CHAPTER I

1.1 Introduction

The simuliids or blackflies, commonly called buffalo-gnats and often called white sacs, are the insects of wide distribution. Running water seems to be preferable habitat of its immature stages from the sea level to about 4,000 m. This family contains about 1700 species in the world, classified into 24 genera of Subfamily Simuliinae and only a few are placed in the genus *Parasimulium* (Subfamily Parasimuliinae) (Crosskey 1999, Crosskey and Howard 1997). In the Oriental region, about 340 blackfly species of the genus *Simulium* Latreille s.l. are known so far; the largest group in the world of this family and under it there are nine subgenera (Takaoka 2003).

Blackflies are smaller (1.0 to 5.0 mm) and stout black insects with the hump-backed shiny thorax of black or grey or yellow color. Eye facets present on upper half of the head are equal in female but larger than those on the lower half in males. Antennae are shorter and cylindrical with 7-9 flagellar segments; wings are also shorter but broader with tubular veins only towards the leading edge and first abdominal segment modified to form a prominent flange (basal scale) fringed with hairs.

They are very annoying flies that can get into the eyes, ears and nostrils of many mammals. The flies swarm in the evening hours; attracted to the hosts from a distance by smell, heat and sight; then attack the exposed skin to suck blood. During the process some kind of anticoagulants inject into the feeding site, often cause mild to severe allergic reactions in sensitive individuals. Strong reactions include fever, nausea and allergic dermatitis. If large black-fly population increases and their bite reacts strongly it can be life threatening and is reported to kill domestic animals. Some species are even responsible for reduction in milk production and loss of weight of cattle, fall of egg production in poultry and even death of various domestic and wild animals.

The simuliids of Nepal is one of least known fauna. Lewis (1964, 1972) reported altogether four species of *Simulium* including *Simulium indicum* Becher, 1885 as a first report from the Southern slopes of Daulagiri Himal (2100 to 2200m), Nepal, *S. (Gomphostilbia) nepalense* Lewis from Taplejung with 2 unnamed species. Maskey

(1985, 1989 and 1998) also described five species of *Simulium* including *S. bagmaticum* from South-eastern region of Nepal, *S. hillycum* from the various localities in Nepal, *Odagmia vega* from Thankot khola in Kathmandu, *O. vega* A. from Godawari khola, Lalitpur and *O. vega* B. from Kirtipur khola, Kathmandu.

Though Maskey reported some species of *Simulium* from the Kathmandu valley, but no work has been done so far on their immature forms specially from the Shivapuri National Park. Hence present study on blackflies carried out aiming to impart knowledge on faunal distribution and abundance of immature stages of blackflies, species composition, monthly variation, altitudinal effects in distribution of species etc. and the affects in distribution of larvae and pupae regarding to different physico-chemical parameters of water (temperature, water current, DO and CO_2). This study therefore, is hoped to help the people to be aware of its vicious bite and nature of acting as a vector of various disease i.e. medical and veterinary importance to some extent.

1.2 Objectives

The objectives of present study are as follows:

1. Find out the species diversity and abundance of blackflies in the study site.
2. Detect the monthly variation in distribution of its immature stages.
3. Bring out the altitudinal effect on their distribution.
4. Distribution of larvae and pupae in different physico-chemical parameters of water (temperature, water current, DO and CO_2).

1.3 Limitations

1. Due to lack of sufficient taxonomic works in the context of Nepal, it is challenging to conduct study properly.
2. The study period is of shorter duration for complete informations.
3. Difficult to run the programme due to constrain budget.
4. Study site being a National Park, therefore, a great risk to work.

CHAPTER II

2.1 Literature Review

2.1.1 In the of Context SAARC Countries Including Nepal

It seems from the so far available literatures that D.J. Lewis of England was the first person working in these flies from Nepal describing *Simulium (Gomphostilbia) nepalense* in 1964. In 1972 he recorded the *S. (Himalayam) indicum* Becher based on male, female, pupal and larval characteristics and two other un-named species of this genus. Then Maskey (1985, 1989) described two species in the same genus (*S. bagmaticum* and *S. hillycum)* from South-eastern region of Nepal. Next, Maskey (1989) described three species of *Simulium* (*Odagmia vega, O. vega* A. and *O. vega* B.) from the Kathmandu rivers (Thankot khola, Kirtipur khola and Godawari khola) based on male, female, pupal and larval characteristics.

Lewis (1973) also reported *S. (Eusimulium) bulbosum, S. (Simulium) jani* and *S. (S.) rashidi* from Pakistan. In India, Datta (1973, 1974 and 1975) described *S. (E.) gracilis, S. (E.) praelargum, S. (E.) purii, S. (E.) nemorivagum, S. (E.) dasguptai, S. (E.) ghoomense, S. (G.) tenuistylum, S. (G.) darjeelingense, S. (S.) biforaminiferum, S. (S.) dentatum, S. (S.) himalayense* and *S. (S.) nigrifacies* from West Bengal and *S. (G.) bucolicum, S. (G.) fidum, S. (G.) litoreum, S. (G.) unum, S. (S.) kapuri* from Assam based upon male, female, pupal and larval specimens. Datta and Pal (1975) further described *S. (S.) singtamense* from West Bengal. Saito et al. recorded one species of *Simulium* from Northern Pakistan in 1989.

2.1.2 In the Global Context

Delfinado (1969, 1971) reported *Simulium (Gomphostilbia) ambigens, S. (Simulium) atrum, S. (S.) discrepans, S. (S.) forcipatum, S. (S.) palawanense, S. (S.) retusum, S. (S.) tomentosum* and *S. (S.) simulacrum* based on male, female and pupa collected from Philippines. Fryauff and Trpis (1986) worked on the identification of larva and adult *Simulium yahense* and *Simulium sanctipauli* based on species-specific enzyme markers and their distribution at different breeding habitats in Central Liberia. Baba and Takaoka (1990, 1991 and 1992) investigated the development and hatching of

eggs and larval growth for *Prosimulium yezoense* Shiraki, the oviposition habits, larval instars and growth pattern of univoltine blackfly *Prosimulium kiotense* in Kyushu, Japan. Similarly, both also observed the egg dormancy and embryogenesis of the same species in South-western Japan under natural and laboratory condition. Baba (1992) examined the development of immature stages and oviposition habits of *Simulium kawamurae*, with reference to seasonal changes in body size and fecundity from Okutake river a tributary of the Ono River and in the same year he also studied the oviposition site preference and preimaginal growth in *Simulium quinquestraitum* in Kin-un-kyo river, a tributary of the Yamaguni river, Oita Prefecture, Japan. In 1997 Kiel and Frutiger investigated the behavioural changes of four larval blackflies (*Simulium noelleri, S. ornatum (complex), S. trifasciatum* [syn. *S. spinosum*] and *S. variegatum*) to experimental reduction of dissolved oxygen with video techniques in the laboratory, during experiments lasting 120-140 min., dissolved oxygen (DO) was reduced to 0.3-0.6 mg within the first hour and kept constant for another hour. Water current in the laboratory channel was 135 cm/s. The experiment shows blackflies did not engage in undulative movements, and they neither increase locomotory activity, nor actively drift. Oxygen depletion mainly altered filter-feeding activity. This experiment results among the four species, *S. variegatum* was found to be the most sensitive species in respect to oxygen depletion, while *S. noelleri* seems to be the most tolerant species. Takaoka et al. (2003) investigated several *Simulium* species as to their biting habit and natural infections with filarial larvae at Ban Pan Fan, Chiang Mai Province, in Northern Thailand. Takaoka and Choochote (2005) described new species of the rare subgenus *Simulium (Montisimulium)* i.e *Simulium (Montisimulium) angkaense* and *S. (M.) laoleense, S. (M.) merga, S. (M.) surrachaii*, also reported species of *Simulium (Simulium)* i.e. *Simulium (Simulium) manoon, S. (S) doipuiense* (formerly *S. (S.)* sp. reported by Takaoka and Suzuki, 1984), , *S. (S.) lampangens, S. (S.) yuphae,* two new species of *griseifrons* species group of *Simulium (Simulium),*i.e. *S. (S.) phayonense* and *S. (S.) pukaengense* based on observation of females, males, pupae and mature larvae collected from Northern Thailand. They further described a new subgenus *Asiosimulium* within the genus *Simulium* and new species of *Simulium, Simulium oblongum* based on female, male, pupal and larval specimens collected from Thailand in the same year. In 2005, Takaoka and Saito described a new species of the black-fly *Simulium (Nevermannia) izuense* from Izu Islands, Tokyo, Japan;

Sato et al. revised the description of *Simulium (Nevermannia) sasai*, a rare and poorly known species from Kanagawa Prefecture, Honshu, Japan; Cai Ru et al. described a new species of *Simulium (Gomphostilbia) altayense* from Altay, Xinjiang, China; Azeveda et al. gave the identification key to pupae of Simuliidae (Diptera) recorded from South-eastern region of Brazil; Masako et al. studied the natural infection of *Simulium (Nevermannia) uchidai* with infective filarial larvae, probably from a bird were captured in mine collection inside a bear shed in Kyushu, Oita Prefecture, South-western, Japan; Sirin and Yalchin recorded 8 species, among which 6 of them are *Simulium (Wilhelmia) equinum* (Linnaeus, 1758), *S. (Nevermannia) augustipes* (Edwards, 1915), *S. (Boophthora) erythrocephalum* (De Geer, 1776), *S. (Simulium) noelleri* (Friederichs, 1920) and *S. (Simulium) fontanum* (Terteryan, 1952) from the tribe Simuliini and 2 are *Prosimulium (Prosimulium) rufipes* (Mergen, 1830) and *P. (Prosimulium) tomosvaryi* (Enderlein, 1921) from the tribe Prosimuliini for the first time in Turkey; Choohote et al. investigated the seasonal abundance and daily flying activity of blackflies at four different altitudes (400m, 860m,1360m and 2460m) in Doi Inthanon National Park, Northern Thailand while Kiel and Roder performed the gelelectrophoretic studies on labial gland secretions of immature blackflies (Diptera: Simuliidae) and found the secretion works as a biological adhesive which adheres to nearly any substrate surface, even to wood or the integument of other aquatic animals. Takaoka (2005, 2006) described *S. (G.) cagayanense*, three new species of *Simulium (Wallacellum)* i.e. *Simulium (Wallacellum) suyoense.*, *S. (W.) claveriaense* and *S. (W.) molawineme*, four species assigned to the *melanopus* species groups of the subgenus *Simulium* (*Simulium*) Latrella and also described the revise description of *Simulium (Simulium) forcipatum* Delfinado, for the first time and three new closely related species to *S. (S.) forcipatum* based on the samples collected from Ifugao and Mountain Provinces, Luzon Island, Phillippines. In 2006, Day discovered *Simulium (Nevermannia) cryophilum* Rubtsov at high tide mark in Fife; Further, Takaoka and Tenedero (2007) described two new species i.e. *Simulium (Gomphostilbia) mindoroense* assigned to the *ceylonicum* species-group and *S. (Simulium) halconese* assigned to the *melanopus* species-group and four newly recorded species i.e. *S. (S.) baltazarae* Delfinado, *S. (S.) tadense* Takaoka, *S. (Wallacellum) suyoense* Takaoka and *S. (W.) tuyese* Takaoka based on the adult, pupae and mature larvae from Mindoro Island, Philippines.

CHAPTER III

3.1 Study Area

The study site consisted inside and outside the Shivapuri National Park (ShNP) located on the Northern fringe of Kathmandu valley, 12 km away from the centre of capital city and coverage area of 144 sq. km. Before it was declared as National Park, its management remained under the Shivapuri Watershed Development Board established in 1976. The park comes under a transition zone between Subtropical and Temperate climates. Vegetation here consists of a variety of natural forest types including pine, oak, rhododendron, etc. depending on the altitude (DNPWC, 2006).

Water from the watershed is tapped from different streams and channeled through the pipelines to reservoirs. Of the two major watersheds of Shivapuri, Sundarijal subwatershed is bigger and more important for the drinking water supply, though Shivapuri Watershed which provides approximately one third of total water demand of Kathmandu valley.

3.2 Site Selection

The study was conducted at the Panimuhan, Southern Hill of ShNP locally called as Muhan Pokhari, located at upper range of Budhanilkantha. Three study sites were selected as I, II and III on the basis of pollution and shedding of the water and altitude as they are situated at the distance of 100m from one another.

Site I (altitude 1700m.) lies nearly 1.5 km away from site II. The forest *Schima-Alnus* type is dense. The stream is very narrow and fully covered with canopy, so stream is shaded. The substratum consisted of cobbles, gravel, sand with big boulders; numerous fallen leaves were found trailing over the stream.

Site II (altitude 1600m) lies just on the left side of the main entrance gate of the Park. It is a reservoir where water from the site I opens. Further the water from this reservoir is sending to another reservoir of Budhanilkantha and to Pani Pokhari, from where it supplies to Kathmandu valley. The site is open. Forest consisted of *Alnus* sp. The substratum here is composed of sandy bottom and submerged aquatic plant

(*Equisetum*, a Pteridophyte) seen at one edge of the stream, number of fallen leaves and decaying twigs are found floating over the stream while only few of cobbles and gravel were seen in this site.

Site III (altitude 1500m) open and partially shaded and run through human residential area so water remained mild polluted. Solid wastes like plastic, paper, bottles, tin were in the site. Substratum composed of sand, cobbles, gravel with large boulders, submerged aquatic plants and numbers of fallen leaves found trailing over the stream.

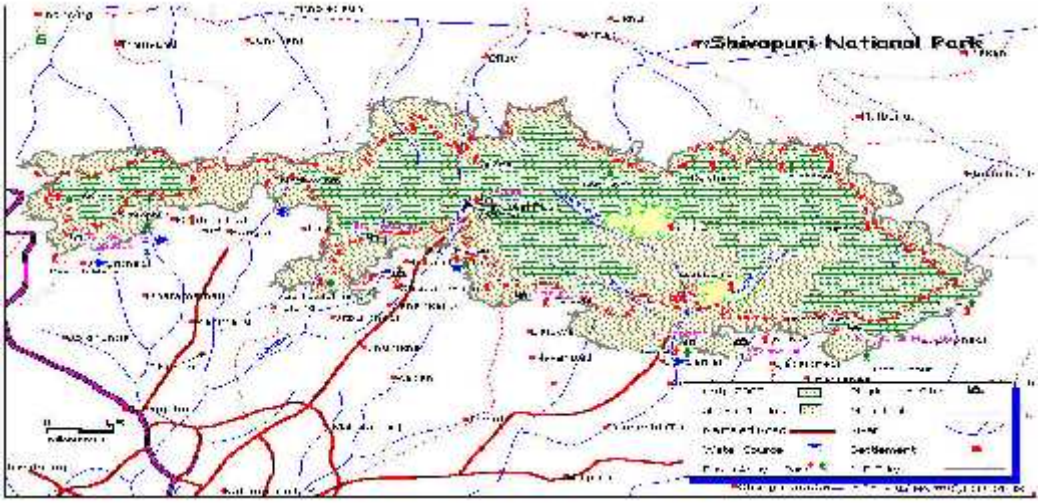

Figure 1: Map of Shivapuri National Park.

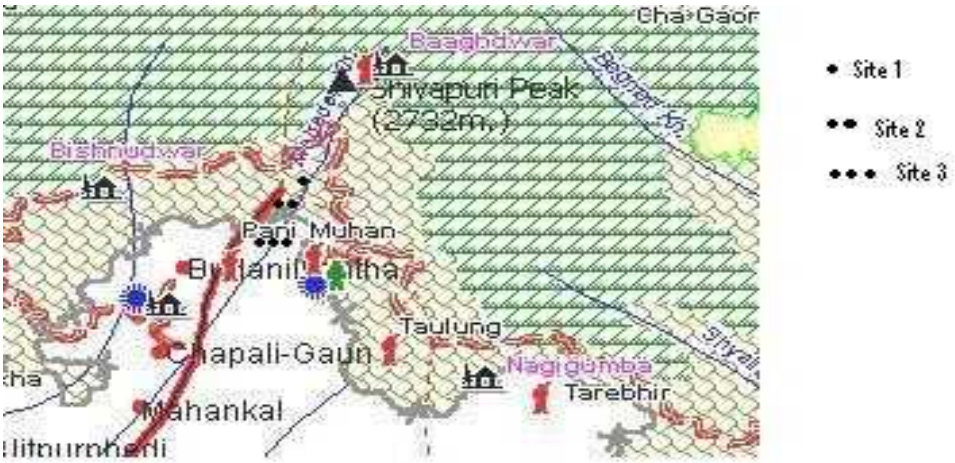

Figure 2: Panimuhan stream showing three study sites.

CHAPTER IV

4.1 Materials and Methods

The study was entirely based on larvae and pupae of blackfly specimens collected from study sites of the Park. Surveys were made during October to June (2007-2008). Following materials and methods are used during the study period.

4.1.1 Materials

The relevant materials are used during the study are listed below.

I.In handling the insects:

1. Plastic tubes and vials (10 × 1.7 cm)
2. Forcep -9
3. Scissors
4. Hand lens
5. Marker
6. Petri-dish
7. Olympus Stereoscopic Microscope
8. Olympus digital camera 7.0
9. Glycerine
10. Camel hair brush -6
11. Needle
12. Tissue paper
13. Altimeter
14. Note copy/ Pen/ Pencil
15. Olympus Lamp
16. Ethyl Alcohol 80%
17. Ethyl Alcohol 70%
18. NaOH or KOH

II. In the study of physico-chemical parameter of water:

1. Measuring tape
2. DO bottle
3. Burette
4. Conical flask
5. Beaker
6. KI
7. NaOH
8. Mercury thermometer
9. Pipette
10. Dropper
11. Measuring cylinder
12. $MnSO_4$
13. Conc.H_2SO_4
14. Phenolphthalein
15. Sodium-thio-sulphate

4.1.2 Methods

The following method was used during the study period .

4.1.2.1 Sampling method

Random sampling method was used for the collection of samples. Larvae and pupae were monthly collected within 30 minutes samplings from the stream of selected sites .Adults were obtained from the reared pupae but few females were collected in the field attracted to bait.

Method used in the field to collect the aquatic immature stages of blackflies and to obtain the reared adults was as follows:

The grass leaves, twigs, stones in running water from the selected sites were searched for larvae and pupae of blackfly within 30 minutes in each sampling period .The larvae if present living together with pupae were first removed and put in a small plastic vials containing 80% Ethyl Alcohol. The substrates were cut gently into pieces so that each single part harbored a single pupa. Then they were placed in plastic test tubes (10cm × 1.7 cm) with tissue paper soaked with little water at the bottom and plastic with small holes made by needle wrapped the test tube in the another end for the passage of air for the rearing pupa. It was made sure that single test tube contained single pupa. Similarly, pupae attached to the surface to stones or rocks were carefully removed by using sharp razor or forceps. Each of them was laid on a leaf and kept in the above-mentioned plastic tube. The tube containing individual pupa was kept in cold or shaded place and then wrapped with a wet towel to avoid exposure to the high temperature. They were kept so in the course of collection until adults emerged. In most of cases adults emerged within 3 days. Time of adult emergence was noted and then allowed to live for at least 24 hours. After coloration of some body parts (legs, hairs, and scutum) was examined by hand lens then these blackflies were each preserved in 70% Ethyl Alcohol with their pupal exuvia.

For identification, detailed morphological study on preserved mature larvae, pupae and adults were done under a stereoscopic dissecting microscope. The collected samples were preliminarily identified by using taxonomic keys published by Takaoka and Davies (1996), Takaoka (2004) and as per guidance of Prof. Dr. Hiroyuki Takaoka of Japan (Dept. of Infectious Disease Control, Oita University). For final reconfirmation of preliminarily identified specimen, the samples were sent to Dept. of Infectious Disease Control, Faculty of Medicine, Hasam, Yufu City, Oita, Japan.

4.1.2.2 Methods applied for the physico-chemical parameter of water

4.1.2.2.1 Water current

The surface float method was used to measure the water current or flow rate. It was measured in meter/second (m/sec)

Formula was used for calculation:

$$\text{Water current} = \frac{\text{Distance fixed (m)}}{\text{Time taken (sec)}}$$

4.1.2.2.2 Temperature

The surface temperature was measured directly by dipping the mercury thermometer bulb (graduated to an accuracy of 0 ± 1^{o} C) in water.

4.1.2.2.3 Width and height

Width and height of the streams were measured by measuring tape in every field visit.

4.1.2.2.4 Dissolved oxygen (D.O) (mg/L)

The D.O content of stream was determined by using Winkler's method. For its BOD (300ml) was filled with the sample water and stopper was placed carefully without trapping air bubbles. The D.O was fixed immediately by adding 2ml of $MnSO_4$ and 2ml of KI using separate pipettes. The stopper was then replaced, taking care to exclude the air bubbles. The bottle was shake thoroughly for well mixing. Brown precipitate was seen which was allowed to settle down. Half of this experiment was done in the field to trap the D.O of water and was brought to the laboratory (Central Department of Zoology), Kirtipur. Next, the precipitate was allowed to dissolve by adding 2ml of conc. H_2SO_4. Then, 50ml of sample water was taken in conical flask and was titrated against sodium-thio-sulphate solution (0.025N) using starch solution as an indicator. At the end point, initial dark blue color changes to colorless. The volume of titrate used in getting the end point was noted. D.O was calculated in mg/L by using the following equation.

$$\text{Dissolved Oxygen} = \frac{(\text{ml} \times \text{N}) \text{ of titrant} \times 8 \times 1000}{\frac{V_2 (V_1 - V)}{V_1}}$$

Where, V_1 = Volume of BOD bottle

V_2 = Volume of titrated sample

V = Volume of $MnSO_4$ and KI added

4.1.2.2.5 Carbondioxide (mg/L)

The sample of 100ml was taken in a clean conical flask and a few drops of phenolphthalein as indicator was added to it. If the color turns pink, free CO_2 is absent. If the sample remains colorless; it was immediately titrated against sodium hydroxide (NaOH - 0.05N) until pink color appears. The mean volume of three readings was taken and converted later into mg/L by using following equation:

$$\text{Free } CO_2 \text{ (mg/L)} = \frac{(\text{ml of titrant} \times \text{N}) \text{ of NaOH} \times 1000 \times 44}{\text{ml. of sample used}}$$

4.1.3 Statistical analysis

4.1.3.1 Species diversity index

Species diversity was calculated by using following the formula:

$(\because\ pi = ni/N)$

$H_{max} = \text{Log } K'$

$H' = -\ \Sigma\, pi\, (\ln pi)$

$e = \frac{H'}{H_{max}}$

Where,

Pi = Proportion of Individual of i th species to the no. of individuals of all the Species (ni/N)

H_{max} = Maximum possible diversity

H' = Shannon – Wiener diversity index

N = Total number of individuals of all species

Ni = No. of individuals of species

K = No. of species.

e = Relative density/Evenness index.

4.1.3.2 Correlation coefficient test:

Correlation coefficient (r) was used to determine the significance of mutual relationship between physicochemical parametes of water (temperature, water current, dissolved oxygen and carbon dioxide) and number of blackfly species collected by using Karl Pearson product moment formula (Gupta, 1990).

$$r = \frac{\Sigma xy - \frac{\Sigma x.\Sigma y}{n}}{\sqrt{\Sigma\, x2 - \frac{\Sigma x2}{n} \times \Sigma\, y2 - \frac{(\Sigma y)2}{n}}}$$

Where,

x = dependent variable (no. of blackfly species)

y = independent variable (temperature, water current, width and height of water, dissolved oxygen and carbon dioxide etc)

r = correlation coefficient

n = pair of observation

CHAPTER V

5.1 Keys for Identification

5.1.1 Keys to subgenera of *Simulium*

Mature larvae:

1. Last abdominal segments lack ventral papillae ….……Subgenus ***Simulium (S.)***

   Last abdominal segments with ventral papillae...…….……. 2

2. Postgenal cleft very small or vestigial ……...Subgenus ***Montisimulium (M.)***

   Postgenal cleft well defined, small to large …….……..…3

3. Postgenal cleft shorter than post genal bridge; lateral serration of hypostomium developed ……..…… …….Subgenus ***Nevermannia (N.)***

   Postgenal cleft longer than post genal bridge; lateral serration of hypostomium undeveloped ……...…..…..Subgenus ***Gomphostilbia (G.)***

Pupae:

1. Gill with 8 or 10 filaments arranged in 3+3+2 (rarely 2+4+2) or 3+3+2+2 from dorsal to ventral; grapnel-like hooklets present on last abdominal segment …….……Subgenus ***Gomphostilbia (G.)***

   Gill with 12 or 14 filaments arranged in pairs ...Subgenus ***Montisimulium (M.)***

   Gill with 4, 6, 9 or 10 filaments arranged in pairs; grapnel-like hooklets absent on last abdominal segment …….2

2. Cocoon with anterodorsal projection ……. Subgenus ***Nevermannia*** (***N.)***

Cocoon with simple wall-pocket-shaped or shoe-shaped ……
……...Subgenus ***Simulium*** (***S.***)

5.1.2 Keys to species of *Simulium* in Nepal:

Pupae

1. Gill of inflated form with 8 slender filaments……***(G.) gombakense***

   Gill of filamentous ……..…….. 2

2. Gill with 4 filaments …….……. ……...***(N.) sp.3***

   Gill with 6 or more filaments …….……...3

3. Gill with 6 filaments …….…… 4

   Gill with 8 or more filaments ……....…… 8

4. Gill filaments much longer than pupal body, arranged in 4+2 filaments from dorsal to ventral ..……..… 5

   Gill filaments as long as or shorter than pupal body, arranged in 2+2+2 filaments ……..……6

5. Cocoon with an anterodorsal projection ……...……. ……***(N.) sp.2***

   Cocoon without such a projection …… ……***(N.) sp.1***

6. Cocoon shoe - shaped ……..…… ……***(S.) indicum***

   Cocoon wall- pocket-shaped ……..…7

7. Cocoon with short anterodorsal projection …….…… ***(N.) aureohirtum***

   Cocoon without anterodorsal projection ……..……..8

8. Angle formed by dorsal most filament and lower most one 90 degrees or less ……..……***(S.) sp.3***

Angle formed by dorsal most filament and lowermost one over 90 degrees
…… ……..***(S.) vegas and /or (S.) sp.1***

9. Gill with 8 filaments ……..……..…….…... 10

Gill with 10 filaments or more …….……..….……11

10. Gill filaments arranged in 3+3+2 filaments from dorsal to ventral
.…….. ……***(G.) sp.1***

Gill filaments arranged in 2+2+2+2 filaments from dorsal to ventral
……..…..…***(S.) hillycum and /or (S.) sp.2***

11. Gill with 10 filaments ……..…. ……..... ***(S.) bagmaticum***

Gill with 12 or 14 filaments ……..……...12

12. Gill with 14 filaments ……..…..……..…...….... ***(M.) sp. 1***

Gill with 12 filaments ……..…….. …….***(M.) sp.2***

*S. (G.) nepalense was not included because its pupa was not known.

Mature larvae

1. Ventral papillae present …….……..…….2

Ventral papillae absent…….……..…… 8

2. Postgenal cleft very small, vestigial ….……..…….…….…….3

Postgenal cleft well defined, small to large ...……..4

3. Pharate pupal gill with 14 filaments .…….……...……..***(M.) sp.1***

Pharate pupal gill with 12 filaments …….……. ……***(M.) sp.2***

4. Postgenal cleft shorter than postgenal bridge ……..…..…….…….. 5

Postgenal cleft nearly as long as or longer than postgenal cleft……. …..……6

5. Abdomen with reddish-brown markings dorsally …....***(N.) sp.1 and (N.) sp.2***

Abdomen without color markings ……. …...***(N.) sp.3***

6. Abdomen with reddish-brown markings dorsally …….....… ..……***(G.) sp.1***

Abdomen without color markings ……..…… 7

7. Head capsule with distinct well-defined head spots ….……...***(N.) aureohirutum***

Head capsule with faint head spots …….. ***(G.) gombakense***

8. Postgenal cleft long, reaching posterior border of hypostoma……..***(S.) indicum***

Postgenal cleft not reaching posterior border of hypostoma ……..……9

9. Abdomen with paired protuberances dorsally…….….***(S.) bagmaticum***

Abdomen without any protuberances dorsally ..……..…..…...10

10. Head capsule yellowish, with well defined head spots……
…….……***(S.) vegas and/or (S.) sp.1***

Head capsule brownish, with indefinite head spots…….…… 11

11. Postgenal cleft narrow, pointed apically …….....…… ***(S.) sp.3***

Postgenal cleft wide, rounded apically……. ***(S.) hillycum and/or (S.) sp.2***

***** S. (G.) nepalense is not included because its larval stage was not known.

***** Given keys were prepared by Prof. Dr. Hiroyuki Takaoka (Dept. of Infectious Disease Control, Oita University, Japan.)

CHAPTER VI

6.1. Results

6.1.1 Distribution and abundance of blackflies during the study period

A total of 1860 larvae grouped to five species belonging to four subgenera, 301 adults reared from pupae belonging to ten species and 8 adults of *Simulium Simulium indicum* attracted to human bait were collected during study period from October, 2007 to June, 2008. Although more or less blackflies were collected in each month, but their distribution varied in three different sites in different months.

To calculate the relative abundance of individual species, the diversity index method was applied. Diversity indices were based upon the relationship between total no. of individual or species. The most frequently used diversity index is Shannon's index (H').

Table: 1. Distribution and abundance of blackfly larvae collected during different months in site I.

S. N	Blackflies Species	Months of Collection									Total *ni*	*pi* = *ni/N*	*pi* ln *pi*	%
		Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun				
1.	*S. (Simulium)* sp.	1	3	10	8	3	19	16	49	6	115	0.300	- 0.361	30
2.	*S.(Nevermannia) feuerborni* gr.	-	-	-	-	-	4	4	-	-	8	0.020	- 0.078	2
3.	*S. (Nevermannia) vernum* gr.	6	5	3	1	10	12	23	22	2	84	0.219	- 0.332	21.9
4.	*S (Gomphostilbia)* sp.	7	2	9	3	3	11	22	51	65	173	0.451	- 0.359	45.1
5.	*S.(Montisimulium)* sp.	-	-	-	-	1	-	2	-	-	3	0.007	- 0.034	1.5
									Total		N=383			
								H'=− Σ *pi* ln *pi*				1.164		
								e				0.72		

In Site I, 383 larvae were sorted to five species belonging to four subgenera i.e *Simulium, Nevermannia, Gomphostilbia* and *Montisimulium* were collected. Among

which 115 larvae of *S. (S.)* sp. under subgenera *Simulium*, 84 *S. (N.) vernum* gr. under *Nevermannia* and 173 larvae under *Gomphostilbia* were collected from all months during study period, where as 8 *S. (N.) feuerborni* gr. under *Nevermannia* were found only in the months March and April and 3 *S. (M.)* sp. under *Montisimulium* were collected from February and April.

From the calculation, S. (*G.)* sp. was found most abundant while *S. (M.)* sp. was found least abundant.

Table: 2. Distribution and abundance of blackfly pupae and adults collected during different months in site I.

State of Coll.	Blackflies Species	Months of Collection									Total *ni*	*pi* = *ni/N*	*pi* ln *pi*	%
		Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun				
Pupae	*S. (G.)* sp.1, *ceylonicum* gr.	-	-	-	-	2	2	2	1	2	9	0.9	- 0.094	90
Pupae	*S.(G.) gombakense*	-	-	-	-	-	-	-	-	-	-	-	-	
Pupae	*S.(N.)* sp.1, *feuerborni* gr.	-	-	-	-	-	-	-	1	-	1	0.1	- 0.230	10
Pupae	*S.(N.)* sp.2, *feuerborni* gr.	-	-	-	-	-	-	-	-	-	-	-	-	
Pupae	*S.(N.)* sp.3, *vernum* gr.	-	-	-	-	-	-	-	-	-	-	-	-	
Pupae	*S.(M.)* sp.1	-	-	-	-	-	-	-	-	-	-	-	-	
Pupae	*S.(M.)* sp.2	-	-	-	-	-	-	-	-	-	-	-	-	
Pupae	*S.(S.)* sp.1, *variegatum* gr.	-	-	-	-	-	-	-	-	-	-	-	-	
Pupae	*S.(S.)* sp.2, *multistriatum* gr.	-	-	-	-	-	-	-	-	-	-	-	-	
Pupae	*S.(S.)* sp.3, *tuberosum* gr.	-	-	-	-	-	-	-	-	-	-	-	-	
Adult	*S.(S.) indicum*	-	-	-	-	-	-	-	-	-	-	-	-	
									Total		N=10			
									H'= - Σ *pi* ln *pi*				0.324	
									e				0.46	

Only 10 blackflies under two species i.e. *S. (G.)* sp.1, *ceylonicum* gr. and *S. (N.)* sp.1, *feuerborni* gr. were collected reared from pupae. Among these two species *S. (G.)* sp.1, *ceylonicum* gr. was collected from the months February to June while only one individual of *S. (N.)* sp.1, *feuerborni* gr. was collected only in May.

From the calculation, *S. (G.)* sp.1, *ceylonicum* gr. was found most abundant while *S. (N.)* sp.1, *feuerborni* gr. was found least abundant.

Table: 3. Distribution and abundance of blackfly larvae collected during different months in site II

S. N	Blackflies Species	Months of Collection									Total *ni*	*pi* = *ni/N*	*pi* ln *pi*	%
		Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun				
1.	*S. (Simulium)* sp.	34	22	22	77	158	105	98	1	-	517	0.672	-0.267	67.23
2.	*S.(Nevermannia) feuerborni* gr.	-	-	-	-	-	-	-	-	-	-			
3.	*S. (Nevermannia) vernum* gr.	-	2	6	-	4	31	11	7	6	67	0.087	-0.212	8.71
4.	*S (Gomphostilbia)* sp.	11	5	4	-	-	20	17	111	17	185	0.240	-0.342	24.05
5.	*S.(Montisimulium)* sp.	-	-	-	-	-	-	-	-	-	-			
									Total		N=769			
								H'=− Σ *pi* ln *pi*				0.821		
								e				0.74		

In Site II, 769 larvae grouped to three species under three subgenera *Simulium* , *Nevermannia* and *Gomphostilbia* were collected. Among which 517 larvae of S. (S.) sp. belonging to subgenera *Simulium* were collected in all months except June, 67 larvae of *S. (N.) vernum* gr. under *Nevermannia* were seen in all except October and January, where as 185 larvae of *S. (G.)* sp. under *Gomphostilbia* were found in all except January and February.

From the calculation, *S. (S.)* sp. was found most abundant and *S. (N.) vernum* gr. was least abundant.

Table: 4. Distribution and abundance of blackfly pupae and adults collected during different months in site II.

State of Coll.	Blackflies Species	Months of Collection									Total *ni*	*pi* = *ni/N*	*pi* ln *pi*	%
		Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun				
Pupae	*S. (G.)* sp.1, *ceylonicum* gr.	-	3	3	1	3	4	-	-	10	24	0.167	-0.298	16.78
Pupae	*S.(G.) gombakense*	-	-	-	-	-	-	-	-	1	1	0.006	-0.030	0.69
Pupae	*S.(N.)* sp.1, *feuerborni* gr.	-	-	-	-	-	-	-	-	-	-		-	
Pupae	*S.(N.)* sp.2, *feuerborni* gr.	-	-	-	-	-	1	-	-	-	1	0.006	-0.030	0.69
Pupae	*S.(N.)* sp.3, *vernum* gr.	-	-	-	-	-	7	-	-	-	7	0.048	-0.145	4.89
Pupae	*S.(M.)* sp.1	-	-	-	-	-	-	-	-	-	-	-	-	
Pupae	*S.(M.)* sp.2	-	-	-	-	-	-	-	-	-	-	-	-	
Pupae	*S.(S.)* sp.1, *variegatum* gr.	-	-	-	-	-	5	-	-	-	5	0.034	-0.114	3.49
Pupae	*S.(S.)* sp.2, *multistriatum* gr.	-	-	-	-	-	-	-	-	-	-	-	-	
Pupae	*S.(S.)* sp.3, *tuberosum* gr.	-	9	10	16	14	47	1	-	-	97	0.678	-0.263	67.83
Adult	*S.(S.) indicum*	-	-	-	-	-	-	-	1	7	8	0.055	-0.159	5.5
									Total		143			
									H'= $-\Sigma$ *pi* ln *pi*				1.039	
									E				0.533	

In site II, 135 blackflies under six species i. e. *S. (G.)* sp.1, *ceylonicum* gr., *S. (G.) gombakense*, *S. (N.)* sp.2, *feuerborni* gr., *S. (N.)* sp.3, *vernum* gr., *S. (S.)* sp.1, *variegatum* gr., *S. (S.)* sp.3, *tuberosum* gr. were collected reared from pupae and only 8 adults of *S. (S.) indicum* were collected attracted to human bait.

Out of 135 pupae, 24 pupae of *S. (G.)* sp.1, *ceylonicum* gr. were collected in all months except October, April and May. Similarly, 97 *S. (S.)* sp.3, *tuberosum* gr., were collected nearly from all months except October, May and June, 1 *S. (N.)* sp.2, *feuerborni* gr., 7 *S. (N.)* sp.3, *vernum* gr. and 5 *S. (S.)* sp.1, *variegatum* gr. were collected from March, while only one individual of *S. (G.) gombakense* was found in June and 8 adults of *S. (S.) indicum* were collected only from the months May and June attracted to human bait.

From the calculation, *S. (S)* sp.3, *tuberosum* gr. was found most abundant while *S. (G) gombakense* and *S. (N.)* sp.2, *feuerborni* gr. *were found* least abundant among all species in site II.

Table: 5. Distribution and abundance of blackfly larvae collected during different months in site III.

S. N	Blackflies Species	Months of Collection									Total *ni*	*pi* = *ni/N*	*pi* ln *pi*	%
		Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun				
1.	*S. (Simulium)* sp.	6	3	15	17	55	67	168	99	129	559	0.789	-0.186	78.95
2.	*S.(Nevermannia) feuerborni* gr.	-	-	3	5	11	-	3	-	-	22	0.031	-0.107	3.10
3.	*S. (Nevermannia) vernum* gr.	-	6	14	24	18	9	-	-	1	72	0.101	-0.231	10.16
4.	*S (Gomphostilbia)* sp.	-	12	4	6	10	3	-	-	-	35	0.049	-0.147	4.94
5.	*S.(Montisimulium)* sp.	-	-	2	2	6	10	-	-	-	20	0.028	-0.100	2.82
									Total		N=708			
								H'=− Σ *pi* ln *pi*				0.77		
								e				0.479		

In site III, a total of 708 larvae grouped to five species under four subgenera *Simulium, Nevermannia a*nd *Gomphostilbia* and *Montisimulium* were collected. Among which 559 larvae of *S. (S.)* sp. belonging to *Simulium* were collected in all months, 22 larvae of *S.(N.) feuerborni* gr. under subgenera *Nevermannia* were found in December, January, February, and April, 72 larvae of *S. (N.) vernum* gr. under subgenera *Nevermannia* were seen in all except October and January, 35 *S. (G.)* sp. under *Gomphostilbia* were found in all except October, April, May and June where as 20 *S. (M.)* sp. under *Montisimulium* were collected from the months December, January, February and March.

From the calculation, *S. (S.)* sp. was found most abundant and *S. (M.)* sp. was found least abundant species.

Table: 6. Distribution and abundance of blackfly pupae and adults collected during different months in site III.

State Coll.	Blackflies Species	Months of Collection									Total *ni*	*pi* = *ni/N*	*pi* ln *pi*	%
		Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun				
Pupae	*S. (G.)* sp.1, *ceylonicum* gr.	-	-	-	-	-	6	2	-	-	8	0.051	-0.151	5.12
Pupae	*S.(G.) gombakense*	-	-	-	-	-	-	-	-	-	-	-	-	-
Pupae	*S.(N.)* sp.1, *feuerborni* gr.	-	-	-	-	-	-	-	-	-	-	-	-	-
Pupae	*S.(N.)* sp.2, *feuerborni* gr.	-	-	-	-	-	8	3	-	-	11	0.070	-0.186	7.05
Pupae	*S.(N.)* sp.3, *vernum* gr.	-	-	-	-	4	6	-	-	-	10	0.064	-0.175	6.41
Pupae	*S.(M.)* sp.1	-	-	-	-	-	-	1	-	-	1	0.006	-0.030	0.64
Pupae	*S.(M.)* sp.2	-	-	-	-	-	5	-	-	-	5	0.032	-0.110	3.20
Pupae	*S.(S.)* sp.1, *variegatum* gr.	-	-	-	-	-	29	59	-	9	97	0.621	-0.295	62.17
Pupae	*S.(S.)* sp.2, *multistriatum* gr.	-	-	-	-	-	5	-	-	-	5	0.032	-0.110	3.2
Pupae	*S.(S.)* sp.3, *tuberosum* gr.	-	-	-	-	3	14	2	-	-	19	0.121	-0.255	12.17
Adult	*S.(S.) indicum*	-	-	-	-	-	-	-	-	-	-	-	-	-
									Total		N=156			
									H'= - Σ *pi* ln *pi*				1.312	
									E				0.630	

In site III, 156 blackflies under eight species i. e. *S. (G.)* sp., *ceylonicum* gr., *S. (N.)* sp.2 *feuerborni* gr., *S. (N.)* sp.3, *vernum* gr., *S. (M.)* sp.1, *S. (M.)* sp.2, *S. (S.)* sp.1 *variegatum* gr., *S. (S.)* sp.2, *multistriatum* gr. *and S. (S.)* sp.3, *tuberosum* gr. were collected reared from pupae. Among which 8 *S. (G.)* sp.1, *ceylonicum* gr. and 11 *S. (N.)* sp.2, *feuerborni* gr. were collected only from March and April, 10 *S. (N.)* sp.3, *vernum* gr. were found in February and March, only one individual of *S. (M.)* sp.1, and *5 S. (M.)* sp.2 were collected from the months April and March respectively. 5 *S. (S.)* sp.2, *multistriatum* gr. were collected only from March. 19 *S. (S.)* sp.3 *tuberosum*

gr. were collected from the months February, March and April where as 97 *S. (S.)* sp.1, *variegatum* gr. were collected from March, April and June.

From the calculation, *S. (S.)* sp.1 *variegatum* gr. was found most abundant while *S. (M.)* sp.1 was found least abundant species in site III.

6.1.2 Monthly variation in distribution of blackflies in different sites

A total of 1860 larvae were categorized into five species under four subgenera and 301 (adults and adults reared from pupae) under eleven species were collected from three different sites during the study period. The variation in the distribution of blackflies were shown in figures 3, 4, 5 and 6 shows the no. of blackflies (larvae), no. of species on the basis of larvae collected, no. of blackflies (pupae and adults) and no. of species on the basis of pupae and adults collected in different months.

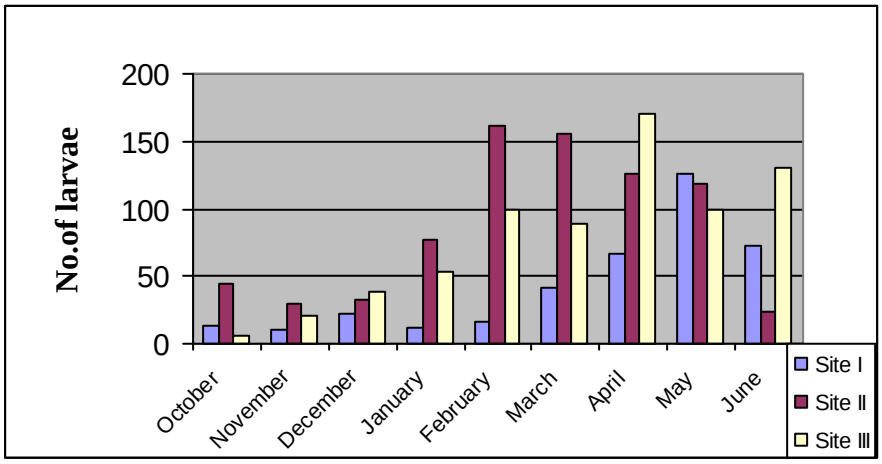

Figure: 3. No. of larvae collected in different months from study sites.

A total of 1860 larvae were collected from different sites. Number of larvae were found larger in site II in compare with site I and site III in October, November, January, February and March. Similarly, number of larvae were relatively larger in site III in the months December, April and June where as their number were found least in site I except in May among to that of site II and site III.

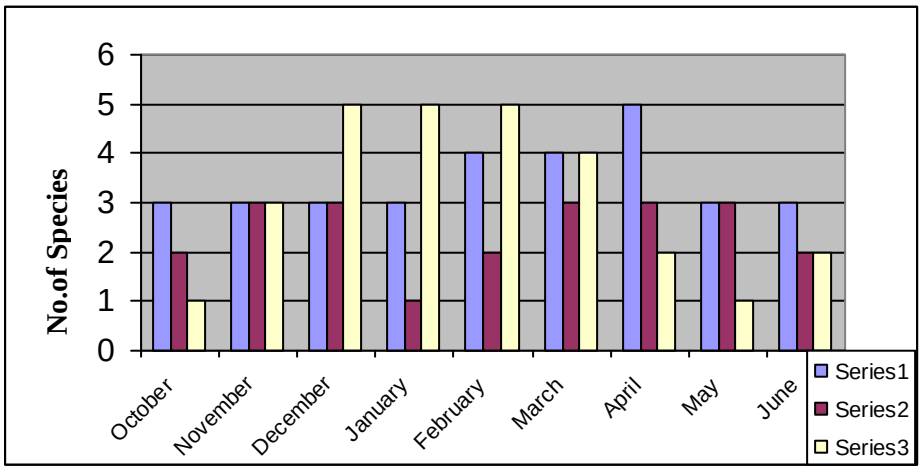

Figure: 4. No. of species sorted on the basis of larvae collected in different months from the study sites.

Five species of blackfly were grouped under four subgenera on the basis of larvae collected from the study sites during the study period, all the five species were collected in December, January and February from the site III and in April from site I, while only one species was recorded in October and May from site III and in January from site II.

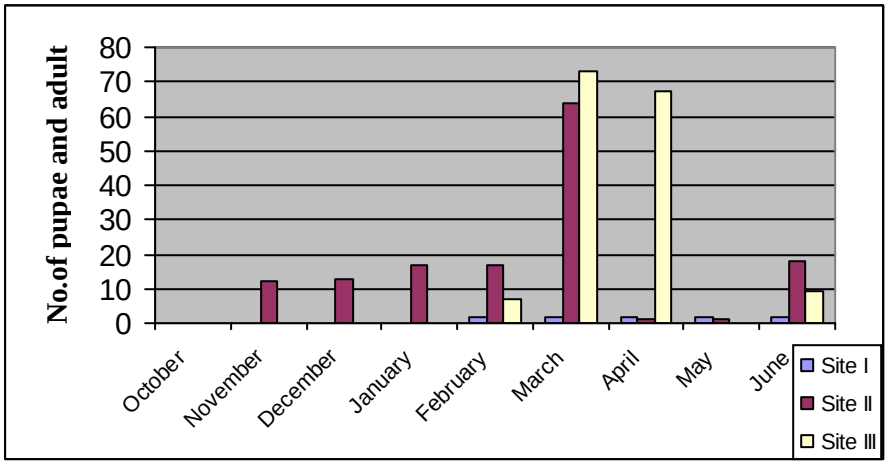

Figure: 5. No. of pupae and adults collected in different months from the study sites.

A total of 309 blackflies (pupae and adult) were collected from different sites. In March and April larger no. of blackflies were collected from site III, similarly in site II, their maximum number were appeared in March and average number were found

in the months November, December, January, February and June respectively, while only a few number of blackflies were found in site I in the months February, March, April ,May and June.

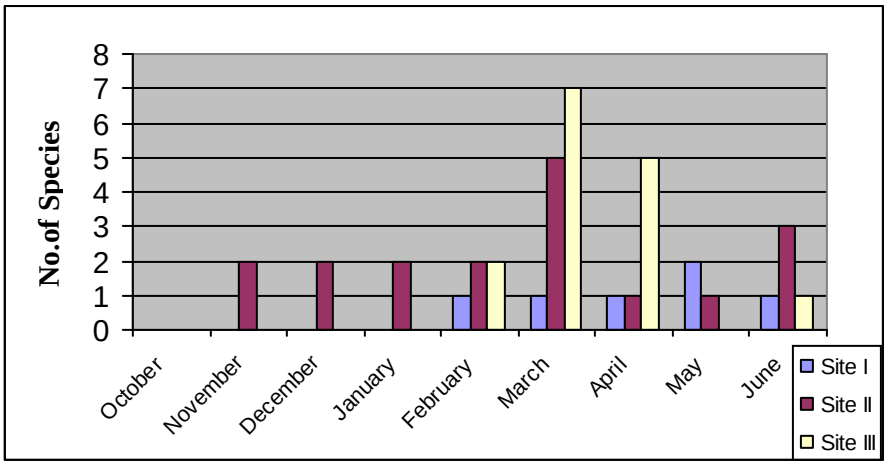

Figure: 6. No. of species recorded on the basis of pupae and adult collected in different months from the study sites.

A total of eleven species were recorded from the study sites based on collected pupae and adults during the study period. In March and April, larger number of species were recorded from site III. Similarly, from site II number of species were found larger only in month March, where as number of species in site I were found least among site II and site III in different months except in May.

6.1.3 Altitudinal distribution pattern of black-flies

Distribution of blackflies (larvae, pupae and adults) were studied in the range of altitude in between 1500m -1700m from sea level.

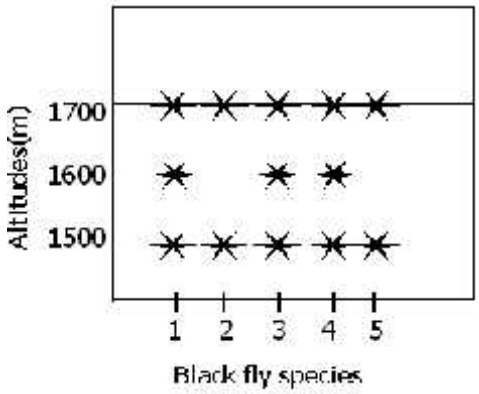

Figure: 7. Altitudinal distribution of blackfly species on the basis of larva

1. *S. (S.)* sp.
2. *S. (N.) feuerborni* gr.
3. *S. (N.) vernum* gr.
4. *S. (G.)* sp.
5. *S. (M.)* sp.

A total of 1860 larvae grouped to five species belonging to four subgenera were collected during study period. Among which larvae of *S. (S.)* sp., *S. (G.)* sp. and *S. (N.) vernum* gr. were found common in all altitude i.e. 1700m, 1600m and 1500m, where as larvae of *S. (N.) feurborni* gr. and *S. (M.)* sp. were found absent from site II i.e. 1600m.

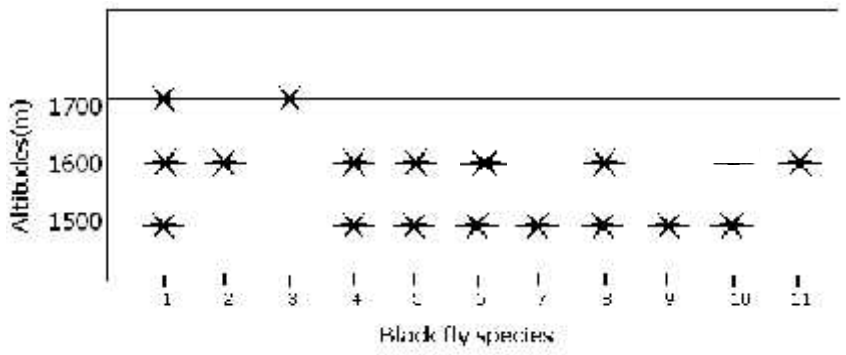

Figure: 8. Altitudinal distribution of blackfly species on the basis of pupa and adult

1. *S. (G.)* sp.1, *ceylonicum* gr.
2. *S. (G.) gombakense*
3. *S. (N.)* sp.1, *feuerborni* gr.
4. *S. (N.)* sp.2, *feuerborni* gr.
5. *S. (N.)* sp. 3, *vernum* gr.
6. *S. (S.)* sp.1, *variegatum* gr.
7. *S. (S.)* sp.2, *multistriatum* gr.
8. *S. (S.)* sp.3, *tuberosum* gr.
9. *S. (M.)* sp.1
10. *S. (M.)* sp.2
11. *S. (S.) indicum*

Eleven species belonging to four subgenera were recorded from collected pupa and adults during the study period from the three different sites at the altitude of 1700m, 1600, and 1500m .

Among these species, *S. (G.)* sp.1, *ceylonicum* gr. was observed common in all the altitude, *S. (N.)* sp.1, *feuerborni* gr. was collected only from site I, *S. (G.) gambakense* and *S. (S.) indicum* were collected only from site II, similarly *S.* (*S.*) sp. 2, *multistritum* gr., *S. (M.)* sp.1 and *S. (M.)* sp.2 were collected only from site III whereas *S. (N.)* sp.2 *feuerborni* gr, *S. (N.)* sp.3, *vernum* gr., *S. (S.)* sp.1 *variegatum* gr. and *S. (S.)* sp.3, *tuberosum* gr. were found common in site II and site III. (i.e. 1600m, 1500m)

6.1.4. To assess the pupae and larvae with the physico-chemical parameter of water

The results of physico-chemical parameters of water at different sites are given in Table 7. Temperature, water current, D.O, free carbon dioxide, width and height of the stream were measured during the study period from October, 2007 to June, 2008. The average values of physico-chemical parameters during the study period were calculated. Temperature of was found 12.26-16.16^{0}C, while width and height were 71.67-77.71 cm and 7.43 - 9.46 cm respectively. Current of water was 0.31- 0.58 m/sec, D.O was found 7.41- 8.06 mg/l and Free Carbon dioxide was found 3.76 - 4.23 mg/l.

Table: 7. Average values of physico-chemical parameters of different sites.

S.N	Physico-chemical parameters	Site I	Site II	Site III
1.	Temperature	12.26	13.27	16.16
2.	Water current (m/sec)	0.36	0.58	0.31
3	Width (cm)	76.4	71.67	77.71
4.	Height (cm)	8.12	9.46	7.43
5.	D.O (mg/L)	8.06	7.82	7.41
6.	Free CO_2 (mg/L)	3.76	3.97	4.23

Correlation coefficients of different physico-chemical parameters and no. of pupae and larvae collected in different sites were calculated and are shown in Table 8.

The value of r for pupae and larvae in site I and site III was found to be positive with D.O, value of r for larvae in site I and site III was also noticed positive with temperature, shows the significant result with D.O and temperature, where as the value of r for larvae in site I was found negative with water current, implies the increase in number of larvae at lowering the water current.

But in site II, the value of r for pupae and larvae with physico-chemical parameter of water (temperature, water current, D.O and free carbon dioxide) were found near to zero shows, no. of pupae and larvae were not affected by temperature, water current, D.O and carbon dioxide.

Table: 8. Coefficient of Correlation between the variables.

S.N	Physico-chemical parameter	No. of pupae			No. of Larvae		
		Site I	Site II	Site III	Site I	Site II	Site III
1.	Temperature	0.31	-0.27	0.36	0.50	-0.21	0.49
2.	Velocity	-0.02	0.026	0.224	-0.74	-0.008	-0.061
3.	Dissolved oxygen	0.518	0.068	0.562	0.484	0.386	0.450
4.	Free Carbon dioxide	-0.29	0.153	0.299	0.040	0.155	-0.223

CHAPTER VI

6.1 Discussion

In present study, blackflies fauna in the stream of Shivapuri National Park were explored. During the study period from October, 2007 to June, 2008, a total of 1860 larvae categorized under five species i.e. *S. (S.)* sp., *S. (N.) feuerborni* gr., *S. (N.) vernum* gr., *S. (G.)* sp. and *S. (M.)* sp., 301 pupae sorted to ten species i. e. *S. (G.)* sp.1, *ceylonicum* gr., *S. (G.) gombakense*, *S. (N.)* sp.1, *feuerborni* gr., *S. (N.)* sp.2, *feuerborni* gr., *S. (N.)* sp.3, *vernum* gr., *S. (S.)* sp.1, *vareigatum* gr., *S. (S.)* sp.2, *multistriatum* gr., *S. (S.)* sp.3, *tuberosum* gr., *S. (M.)* sp.1, *S. (M.)* sp.2 and 8 adults of *S. (S.) indicum* under four subgenera *Simulium*, *Nevermannia*, *Gomphostilbia* and *Montisimulium* were collected from three different sites at the altitude of 1700m, 1600m, and 1500m respectively.

Distributions and abundance of species were varied on the basis of larvae, pupae and adults collection in three different sites in different months during study period. In site I, April and May became the most suitable months for larvae as max. number of species and max. no. of larvae were observed while for pupae variation in month wasn't observed as single species of blackfly in equal number was appeared from February to June, though two species were observed in May. Hence from no. of collection of larvae and pupae, it results *S. (G.)* sp. and *S. (G.)* sp.1, *ceylonicum* gr. were most abundant species in site I where as Shannon's diversity index was calculated as H' =1.16 for larvae and H' = 0.32 for pupae. In site II, March, April, and May were found the most favorable months for larvae and only March for pupae as max. no. of species and number of larvae and pupae were collected while only a few adults were collected in May and June from the same site. Hence from the no. of collection of larvae and pupae, it showed *S. (S.)* sp. and *S. (S.)* sp.3, *tuberosum* gr. were the most abundant species where as Shannon diversity index was calculated as H'= 0.82 for larvae and H'= 1.03 for pupae.

In site III, Feb and April appeared to be the most suitable months for the distribution of species for larvae and March and April for pupae. Based on the no. of collection of larvae and pupae *S. (S.)* sp. and *S. (S.)* sp.2, *vareigatum* gr. were found the most

abundant species, where as Shannon diversity index was calculated as H'= 0.77 for larvae and H'= 1.16 for pupae.These value indicate species diversity of blackflies based on larvae were high in site I and low in site III while the opposite results indicate species diversity based on pupae were found high in site III and low in site I.

Relationship between Simuliidae species and environmental variables were explored by canonical correspondence analysis (Ter Braak, 1987). *Simulium (S.) variegatum* appears to be dependent on high current velocities and oxygen levels (Feld et al., 2002) and thus frequently occurs at higher altitudes. *S. (S.) bukovskii* prefers shaded regions in running waters and dense riparian vegetation, while *S. (S.) kerisorum* and *P. (P.) pronevitschae* prefer cold mountain streams (Rubstov, 1956). *Tetisimulium bezzii* occurs over a wide range of altitudes from lowlands to about 2500 m in Morocco (Guidicelli and Dia, 1986). This species is hemi-stenothermal and eurystenothermal and it is widely distributed over Mediterranean Europe in middle and lower regions of mountain rivers in Italy and France (Guidicelli and Dia, 1986; Dorier, 1963). Specimens of *T. bezzii* were collected from the hyporhitron of streams between altitudes of 650 and 900 m in this study. Thus, altitudinal variation may be the one of most important factor for the distribution of blackfly fauna. And abundance or dominance of single species in three different sites shows the richness of same species in same locality. Each species of blackfly is associated with a particular habitat and geographic area (Adler, P.H. et al., 2004).

Increase in no. of larvae with increase temperature and increase in no. of both larvae and pupae with increase in D.O in site I and site III may be due to its significant relation with these factor, while change in no. of larvae and pupae in site II was not remarkable with variation in temperature and D.O. Behavior of 4 larval blackflies were found to cease when D. O was reduced to 0.3-0.6 mg but their tolerance was found differ in different species was investigated by Kiel, E. and Frutiger, A. (1997). In present study water current didn't show any significant relation except larvae of site I which was negatively correlated implies increase in no. of larvae with lowering water current. It is known that some species, such as *Simulium costatum*, are very resistant to low stream velocity (Jensen, 1997), but blackfly larvae commonly do not develop in streams with a velocity of less than 0.1 – 0.2 m/s (Rubstov, 1956). Simuliidae species are an important component of the fauna of aquatic insects in most

types of running waters and can serve as indicators of structural and physico-chemical quality of habitats (Feld et al. 2002). Hence, physico-chemical parameter has significant role in the distribution of black-fly fauna.

Among these eleven species, *S. (G.)* sp.1, *ceylonicum* gr. can be compared with *S. (G.) nepalense* Lewis,1964, *S. (S.)* sp.1 *variegatum* gr. with *Odagmia vega* Maskey, 1989, *S. (S.)* sp.2, *multistriatum* gr. with *Simulium hillycum* Maskey, 1989 and *S. (S.) indicum* with *S. (Himalayum) indicum* Becher, 1885 (subgenus *Himalayam* has been suck as a synonym of *Simulium* by Ostuka et.al. 2003, therefore, it is now shown that *S.* (*S.*) *indicum*) on the basis of pupal and adult characteristics while, *S. (G.) gombakense*, pupae of this species was found to have a pair of gills of inflated form with 8 slender filaments arising on the inflated portion, *S. (N.)* sp.1 *feuerborni* gr., pupae of this species having 6 gill filaments arranged in 4+2 from dorsal to ventral and cocoon is simple, *S. (N.)* sp.2, *feuerborni* gr., pupae of this species having 6 gill filaments arranged in 4+2 from dorsal to ventral and cocoon with an long anterodorsal projection, *S. (N.)* sp.3, *vernum* gr., pupae of this species having 4 gill filaments arranged in 2+2, *S. (S.)* sp.3, *tuberosum* gr., pupae of this species having 6 gill filaments arranged in 2+2+2, dorsal most gill pair make an angle 90 degree or less with the lower most ventral pair, *S. (M.)* sp.1, pupae of this species having 14 gill filaments arranged in (2+2+2)+(1+1[2+2])+2 and *S. (M.)* sp.2, pupae of this species with 12 gill filaments arranged in 2+([1+2]+[2+1])+(2+2) are completely new recorded species for Nepal.

Blackflies are of considerable medical importance and cause a lot of distress to mankind, due to blood feeding nature of females, this family includes serious pest of man and animals in many countries. Females of some species transmit filarioid nematodes of the genus *Onchocerca* to human, cattle and deer, the genus *Dirofilaria* to bears, the genus *Splendidofilaria* to ducks and blood protozoans of the genus *Leucocytozoon* to birds (Crosskey, 1990; Kettle,1990). In Asia, five species were reported as vectors of three bovine *Onchocerca* species in Japan (Takaoka et al. 1992; Takaoka 1994) and one species as vector of an unknown *Onchocerca* species in Thailand (Takaoka et al. 2003).

In the present study, out of these 11 species, *S. (G.)* sp.1, *ceylonicum* gr. close to *S. (G.) asakoae*, which has been reported as a vector of unknown filaria in Northern

Thailand (Ishii et al. 2008), *S. (N.)* sp.3, *vernum* gr. close to *S. (N.) uchidai* which has been reported as a vector of unknown filaria in Japan (Fukuda et al. 2005), *S. (S.)* sp.1, *vaiegatum* gr. close to *S. (S.) aokii*, which has been reported as vector of bovine filaria in Northern Japan (Takaoka et al. 1992) and *S. (S.) indicum* Becher, 1885 close to *S. (S.) nigrogilvum,* which has been reported as a vector of unknown filaria in Northern Thailand (Fukuda et al. 2003). Hence further research and investigation of these species should be conducted to determine whether they play as a vector of filariae in Nepal.

CHAPTER VII

7.1 Conclusion

Based on the study of blackflies from the study sites following conclusions were made.

1) Five species based on larvae, ten species based on pupae and only one species based on adult were observed from samples collected during study period.
2) Out of eleven species , *S. (G.) gombakense*, *S. (N.)* sp.1, *feuerborni* gr., *S. (N.)* sp.2, *feuerborni* gr., *S. (N.)* sp.3, *vernum* gr., *S. (S.)* sp.3, *tuberosum* gr., *S. (M.)* sp.1 *and S. (M.)* sp.2 are new records to Nepal.
3) Species diversity was calculated on the basis of larvae and pupae collected from different sites showed the highest value of H'= 1.16 for larvae in site I showing greater diversity of blackfly (larvae) while, highest value of H'= 1.31 for pupae in site III, showing greater diversity of blackfly (pupae).
4) Abundance of individual species in different sites showed richness of same kind of species in same locality.
5) March, April and May appeared the suitable months for the study of blackfly as maximum no. of larvae and pupae were observed during the study period from October to June.
6) No. of species based on larvae and pupae were varied in different sites, probably due to altitudinal variation.
7) No. of larvae in site I and site III showed significant relation with water temperature and dissolved oxygen, while no. of larvae and pupae in site II showed insignificant relation with water temperature and dissolved oxygen.

CHAPTER VIII

8.1 Recommendation

From the study on the blackflies in the stream of Shivapuri National Park, following recommendation were made to the concerned authorities and further researches in this field.

1. Nepal is a beautiful Himalayan country with both Oriental and Palearctic climates, thus rich in insect fauna. Hence, further research works should be carried out to document the blackfly fauna all over the country.
2. Blackflies are reported as a vector of many unknown filaria in different countries, so further investigation of the relevant species to determine whether they play as a vector of filaria in Nepal should be carried out.
3. Blackflies being a vector, thus detail study on biology and habitat distribution is strongly recommended.
4. Awareness program among the people about blackfly and its role as vector should be launched.
5. Government should give emphasis for the research of vectors on National level.

CHAPTER IX

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ANNEX
Annex 1.

Table: 9. Distribution of blackflies (larvae) in different months.

Site/Month	Site I	Site II	Site III	Total
October	14	45	6	65
November	10	29	21	60
December	22	32	38	92
January	12	77	54	92
February	17	162	100	279
March	42	156	89	287
April	67	126	171	364
May	126	119	99	344
June	73	23	130	226
Total	383	769	708	1860

Table: 10. Species (on the basis of larvae) distribution in different months.

Site/Month	Site I	Site II	Site III	Total
October	3	2	1	6
November	3	3	3	9
December	3	3	5	11
January	3	1	5	9
February	4	2	5	11
March	4	3	4	11
April	5	3	2	10
May	3	3	1	7
June	3	2	2	7
Total	31	22	28	81

Table: 11. Distribution of black-flies (pupae and adults) in different months.

Site/Month	Site I	Site II	Site III	Total
October	0	0	0	0
November	0	12	0	12
December	0	13	0	13
January	0	17	0	17
February	2	17	7	26
March	2	64	73	139
April	2	1	67	70
May	2	1	0	3
June	2	18	9	29
Total	10	143	156	309

Annex 2.
Plates

i. Larva of *Simulium* (*Simulium*) sp.

ii. Larva of *S.* (*Gomphostilbia*) sp.

iii. Larva of *S.* (*Montisimulium*) sp.

iv. Larva of *S.* (*Nevermannia.*) *vernum* gr.

v. Larva of *S.* (*N.*) *feuerborni* gr.