
12		Mangde chu	River channel	27°32'25.8"N 90°26'32.7"E	1903
13		Streams in and around Sephu	Running streams	27.30'51.7"N 90.16'52.7"E	2744
14		Streams in and around Sephu	Running streams	27.31'36.6"N 90.18'10.4"E	2639
15		Streams in and around Sephu	Running streams	27.30'08.9"N 90.18'34.5"E	2546
16		Streams in and around Sephu	Running streams	27.28'30.5"N 90.21'08.0"E	2241
17	Wangdue	Zowzasa valley-stream	Running streams	27.38'56.4"N 90.18'27.8"E	3774
18		Sombji-Dortsho lake	Lake	27.39'28.2"N 90.19'54.2"E	4184

Appendix B | Data collection format for the sampling sites

Major aspects provided in the ecosystem and organism/species data collection
Date and place (Country/State/Districts/Protected area/Locality
Ecosystem category/ Habitat - River/lake/wetland
Location **–** GIS location of the ecosystem
Area – Length, width and Depth of River/stream/lakes/wetlands
Water flow – Velocity of flowing water; it is directly related to abundance and site specificity of individual faunal groups on account of their flow preference and acclimatization.
Bottom substrates – muddy/sandy/clay/rocky
Riparian vegetation/land use – Forest/secondary forest/agricultural land/ human locality or urban area; type of land use indicates the influence of external inputs (e.g. organic matter in the form of leaves, debris, etc.) to the river, while the absence of vegetation (due to land use) could indicate the source of turbidity of the river water.
Sampling method – types of nets used/habitats sampled
Taxonomic category – classification of organisms/species from Phylum to Family The ***canopy cover*** is generally measured to have a better understanding about the habitat.

Appendix C

Guidelines for data collection under water quality (physico-chemical) parameters

Major aspects provided in the water quality data collection
Air temperature: Determines the slice of environment and riparian vegetation.
***Water temperature*:** Important as to determine the slice of aquatic environment by regulating water chemistry and directly influence the biodiversity. Also, strongly determine the physiological and metabolic activity of animals present in the aquatic habitat.
Transparency: Transparency or visibility of the water depth defines the light penetration and determined by means of using a secchi disc. Important determinant for water quality and productivity.
pH: It is the measure of the water's relative acidity based on a logarithmic scale of 7-14. On this scale 7 is neutral and less than 7 is acidic, while above 7 is alkaline**.** The largest varieties of freshwater aquatic organisms prefer a pH range between 6.5 to 8.0.
Dissolved Oxygen: Considered as most important parameter in the aquatic environment and critical in maintaining a stable ecosystem. Cold water holds more DO2 than warm water - naturally. Dissolved oxygen is measured in mg/L. 0-2 mg/L: not enough oxygen to support life. 2-4 mg/L: only a few fish and aquatic insects can survive. 4-7 mg/L: good for many aquatic animals, low for cold water fish 7-11 mg/L: very good for most stream fish
Salt concentration: This indicates inorganic salt concentration and pond's productive health.
Total dissolved solid: This indicates the loads of organic and inorganic substances in the water column. It is measured in mg/L. In freshwater it ranges between 100-1000mg/L.
***Total hardness*:** It represents primarily the total concentration of metal ions (mainly Ca^{++}& Mg^{++}). Natural hardness of water depends upon the geological nature of the catchment area.
***Total alkalinity*:** TA of water is related to the actual number of base components and considered as acid neutralizing capacity. It contributes to the chemical balance in aquatic systems.
***Total acidity*:** Acidity of water is its quantitative capacity to neutralise a strong base to a designated pH. Acidity is important because acid contributes to corrosiveness and influences certain chemical and biological processes.
***Iron*:** Important as micronutrient and regulates cellular reactions.
***Inorganic phosphate (ortho-phosphate)*:** Limiting factor for algal production. Coupled with nitrate-nitrogen, it forms the major nutritional building block of aquatic habitat. It is measured in mg/L. Larger streams may react to phosphate only at levels approaching 0.1 mg/L, while small streams may react to levels of PO4-3 at levels of 0.01 mg/L or less. In general, concentrations over 0.05 will likely have an impact while concentrations greater than 0.1 mg/L will certainly have impact on a river.
***Nitrite-nitrogen*:** Intermediary nitrogenous compound, naturally very unstable in the system. Highly harmful to aquatic fauna in higher concentration
***Total ammonical nitrogen*:** Another very important nutritional component which may turn into toxic in anoxic condition. Reflects overall nutrient status and water quality of the aquatic habitat.
***Nitrate-nitrogen*:** Primary nutritional requirement for algae. Control the productive potential of the water body. Regulated by nitrogen cycle in the aquatic system. Nitrate is measured in mg/L. Natural levels of nitrate are usually less than 1 mg/L. Concentrations over 10 mg/L will have an effect on the freshwater aquatic environment.

Appendix D | Family distribution of species at different sites in WCP

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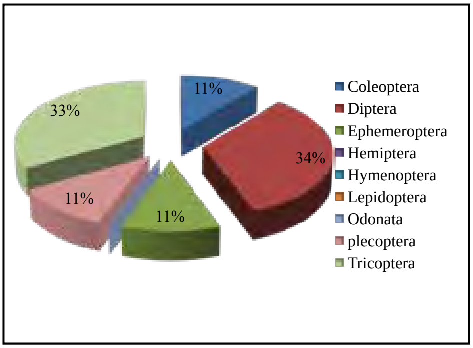

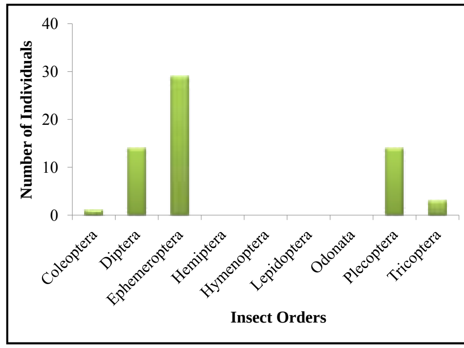

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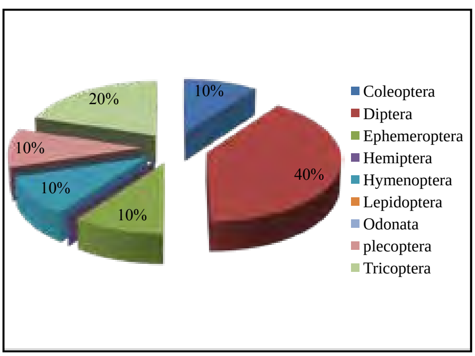

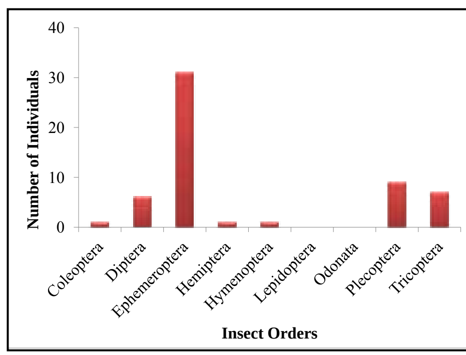

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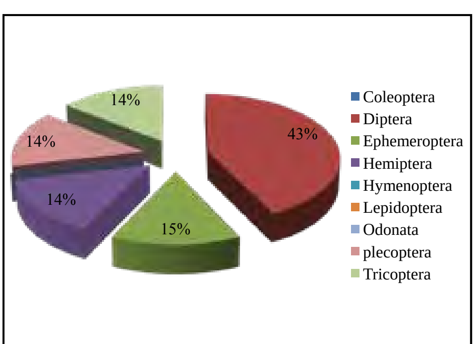

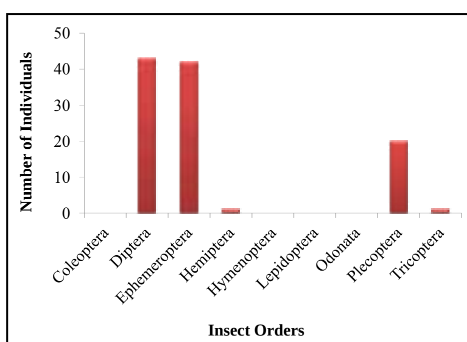

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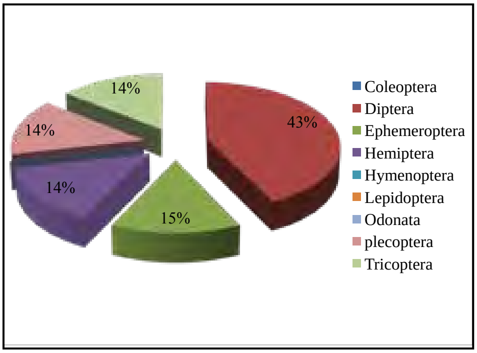

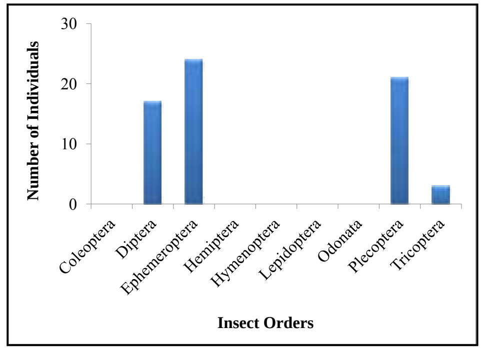

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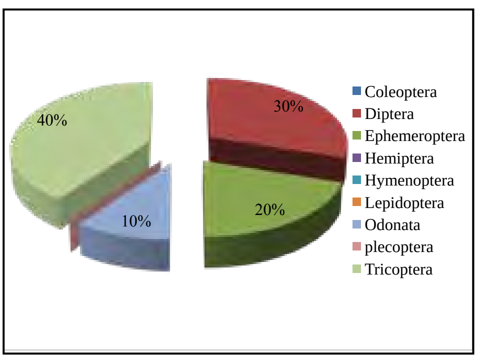

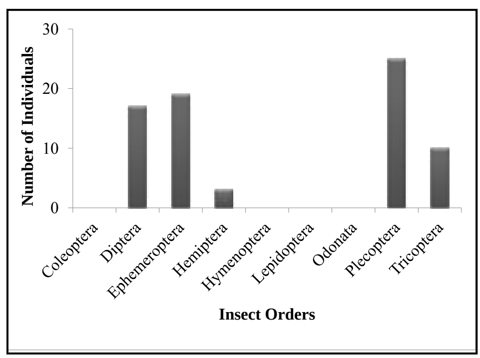

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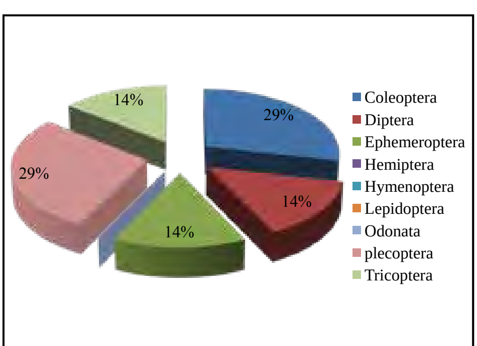

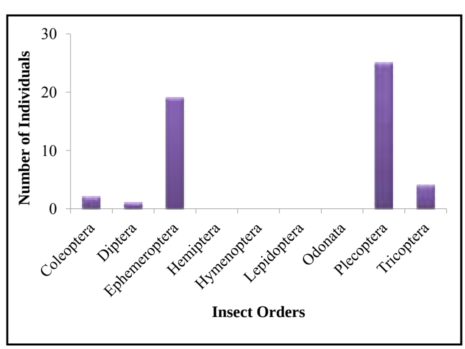

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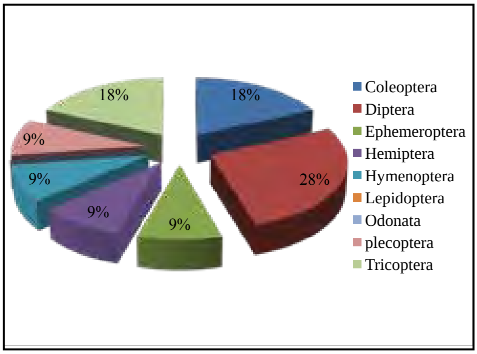

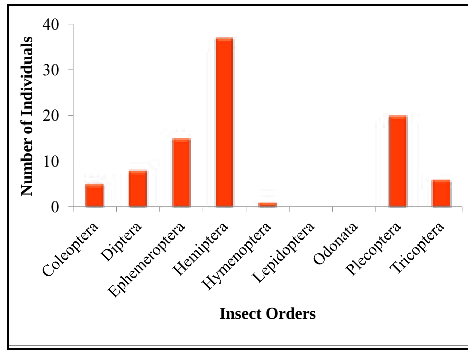

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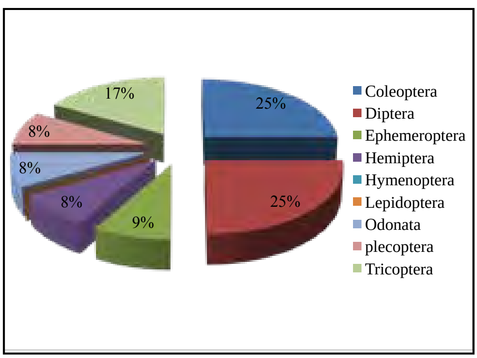

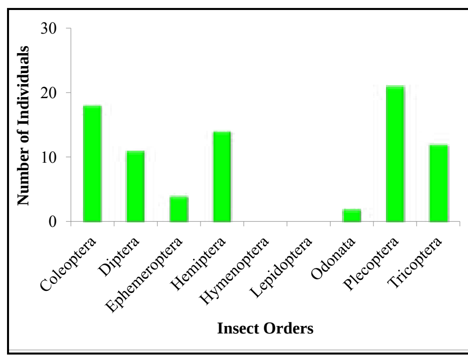

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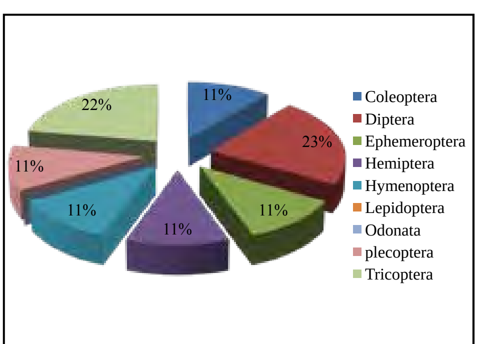

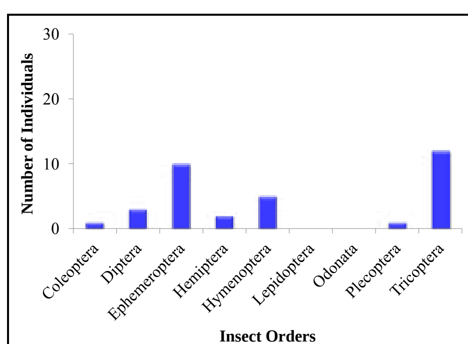

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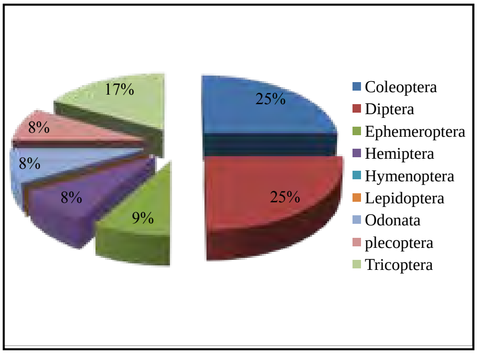

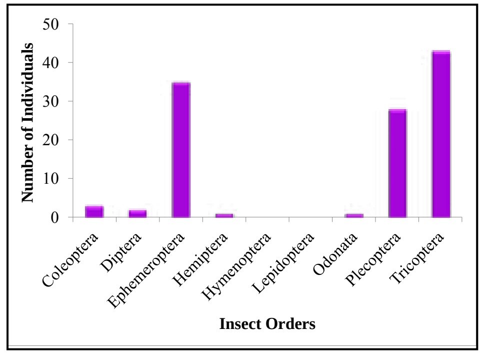

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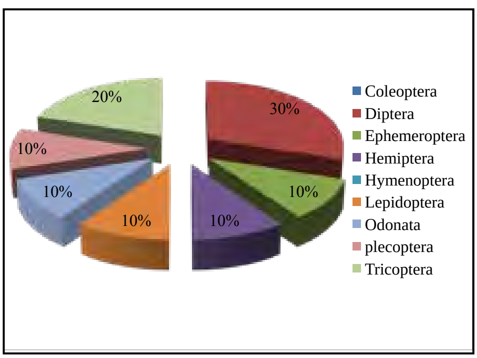

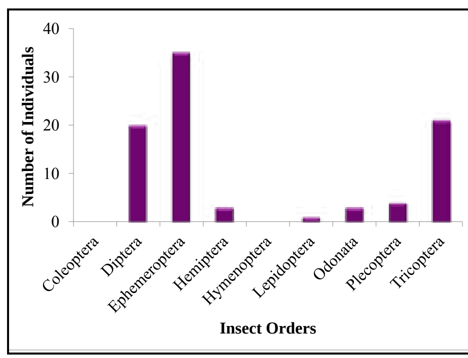

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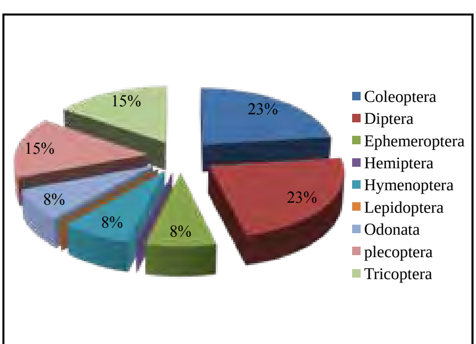

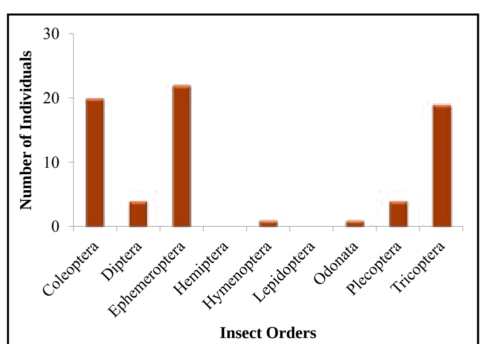

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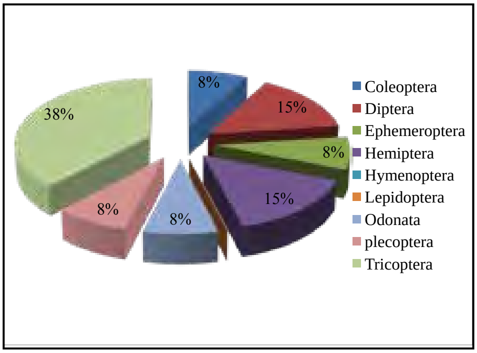

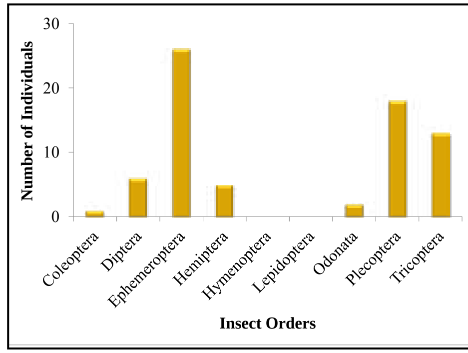

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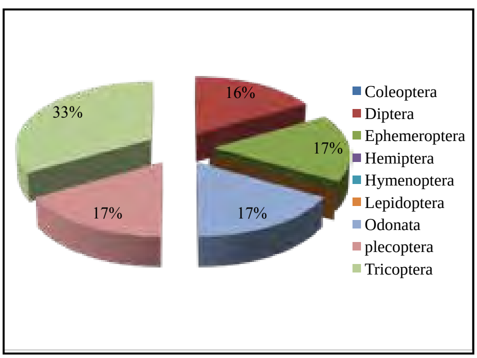

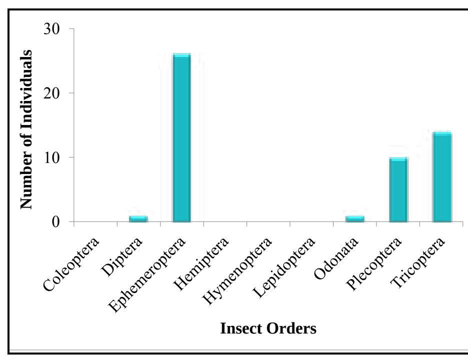

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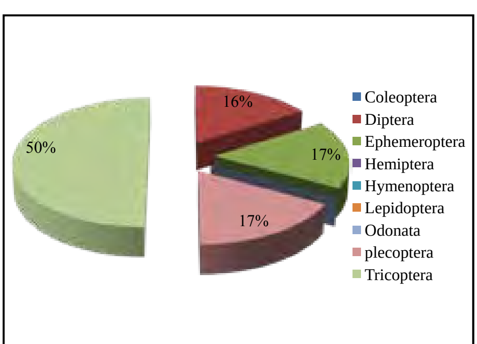

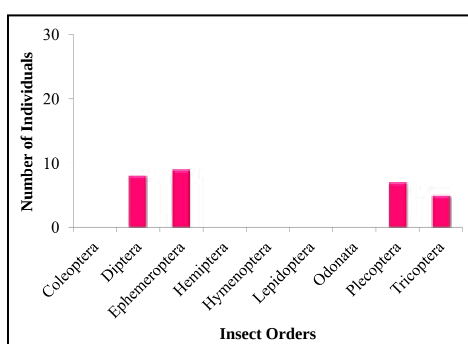

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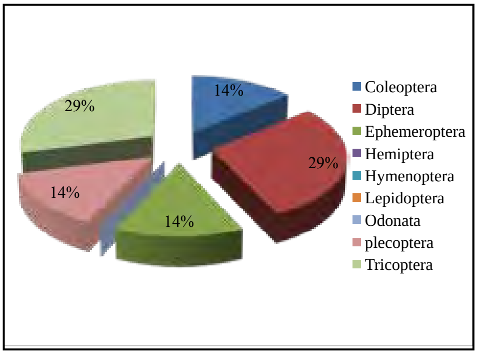

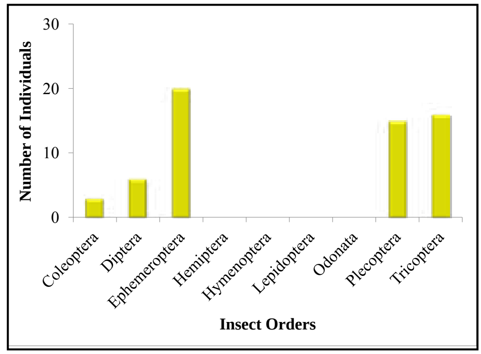

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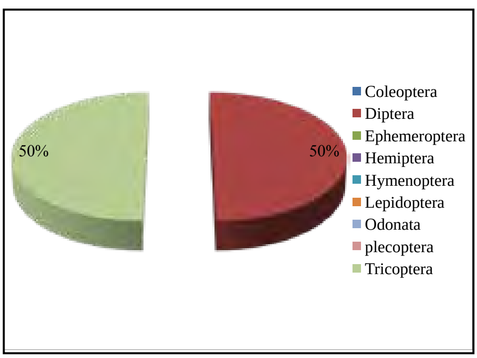
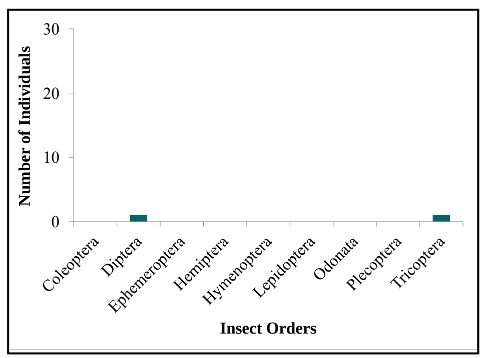




Appendix E

The correlation matrix among abundance of different taxonomic groups

	Col	Dip	Eph	Hem	Hym	Lep	Odo	Ple	Tri	Moll	Ann	Aci	Alk	pH	Amm	Nit	Nitr	Phos	Iron	DO	TDS	WT	Alt
Col	1.00	0.13	-0.19	0.27	0.05	0.13	0.33	0.04	0.25	-0.17	-0.11	-0.15	-0.17	0.17	-0.41	0.19	0.26	-0.12	.573*	-.484*	0.346	.519*	-.483*
Dip		1.00	.536*	0.01	-0.20	0.26	0.05	0.28	-0.22	-0.10	.590**	-0.37	0.25	0.30	.584*	-0.31	-0.06	.808**	-0.15	0.18	0.231	0.059	-0.121
Eph			1.00	-0.18	-0.18	0.30	0.23	0.39	0.38	-0.14	.550*	0.08	0.07	0.22	0.42	0.19	-0.09	.562*	0.03	-0.02	-0.112	0.207	-0.253
Hem				1.00	0.10	0.09	0.08	0.26	-0.05	-0.15	-0.11	-0.04	-0.30	0.13	-0.15	-0.12	0.02	0.04	0.18	-0.35	.593**	0.446	-0.456
Hym					1.00	0.02	-0.18	0.37	0.04	-0.18	-0.13	0.07	-0.30	0.14	0.00	0.02	.573*	-0.10	0.42	-0.38	.592**	0.45	-0.42
Lep						1.00	.662**	0.25	0.25	0.06	-0.08	-0.43	-0.31	0.34	0.34	0.26	0.01	0.08	0.12	-0.32	0.05	0.12	-0.16
Odo							1.00	0.02	.517*	-0.07	-0.06	-0.28	.491*	0.31	-0.16	.480*	-0.05	-0.12	0.16	-0.34	0.05	0.42	-0.28
Ple								1.00	0.23	-0.12	0.37	-0.17	-0.07	0.31	-0.05	-0.03	0.08	0.27	0.16	0.01	0.17	0.26	-0.35
Tri									1.00	-0.02	0.25	-0.01	-0.43	.474*	-0.41	.595**	0.30	-0.17	0.46	-0.42	0.09	0.42	-.544*
Moll										1.00	-0.17	0.04	-0.09	-0.20	-0.09	-0.10	-0.15	-0.19	-.469*	0.27	-0.35	-0.10	0.10
Ann											1.00	-0.12	0.17	0.46	0.32	-0.14	0.18	.787**	0.01	-0.02	0.06	0.09	-0.16
Aci												1.00	0.34	.634**	-0.15	0.16	-0.46	-0.21	-0.39	0.34	-0.39	-0.17	0.20
Alk													1.00	-.573*	0.42	-.497*	-.561*	0.24	-0.36	0.45	-0.44	.660**	.496*
pH														1.00	-0.06	0.18	.743**	0.35	.556*	-.476*	.602**	0.46	-.555*
Amm															1.00	-0.41	-0.14	.688**	-0.14	0.06	-0.08	-0.18	0.16
Nit																1.00	0.09	-0.37	0.29	-0.18	-0.08	0.37	-0.24
Nitr																	1.00	0.09	.701**	-.575*	.640**	.501*	-.568*
Phos																		1.00	-0.06	0.05	0.17	0.06	-0.08
Iron																			1.00	.808**	.601**	.578*	.694**
DO																				1.00	-.505*	.661**	.708**
TDS																					1.00	.642**	.728**
WT																						1.00	.886**
Alt																							1.00

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

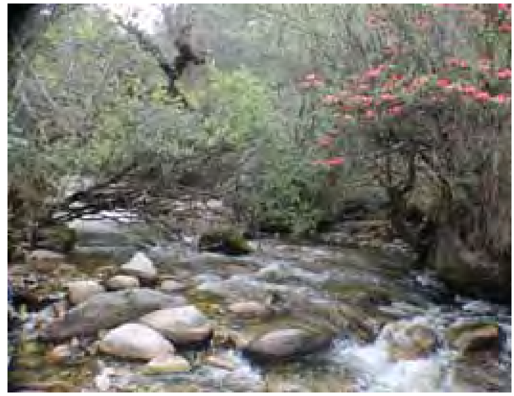
Correlation Matrix among abundance of different taxonomic groups and physico-chemical parameters of WCP. Abbreviations: Col= Coleoptera, Dip= Diptera, Eph= Ephemeroptera, Hem= Hemiptera,Hym= Hymenoptera, Lep= Lepidoptera, Odo= Odonata, Ple= Plecoptera, Tri= Tricoptera, Moll= Mollusca, Ann= Annelida, Aci= Acidity, Alk= Alkalinity, Amm= Ammonia, Nit= Nitrate, Nitr= Nitrite, Phos= Phosphate, DO= Dissolved Oxygen, TDS= Total Dissolved Solute, WT= Water temperature, Alt= Altitude.

Appendix F | Different physical parameters of the 18 sites in WCP

	Habitat	Microhabitat	Riparian Land use	Canopy Cover (%)	Altitude (m)	Bottom Substrate	Temperatute (°C)		pH	Width (m)	Depth (m)	TDS (ppt)	Transperency (m)	Conductivity (ppt)	Water current (m/s)	Dissolved oxygen (mg/l)
							Air	Water								
Site 1	River	Main River	Forest	35-40	2642	Rocky	23.7	11	6	10.4	0.457	0	0.457	47.5	1	8.3
Site 2	Stream	River flow	Forest	70	2770	Pebbles	21.5	10.1	6	15.7	0.406	0	0.406	52.1	0.9	10.2
Site 3	River	Buffer Zone	Community Forest	20-30	2694	Rocky, Pebbles	16.4	10.4	8.94	5.8	0.1778	34.9	0.1778	48.9	0.231644	10.2
Site 4	Dhur River	Rift/pool	Forest	10-11	2690	Rocky	19.6	6.7	8.43	15.19	0.9398	33.3	0.3302	46.9	0.63	9.47
Site 5	Minchu River	Confluence of two river, Minchu and Dhur	Forest covered, Small locality	5	2737	Rocky/ Algal growth on stones	15.4	7.4	8.6	10.24	0.3302	48.2	0.3302	67.8	0.5	12.2
Site 6	River	Steady flow	Natural	63	2742	Pebbles	20.09	12.1	8.4	20.7	0.5334	12.2	0.5334	18.1	0.6173	7.14
Site 7	Gangror stream	Small pool	Disturbed	60	1309	Rocky, sandy in some places	28	17	8.2	2.2	0.2032	77.5	0.2032	108.5	0.4	6.42
Site 8	Main river	River flow	Natural	38	1267	rocky	28.5	20	8.2	7.49	0.381	59.3	0.381	12.5	0.5357	6.8
Site 9	Main river	River flow	Natural	48	1289	rocky	25.5	19	8.7	15.74	0.6858	84	0.6858	115.8	0.4615	6.3
Site 10	Stream	Stream/ water pool	Bit disturbed	10	1302	rocky	29.2	15.2	9.9	6.5	0.3302	20.2	0.3302	28.4	0.2913	6.34
Site 11	Stream	Running stream	Bit disturbed	78	1975	small pebbles	21.4	13.5	9.4	4.6	0.2286	30.2	0.2286	42.4	0.4	6.23
Site 12	Stream-river confluence	Flowing water	Bit disturbed	0	1903	rocky-sandy mixture	20	15.5	8.5	4.4	0.3302	34.6	0.3302	48.2	0.4154	6.55
Site 13	Stream	Running stream	Bit disturbed	72	2744	Rocky, muddy in some places	17.5	12.6	7.01	6.45	0.406	11.3	0.406	0	2	10.6
Site 14	Main river	River flow	Natural	51	2639	Rocky, muddy in some places	19.1	9.1	7.12	10.18	0.609	16.8	0.559	0	0.66	10.5
Site 15	Main river	River flow/ water pool	Bit disturbed	13	2546	Rocky, muddy in some places	20.3	10.9	7.52	14.44	0.304	21.4	0.304	0	1.12	10.9
Site 16	Spring pool	Small pool	Natural	86	2241	Rocky, muddy in some places	15.3	11.4	6.14	5.4	0.214	33.1	0.305	0	0.18	9.2
Site 17	Main river	River flow	Natural	27	3774	Rocky	4	0	6.31	8.38	0.609	6.08	0.356	0	0.45	9.5
Site 18	Lake	Lake	Natural	0	4184	Rocky	3	0	7.22	500	0.356	5.49	xxx	0	0.04	10.5

PLATE 1 STUDY SITES

Site 1- Bumthang (below Thangbi)



Site 2 -Bumthang (Nasphel)

Site 3- Bumthang (Tang Pralang)

Site 4 -Bumthang (On the way to Dhur)

Site 5- Bumthang (Dhur Menchugang)

Site 6 -Bumthang (Dhur)

PLATE 2 STUDY SITES

Site 7- Lhuntshi (Gangzur)

Site 8 - Lhuntshi (Kurichu)

Site 9 - Lhuntshi (below Kurichu)

Site 10 - Lhuntshi (Stream joining Kurichu)

Site 11 - Trongsa (Nubi)

Site 12 - Trongsa (Mangdechu)

PLATE 3 STUDY SITES

Site 13 - Wangdue (Sephu)

Site 14 - Wangdue (Sephu)

Site 15 - Wangdue (Sephu)

Site 16 - Wangdue (Sephu)

Site 17 - Wangdue (Zowzasa valley)

Site 18- Wangdue (Dortsho)

PLATE 4 ORDER : COLEOPTERA

PLATE 5 **ORDER : DIPTERA**

Adult

PLATE 6 **ORDER : DIPTERA**

LARVAE

PLATE 7 **ORDER : TRICHOPTERA**

LARVAE

PLATE 8 **ORDER: EPHEMEROPTERA**

PLATE 9 ORDER : HEMIPTERA

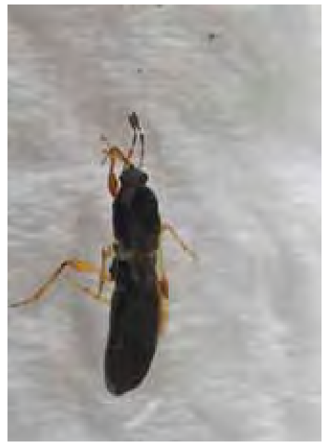
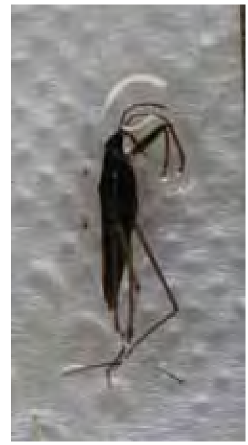
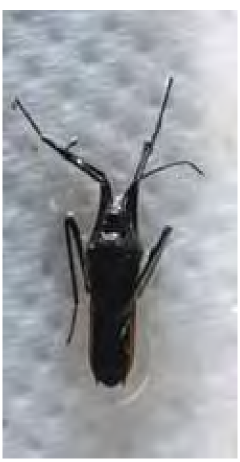






PLATE 10 ORDER : HYMENOPTERA

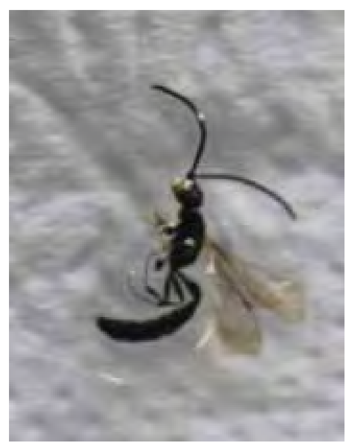


PLATE 11 **ORDER : LEPIDOPTERA**

PLATE 12 **ORDER : ODONATA**

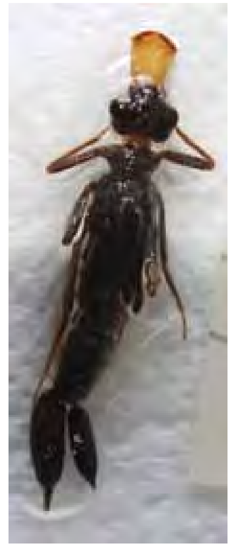
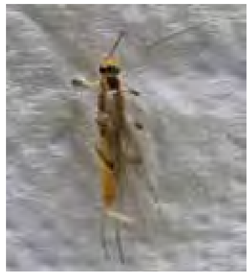
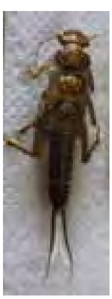
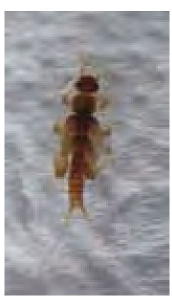


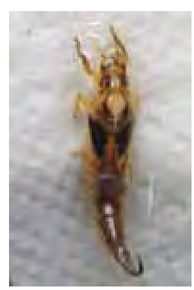
PLATE 13 ORDER : PLECOPTERA

Adult

LARVAE



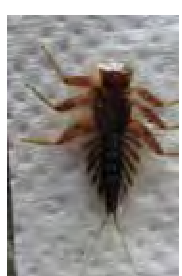
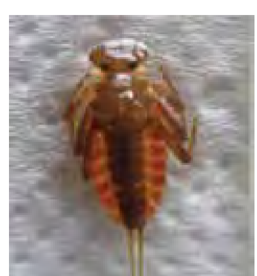




PLATE 14 **ORDER : MOLLUSCA**

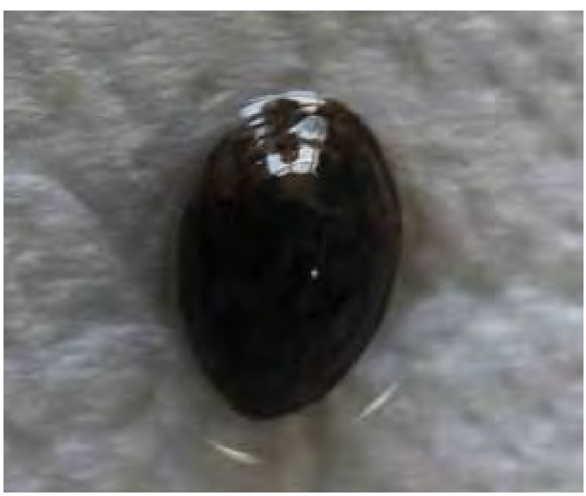


PLATE 15 **ORDER : ANNELIDA**

PLATE 16 ORDER : SALMONIFORMES

Family: ***Salmonidae***

Salmo trutta trutta **Linnaeus, 1758**

PLATE 17 ORDER : CYPRINFORMES

Family: ***Cyprinidae***

Schizothorax richardsonii **Gray, 1832**

PLATE 18 **ORDER : SILURIFORMES**

Family: ***Sisoridae***

Oreoglanis insignis **Ng and Rainboth, 2001**