CHAPTER I

1. INTRODUCTION

1.1 Background of the black-flies

Common name of the simuliids is black-flies, even called buffalo gnats or Turkey's gnat. These flies are common inhabitant of the most flowing water bodies and distributed widely in all the Zoogeographical regions occurring from sea level to over 4,000m. The simuliids include about 1,700 species in the world (Crosskey, 1990) but there about only 340 recorded species in the Oriental region mostly of the genus *Simulium* (Takaoka et al. 2003). In Nepal, therefore, it occurs in Terai to the mid hills up to the Mahabharat Lekh and like mosquitoes to which they are closely related, the females are annoying pest sucking the blood of other animals (Coscavon and Laura, 2003), although the males feed mainly on nectars.

The adult fly is smaller (1.0#5.0mm), dark grey or yellow in colour with a characteristic humped- thorax, therefore, its appearance gives them their common name buffalo gnat. Wings are shorter, broader with tubular veins only towards the leading edge and antennae are shorter. Eye facets are present on upper half of the head and equal in females but larger on the lower half in male. Thorax is ovate, short, highly arched, bare or with sparse hairy processes. Abdomen is broader with 7# 8 segments.

Immature stages (egg, larva and pupa) are aquatic in exclusively lotic water. Larva is a soft skinned, cylindrical but with enlarged basal portion. It attaches on substratum by the radial rows of strong hooks as well as labial secretion (Kiel and Roder, 2005). Cephalic fine fans on the anterior part of the body help them to feed on microscopic diatoms and other similar microbes.

The black-flies pupate in a silken cocoon usually of brown colour. Fully-grown adult emerges out from the pupal cage, which is carried up to the surface in air bubble, and it is able to fly off at once. The flies swarm in the evening hours. Females of certain species are vector of the filarial nematods (Reeves,et al.2007) and certain protozoans (*Leucocytozoan* spp, *Trypanosoma* spp and *Wolbachia*

spp etc) . Therefore, they play an important role in carrying certain parasitic organisms like *Onchocera, Dirofilaria, Spendidofilaria* or the protozoans.

1.2 Need of the present study

The black –fly being the important freshwater fauna of the running streams which serves as indicators of structural and physico – chemical quality of habitat (Feld et al.,2002). And, realizing economic importance of these flies as mentioned, present study was carried out in three different rivers (Godawari, Nakkhu and Karmanasa) of Lalitpur. Its diversity in the district was not formerly recorded from the river though Maskey (1998) reported *Odagmia vega* (veriety A) from the Godawari (Lalitpur) river and other species from Kathmandu (Gokarna and Kirtipur) but she did not include the present sites for her study. At the same time, the black-flies being a common vector in Kathmandu, so its diversity and abundance was important to know and physico-chemical parameters of the present study sites are little different from other flowing rivers of Kathmandu. Moreover, the research sites were accessible for the investigator to work.

Present work on black-fly is about its status, species distribution, composition as well as the relation of the physico-chemical parameters with the biology of flies which could be the foundation step to spread knowledge on this group. The study was also emphasized on distribution of immature stages and their monthly variations, etc. Therefore, the study hopes to bring awareness about medical and veterinary importance of the fly among the people.

1.3 Limitation of study

1. Due to lack of sufficient time complete information on the chosen topic could not be collected.
2. Difficult in field collection due to human activities in rivers where study was carried out.
3. Budget constrain, so difficult to carry the detail study.
4. Challenging taxonomic works due to lack of sufficient works done in Nepal.
5. Could not identify most of the collected specimens due to lack of standard identifying keys and subject experts both nationally and internationally.

1.4 Objectives of study

Main objective of the present study was to find out the monthly variation of larval, pupal and adult populations of black-fly in Lalitpur District. Other specific objectives are to:

1. Find out the diversity of these flies in the study sites.
2. Compare the population of immature and adults stages in the three study sites and
3. Find out the relations of black-flies in different aquatic parameters (temperature, water current, Dissolve Oxygen, free Carbon - dioxide in water).

CHAPTER II

2. LITERATURE REVIEW

Broad study about the black- flies has been done throughout the world but it is at the minimum point in Nepal.

2.1 Nepal Publications.

From all the literatures on black-flies, it has been known that Lewis, D.J (1964) of England was the Pioneer to study from Nepal and described *Simulium (Gomphostilbia) nepalensis* for the first time. Then in 1972, he reported *Simulium (Himalayam) indicum* Becher and un-named species of the same genus on the basis of male, female, larval and pupal characteristics. Maskey (1985, 1989) was next personnel to explain two species of the same genus (*Simulium bagmaticum* and *S. hillycum*) from the south-eastern part of Nepal. Furthermore in 1998, Maskey reported *Odagmia vega*, *O.vega A* and *O.vega* B of the same genus from Thankot, Kirtipur and Godawari streams on the basis of characteristics of both males and females as well as the larval and pupal characteristics.

2.2 Asian publications.

From Pakistan, Lewis, D.J (1973) reported the *Simulium. (Eusimulium) bulbosum, S. (Simulium) jani* and *S. (S.)rashidi.* In India, Datta (1973, 1974 and 1975) collected specimen from different regions and studied the male, female, larval and pupal characteristics. He identified them as *Simulium (E.)gracilis, S.(E.)praelarum, S.(E.)purii, S.(E)nemarivagum, 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.) fidecum ,S.(G.) litoreum , S.(G.) unum, S.(S.) kapuri* from Assam. Again in 1975, Datta and Pal described *S. (S.) singtamense* from West Bengal.

Baba (1992), Takaoka (1990, 1991 and 1992), Takaoka (1994, 2003, 2005, and 2006) and Hirako (2005) were the distinct workers on black-flies from Japan. They did an experiment on the development of eggs and larval growth of *Prosimulium yenzoense* Shiraki, the *oviposition* habits, larval instars and growth patterns of the univoltine black-fly, *Prosimulium kiotense.* Baba (1992) worked on the development of immature stages and ovipositions of *Simulium kawamure* with reference to the seasonal body size changes. In the same year, he worked on oviposition site and pre-imaginal growth of *S. quinquestraitum* in Kinunkyo river of Japan. Hirako et al (2005) revised the description of a rare and poorly known species i.e., *S. (N.) sasai* from Japan. Takaoka and Saito (2005) collected the new species *S. (N) izuense* from Japan.

In 2003, 2005 unlimited works were carried out in other parts of Asia like Takaoka et al. (2003) recorded several *Simulium* species with filarial larva in Northern parts of Thailand. Similarly, Takaoka and Choochote (2005) described new species of black-flies on the basis of female, male, pupa and mature larva from North Thailand. Choochote et al. (2005) explain the seasonal abundance and daily activities of adult black-flies at four different altitudes (400m, 860m, 1360m and 2,460m) in Doi Inthanon National Park, North Thailand.

2.3 Publications from Europe and South America

Kiel and Frutiger (1997) examined the behavioral changes of larvae of four black-fly species (*Simulium noelleri, S. ornatum* complex*, S.trifasciatum (syn. S.spinusum))* and *Simulium variegatum* in the laboratory condition. Experiment resulted the *S. variegatum* as the most sensitive species in respect to oxygen depletion and the *S.noelleri* as the most tolerant species. In 2003, Pond and Werner collected *Simulium chutteri* Lewis 1965, associated with *Xenomyia osculate* (Diptera: muscidae) from Brazil. Coscavon and Laura (2003) described the black-flies of European countries as notorious worldwide pest of human beings, livestock's and poultry.

In 2004, Creadie et al. investigated the spatial temporal distribution of pre-imaginal black-flies in Brazil and Venezuela. Furthermore Malmqvist did

hypothesis testing on egg, number and size of black-flies and concluded over wintering larvae produced more eggs than over wintering egg of black-flies. At the same time Adler et al. (2004) reviewed on ecological role of black-flies and option available for their management. Credie et al. (2005) of Europe calculated correlation between immature stages of black-flies with different aquatic parameters. In the same year, Azeveda et al of Southern Brazil formulated the identification key to pupa of *Simulium*. Kiel and Roder (2005) conducted an experiment on the gelectrophoretic studies on the labial gland secretion of immature black-flies. Sirin and Yalchin (2005) recorded eight species from Turkey among which six were *Simulium* and two were *Prosimulium* which were reported for the first time from that country.

CHAPTER III

3. STUDY AREA

It is Lalitpur (alt.1, 375m) located in the Southern part of Kathmandu Valley where there are Godawari, Nakkhu and Karmanasa as local slow running streams all the year around. These are smaller rivers at Godawari, Nakkhu and Saibu respectively.

The Godawari river is at Godawari VDC (1,400m) (27° 35″N, 85° 20” E) Nagargaon,Ward no.5, about 11.2 km away from Lalitpur municipality and South-eastern corner about 15 km away from Kathmandu municipality. The Godawari river flows with a clean and fresh water and originates from Godawari Kunda and merges in Manohara river, near Sanogaon. Its surroundings are richly filled up with natural greeneries and sparse human population. River beds are covered with aquatic flora (phytoplanktons, mosses as bryophytes, trailing leaves, etc.) and the substrata are the rocks, pebbles, etc.

The Nakkhu river is next well recognized stream starting from Tika Bhairav at Lele and opens into Bagmati river at Chobbar. This river is at Saibu VDC (Nakkhu Dole, Ward no. 3) (1,286 m) (27^{o}39.007” N, 85^{o}18.754”E). It is in the southern part of the Kathmandu city and vegetation in this area includes both cultivated and natural flora. The river beds consist of gravel, stones, twigs, fine roots and trailing grasses. Water is polluted due to human activities like direct sewage discharges and others.

The next river Karmanasa river is flowing from South to North. It is located at Imadol VDC (1,283 m) (27^{o}39.708” N, 85^{o}20.117”E). Surrounding areas are open, so became residential in recent years, and poor vegetation and partly cultivated. Due to excessive human activities and chemical discharges from local garment factories the water has been highly polluted and river beds contain mosaic of pebbles, stones, plastic and rare aquatic plants.

CHAPTER IV

4.1 Materials

For the study of black-fly species at Lalitpur district of Kathmandu valley following methods were adopted as most senior workers followed. The black-fly larva pupa and adult stages collection were made in the study sites during Oct, 2007 to march, 2008 and equipments used for the same were based on purpose of study whether the sample species was meant for identification or for rearing the immature forms or for determining the physico-chemical parameter of water, etc.

Collecting materials used in the fields are listed as:

1. Plastic vials (8ml, about three dozen)
2. Hand lens (single)
3. Forceps (no. 00,single)
4. Needle (no.0, single)
5. Scissors
6. Tissue papers (single roll)
7. Camel – hair brush (no. 0, two)
8. Insect net (30 cm diameter, mesh size of about 1.5 mm, single)
9. Marker pen black (Permanent, one)
10. Petri-dish (90mm about half dozen)
11. Note book/Pen/pencil
12. Digital camera (7.2 mega pixel)

Chemicals:

1. Ethyl alcohol (70%)
2. Glycerin

Equipments used in the laboratory for the measurement of physico -chemical parameters of river were;

1. Mercury thermometer (ºc, single)
2. Measuring tape (100 feet, single roll)
3. Thread – nylon (about 100 meters)
4. DO bottle (300 ml, two)

5. Pipette (10 ml, single)
6. Burette (50 ml, single)
7. Rubber dropper (single)
8. Measuring cylinder (100 ml)
9. Conical flask (250ml, single)
10. Beakers (250 ml, about half dozen)
11. Borocil Test –tubes (15 ml. about a dozen)
12. $MnSo_4$
13. KI
14. NaOH
15. Conc. H_2SO_4
16. Binocular Microscope (Olympus)
17. Lamp (electric)
18. Stereoscopic dissecting microscope
19. Needles (two)

4.2 Method

The three streams (Godawari, Nakkhu and Karmanasa) of Lalitpur District (Kathmandu) were selected for the present study based on accessibility, occurence and values of flies. Collections of sample species were done in these streams once in every month (third week) during the study period of six months. First of all, two sample spots of about 200 meters apart were selected in each river and collections were done for about half an hour everyday on each spot. Collection of the larval, pupal and adult stages was done randomly from these spots as follows.

a. Larval Collection.

Larvae were collected mostly from the stones and debris including twigs. (Photo no. 3), plastics and sacks (garbage). The stones were lifted up slowly and dropped the larvae into the plastic vial by the help of camel hairbrush then killed and preserved by 70% ethyl alcohol for their morphological studies.

b. Pupal Collection

Pupae also lie attached to the twigs (Photo no. 7) or on trailing grasses, plastic threads, stones or other debris, etc. So they were collected with their substrates. The twig with pupae was cut into smaller pieces (about 2-3cm long) by a scissor and put them separately in individual test – tube (15 ml) for rearing to emerge into adults. Test- tube contained the moistened tissue paper. For aeration purpose, smaller holes, about five were made on plastic covering the test-tube mouth.

c. Adult Collection

Collection of adults for study was done by sweeping the herbages near the river banks but it was very poor. Adults were kept in a test-tube and after 24 hours, they were preserved in 70% ethyl alcohol for their morphological studies.

Rearing and hatching of pupa

In laboratory, the collected pupae were reared in the test- tube (Photo no. 12) for hatching. Careful attention was paid ever day. The tissue paper within test-tube was moistened everyday till adults were hatched. Care was taken not too moist the test- tube wall which could kill the hatched adults as they get stuck to the wall.

Most of the pupae are hatched within 3-4 days as Maskey, 1985 recorded. In the present study the adults of both sexes were allowed to hatch in the lab condition, which was then kept alive for about 1-2 days. During alive condition, their morphological coloration, body size, legs, scutum, etc study was done. After that the flies were killed and transferred into vials and preserved in ethyl alcohol 70% with their pupal exuvie.

4.3 Methods used for the study of physico-chemical parameters of water

a. Water temperature

Water temperature in ^{o}C was measured directly by using the mercury thermometer.

b. Water depth

River depth was measured in every visit. For it the depth was measured by using a stick in water and compared with the measuring tape.

c. Water velocity

Water current was measured in meter/second. For it the cork was used (surface floating method).

Formula:

$$\text{Water Current} = \frac{\text{Fixed distance (m)}}{\text{Time taken (sec)}}$$

d. Dissolve oxygen (DO)

The DO in water was determined by using Winkler's method (Winkler, 1888 A.D.) Water sample was taken in BOD glass bottle (300ml). About 2ml of $MnSO_4$ solution was added in it, and then followed by the addition of 2ml of KI by micro-pippette (10ml) at an interval of 2 minutes. Then the ppt. was formed. The bottle went on shaking well and precipitation was allowed to settle down for few minutes. Then about 2ml of Conc. H_2So_4 was added to dissolve the precipitate. Oxygen was then fixed.

50ml of fixed dissolve oxygen sample was taken in a conical flask and few drops of starch solution were added as an indicator with constant shaking of the flask till the colour changed into blue. Then the solution was titrated against 0.025N Sodium Thiosulphate solution with constant shaking of the flask till the blue colour disappeared. The burette reading was noted. DO was calculated in mg/ltr by using the following equation.

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

Where, V_1 - volume of BOD bottle

V_2 - volume of titrated sample

V - Volume of $MnSO_4$ and KI added

e. Free CO_2

For the measurement of free CO_2, about 100ml of water sample was taken in a conical flask. Then, two drops of phenolphthalein indicator was added. The water sample remained colourless proving the presence of CO_2 and the same was titrated with 0.05N. Sodium hydroxide (NaOH) solution till faint pink colour appeared. Amount of NaOH used was noted and calculation was made by using the following formula.

$$\text{Mg of } CO_2\text{/litre} = \frac{\text{(ml x N) of NaOH X 1000 X 44}}{\text{Volume of Sample}}$$

4.4 Statistical Analysis

Collected data was analyzed by using following statistical tools.

a. Correlation Coefficient test

Coefficient of correlation value was calculated by using the formula given by Karl Pearson (Biologist and Statistician). According to him, the coefficient of correlation between two variables is obtained by dividing the products of the corresponding deviation of the various items of two series from their respective means by the product of their standard deviation and number of pairs of observations.

Karl Pearson's formula was used for the calculation of correlation coefficient. It is also known as product moment formula of coefficient of correlation which is

$$\text{Coefficient of correlation (r)} = \frac{\sum XY - n.\,\bar{X}.\,\bar{Y}}{\sqrt{\sum x^2 - n\,(\bar{X})^2}\ \sqrt{\sum y^2 - n\,(\bar{Y})^2}}$$

Where, r = coefficient of correlation

X = deviation of various items of the first variable.

$\bar{X}$ = Average of deviation of various items of the first variable i.e., X

Y = corresponding deviations of the second variable.

$\bar{Y}$ = average of the corresponding deviation of the second variable i.e., Y

n = Number of observations.

b. Species Diversity Index

Species Diversity was calculated by using the formula (Shannon- Wiener, 1949):

$$\bar{H} = - \sum p_i \log_e p_i \qquad (\because p_i = n_i / N)$$

Where, $\bar{H}$ = Shannon's Index

p_i = Probability function

n_i = number of individuals

N = Total no. of individuals in all species

To know the diversity is whether higher or not, equitability is calculated as;

$$e = J = \bar{H} / \log_e (S)$$

Where, S = total no. of species

The value of 'e' generally lies between o-1. If the value is near to zero, the diversity said to be uneven and if the value is near to 1, then the distribution is said to be even.

4.5. Identification

For identification, detailed morphological studies of the larvae, pupae and adults were done under the stereoscopic dissecting microscope (Olympus) at laboratory of Central Horticulture Center, Kirtipur. The histoblast of gill (Photo no. 2) of mature larva was also dissected with the help of needle under the dissecting microscope for detail study. Samples were then compared by using taxonomical keys published by Takaoka and Davies (1996).

4.6. Keys for identification

4.6.1. Keys to sub genera of *Simulium*

Mature larvae:

1. Lacks ventral papillae in last abdominal segment ……*Simulium*

   Presence of ventral papillae in last abdominal segment…… (2)

2. Very small or vestigial post genal cleft…… *Montisimulium*

   Well defined, small to large post genal cleft…… (3)

3. Post genal cleft shorter than post genal bridge; lateral serration of hypostomium developed…….. *Nevermannia*

   Post genal cleft longer than post genal bridge; lateral serration of hypostomium undeveloped……. *Gomphostilbia*

Pupae:

1. 8 or 10 gill filaments arranged in 3+3+2 (rarely 2+4+2) or 3+3+2+2 from dorsal to ventral; grapnel- like hook lets present on last abdominal segment……. *Gomphostilbia*

   12 or 14 gill filaments arranged in pairs……..…. *Montisimulium*

   4, 6, 9 or 10 gill filaments arranged in pairs; grapnel – like hook lets absent…….. (2)

2. Cocoon with anterodorsal projection……. *Nevermannia*

Cocoon with simple wall - pocket - shaped or shoe – shaped ……. *Simulium*

Adult female:

1. Katepisternum haired…….…….... *Gomphostilbia*

   Katepisternum bare…….…… (2)

2. Tarsal claw with large basaltooth……..…….*Nevermannia*

   Tarsal claw simple or with small sub basal tooth…….…… *Simulium.*

Adult males:

1. Katepisternum haired…….…….... *Gomphostilbia*

   Katepisternum bare…….…… (2)

2. Basal section of radius haired……..…… *Nevermannia*

   Basal section of radius bare……..…… *Simulium*

4.6.2. Key of species of Sub genus *Simulium*

Larvae:

1. Yellowish head with well defined head spot…….…….. *S. variegatum*

   Different colour…….…… (2)

2. Post genal cleft, wide rounded apically……..…….. *S. multistriatum*

   Well defined, apically different post genal cleft…….…… (3)

3. Post genal cleft narrow and pointed apically…….…….. *S. tuberosum*

   Post genal cleft deep and reaching posterior boarder of hypostomium ……..…… *S. indicum*

Pupae:

1. Gill with 6 filaments (2+2+2) and scattered……. *S. variegatum*

   Gill with 6 filaments and different arrangement……..…….... (2)

2. Gill with 6 filaments (2+2+2), dorsal pair making angle of 90° with the lowermost ventral pair……..…... *S. tuberosum*

   Gill with 8 filaments……..…….. (3)

3. Gill with 8 filaments arranged in pair as 2+2+2+2 from dorsal to ventral……..…... *S. multistriatum*

CHAPTER V

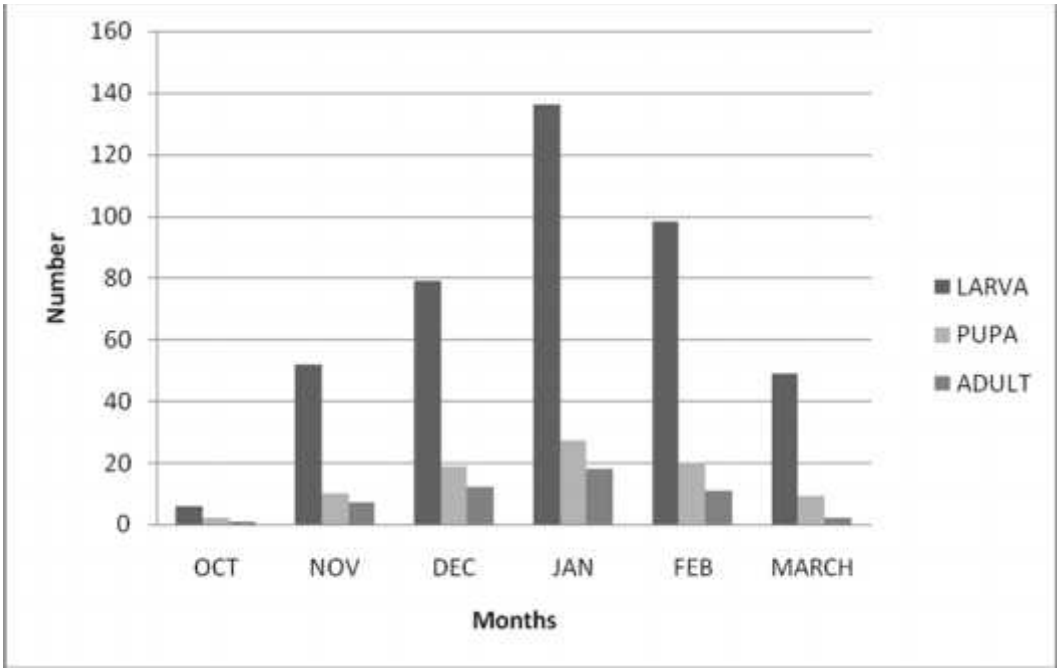
5. RESULT

5.1 Monthly variation

During field work, the larva, pupa and adults of black-flies were collected separately from the three different rivers (Godawari, Nakkhu and Karmanasa) of Lalitpur. The number of collected samples (larva, pupa and adult) varied in each of the three different study sites in relation to different physico – chemical parameters (temperature, DO, water current, free carbondioxide). Results for each study site are:

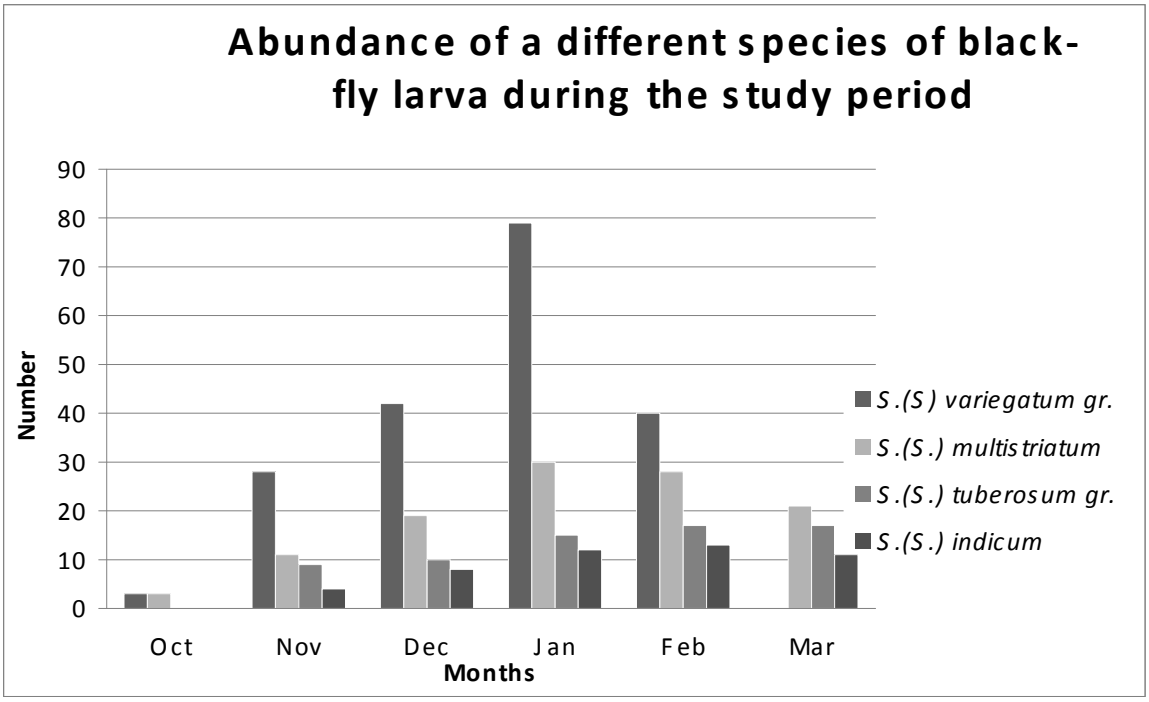
SITE I: Godawari River

Fig.1 No. of larvae, pupae and adults collected in different month



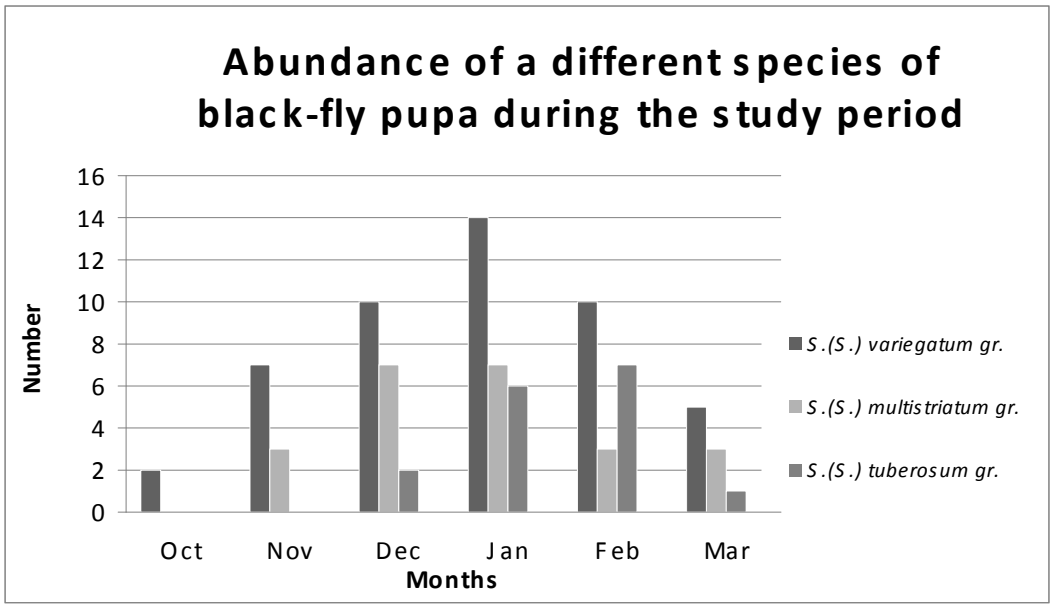
Thus the total number of larva, pupa and adult stages of *Simulium (Simulium*) species collected throughout the study period in site I (Table no. 3) shows the highest number of these flies in all the developmental stages was recorded in January and lowest in October.

Fig. 2



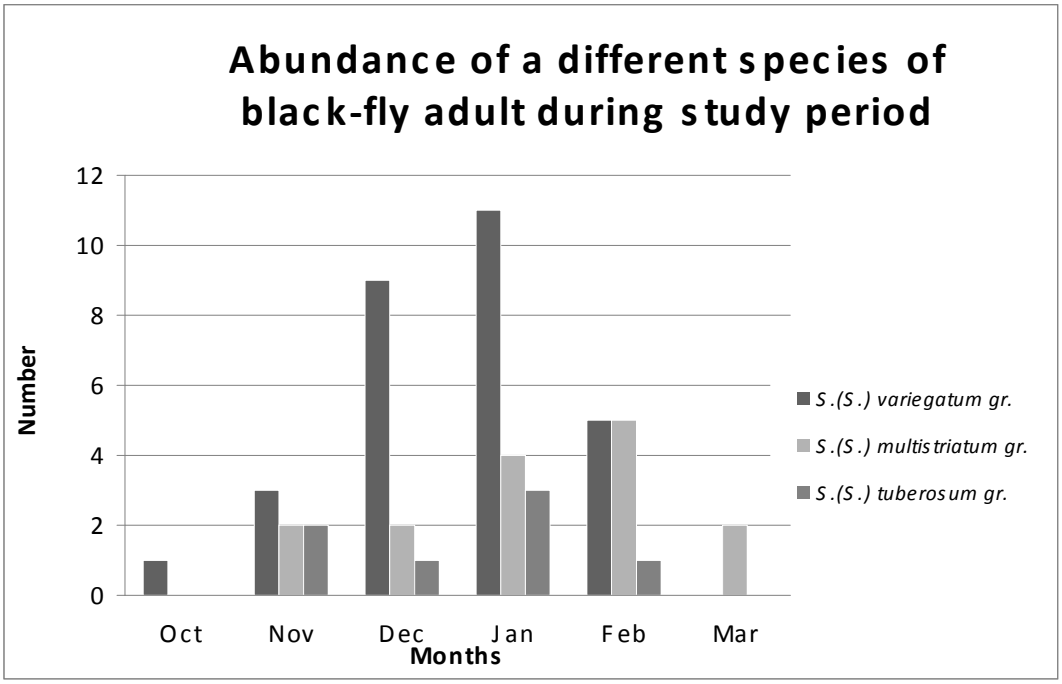
Larvae of four different species collected during six months of study period.

Fig. 3



Ratio of pupal number of three different species; *Simulium variegatum gr. Simulium multistriatum gr.* and *Simulium tuberosum* gr.

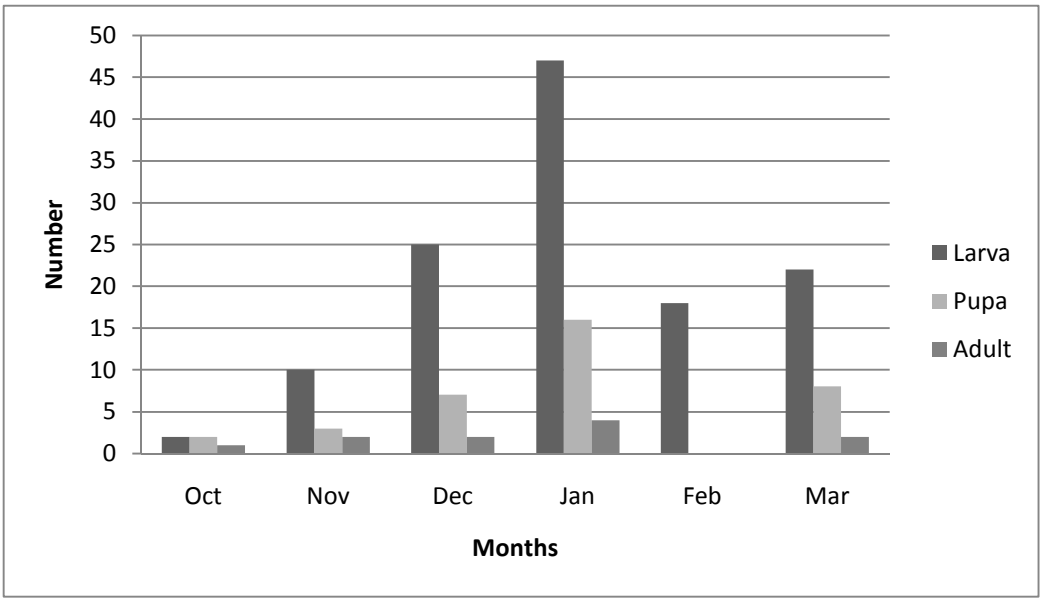
Fig. 4



The total number of adults (collected as well as rared) were 51 (Table no. 6) and they belong to 3 species; *Simulium variegatum gr*, *Simulium multistriatum gr.* and *Simulium tuberosum gr.*

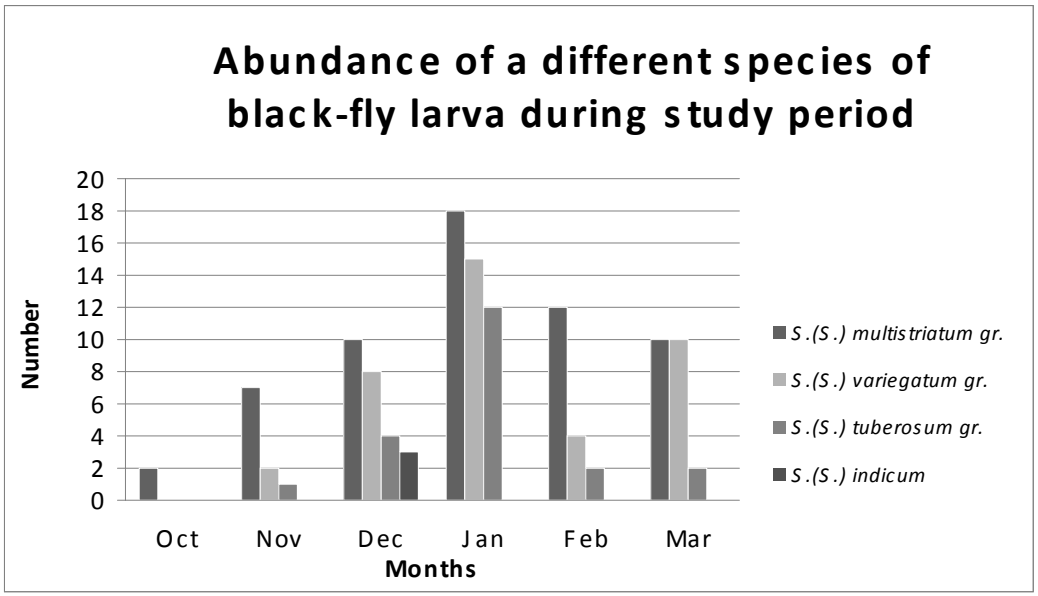
SITE II: NAKKHU RIVER

Fig.5. No. of different larvae,pupae and adult collected in different month.



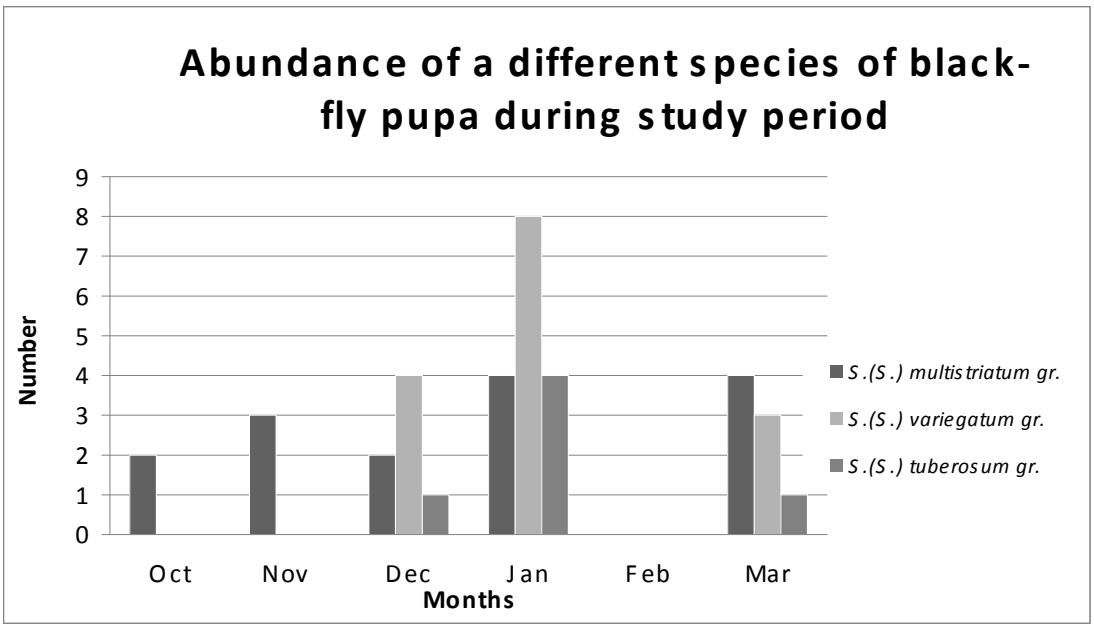
All the collected specimens identified. In this field also the highest simuliids recorded in all developmental stages was in the month of January while least in October (Table no. 7).

Fig. 6



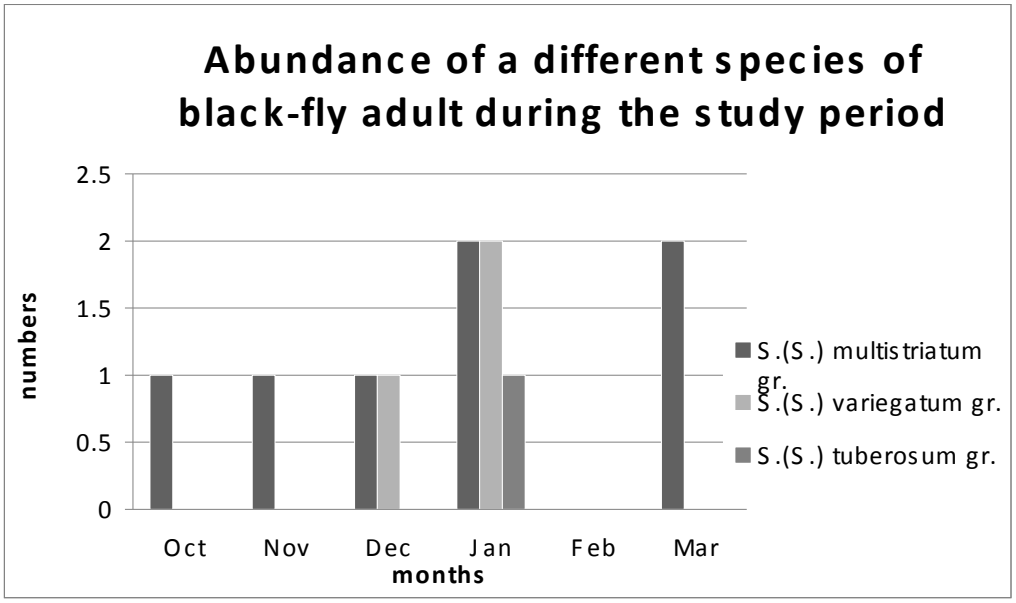
Among 124 larvae (Table no. 8) collected were identified as; *Simulium multistiatum gr.Simulium variegatum* gr, *Simulium tuberosum gr.* and *Simulium indicum* .

Fig. 7



Among 36 pupae (Table no. 9) collected were identified as *Simulium multistriatum gr.*, *Simulium variegatum* gr.and *Simulium tuberosum* gr.

Fig. 8



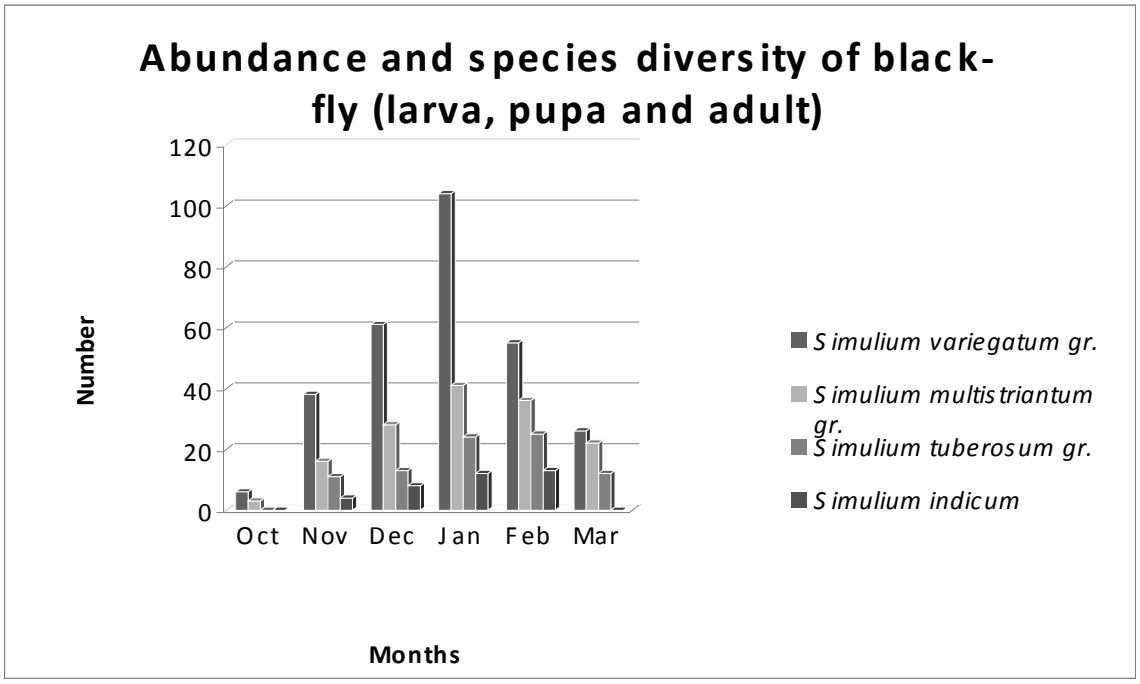
Among 11 adults (rared) (Table no. 10), were identified as *Simulium multistriatum gr.*, *Simulium variegatum* gr. and *Simulium tuberosum gr.*

5.2 DIVERSITY OF BLACK – FLY SPECIES IN THE STUDY AREA

To calculate the relative abundance of black – fly species in the study areas, the Diversity Index Method was applied. The commonly used diversity index is Shannon's Index ($\bar{H}$).

SITE I

Fig. 9

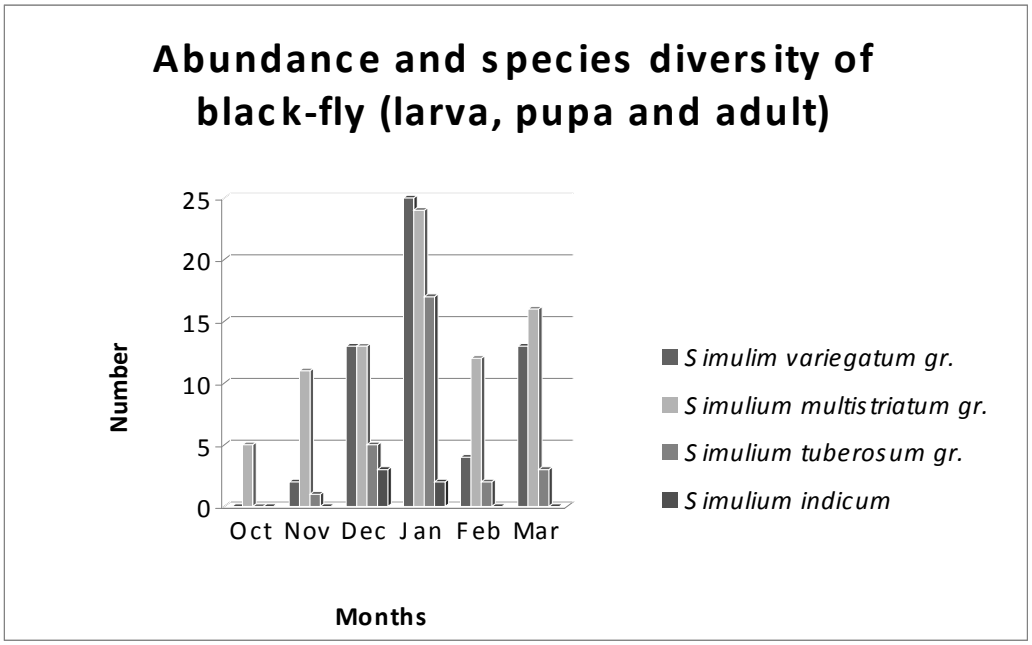


Among four different species (sorted from larva, pupa and adult) collected during the study period was recognized into *S. variegatum gr.*, *S. multistriatum gr., S. tuberosum gr. and S. indicum* of genus *Simulium*. The most dominant species was *S. variegatum* gr. (290) followed by *S. multistriatum gr.* (146), *S. tuberosum gr.* (85) and *S. indicum* (37) (Table no. 11).

Shannon's index (**H̄**) = 1.154 and Evenness (**e**) = 0.83

SITE II

Fig. 10



A total of 171 specimens were categorized into four species of genus *Simulium; S. variegatum* gr. (57), *S. multistriatum gr.* (81), *S. tuberosum* gr. (28) and *S. indicum* (5) (Table no. 12). The most abundant species was *S.multistriatum gr followed by S. variegatum gr., S. tuberosum gr.* and *S. indicum.*

Shannon's index (**H̄**) = 1.115 and Evenness (**e**) = 0.80 (Table no.12)

5.3 RELATION OF BLACK – FLY SPECIES IN THE STUDY AREA

The different physico - chemical parameters (water temperature, DO, free carbon dioxide, water current and breadth of river, etc.) were measured to show the relation between black-fly species with them. The average values of different sites are tabulated in table no.1.

Table 1: Average value of aquatic parameters

S/N	Parameters	Site I	Site II	Site III
1.	DO (mg / ltr)	5.8	5.8	1.2
2.	Temperature (˚c)	15.8	17.3	17.4
3.	Water Current (m / sec)	0.37	0.29	0.2
4.	Breadth (m)	4.1	4.9	3.0
5.	Depth (cm)	20	18	17
6.	Free CO_2 (mg / l)	3.06	3.8	3.10

5.4 Coefficient of correlation (r) between aquatic parameters and number of species in different sites

The calculated values of coefficient of correlation (r) (Table no.2) show the +ve correlation between DO and number of species but –ve correlation between the temperature and species number in the both study areas. The values in the table also shows perfect positive correlation between water current and species in Site I but negative result in Site II.

Table 2

S / N	Parameters	Value of r	
		Site I	Site II
1.	DO	0.8	0.62
2.	Temperature	-0.8	-0.72
3.	Water Current	1	-0.01

5.5 RESULT OF SITE III (KARMANASA RIVER)

No records of *Simulium* fauna at the study site were made throughout the study period.

CHAPTER VI

6. DISCUSSION

During six months study period, total sample number of larva, pupa and adult collected were 420, 87 and 51 at site I and 124, 36 and 11 at Site II but none from the Site III. On the basis of larva, pupa and adult's morphological characters, the species identified were *Simulium (Simulium) variegatum gr*., *S. (S.) multistriatum gr*., *S. (S.) tuberosum gr.* and *S. (S.) indicum*.

Great population fluctuation was observed each month in Site I and Site II. In all the developmental stages, the highest recorded (32.3% larva, 31% pupa and 35.2% adult) was in the month of January at Site I when average water temperature was 14˚c and water current 0.33 m/sec. The lowest population was recorded (1.4% larva, 2.2% pupa and 1.9% adult) in the month of October (Table no.3). Similar kind of result was obtained at site II (table no.7).

The figures (1 and 5) show that January supports the peak density of black-fly perhaps due to high availability of breeding places and suitable water temperature, it's current and DO. This result agrees with those recorded by Baba and Takaoka (1992) who concluded that the normal development of eggs occur at 10-14˚c but delays at 18-22˚c. These temperatures were nearly similar to the present recorded value at Site I and II. So, this must be the reason of population variation during the study period.

Number of collection of larvae, pupae and adults was higher in Site I, followed by Site II while no record in Site III perhaps due to poor vegetation and aquatic parameters. The result shows that Site I is the most suitable place for breeding of black-fly population compared to other sites. Most species of *Simulium* use the bryophytes, trailing leaves for oviposition as reported by Baba and Takaoka (1989) as in the Site I which has more trailing leaves, bryophytes and stones compared to II. The Site III is unsuitable for oviposition because of poor physico-chemical parameters.

While comparing the four species of *Simulium, S. (S.) variegatum gr.* was the most abundant at Site I and *S. (S.) multistriatum gr.* at Site II but least abundance species was *S. (S.) indicum* at both sites.

The calculated Shannon's index and evenness are $\bar{H}$ = 1.154, e = 0.83 and $\bar{H}$ = 1.155 and e = 0.80 at Site I and II respectively (Table no. 11 and 12). These values indicate the even species diversity in both sites and no such differences in both the sites. It must be due to more or less similar habitat and aquatic parameters.

In the morphological study, the larvae of *Simulium (S.) variegatum gr.* was recorded as having yellowish head capsule with well defined head spot (Photo no. 4) and the Pupa with six scattered gills filaments (2 + 2 + 2) (Photo no. 8). So, it can be compared with *Odagmia vega*, Maskey (1998), larva of the *Simulium (S.) multistriatum gr.* has a post genal cleft wide, rounded apically (Photo no. 5), pupa with eight gill filaments (2 + 2 + 2 + 2) (Photo no. 9) which is very much similar to *S. hillycum* Maskey (1989). So this Species can be matched with *Simulium hillycum*. Larvae of *S. tuberosum* gr. (Photo no. 6) has pointed post-genal cleft and pupa of (Photo no. 10) has six gill filament (2 + 2 + 2), dorsal pair making angle of 90˚ with the lower most ventral pair. This arrangement of gill filaments makes *S. (S.) variegatum gr.* different from *S. tuberosum* gr. Simulium indicum has larvae with post genal cleft deep and reaching posterior border of hypostomium. Its feature can be compared with *Simulium (Himalayum) indicum* Becher (1885).

Maskey (1998) recorded *Odagmia vega* from the Godawari river with the water temperature of 20˚c and velocity of 0.6m/sec. This given data can be compared with the present study which could be the reason of abundance of *Simulium (S.) variegatum gr. at Site I.*

The aquatic parameters seem to play a significant role in the distribution of black-fly during the study period. Maximum capture of all the stages of black-fly was done at 14˚c in Site I and II. The coefficient of correlation (r) is -0.8 for Site I and -0.7 for Site II. These values show the negative correlation between water temperature and black-fly species.

Another important and critical factor for an aquatic environment is the amount of DO which provides information about the quality of water. Ellis (1937) pointed out that DO content of water must be 5mg/ltr at 20˚c for maintenance of aquatic life and the average DO recorded in the present study at Site I and II was 5.8 mg/ltr (Table no.1) which concurrent with the above observation supporting the presence of black – fly fauna in these study sites due to suitable aquatic parameters. On the contrary, average DO is 1.2 mg/ltr at Site III which does not support life at all (Ellis,1937).

Water current also plays significant role in the biology of *Simulium.* Table.2 shows the perfect positive correlation between water current and black-fly at Site I as the value of r is +1 while –ve at Site II (r = -0.01).

It was recorded by Jensen (1997) that some species of black – fly like *Simulium costatum* are very much resistant to low stream velocity. The immature stages of these flies commonly do not develop in water current having less than 0.1 – 0.2 m/sec (Rubstov, 1956) which agrees with the present study at site III and this must be reason why the black – fly was not recorded at Site III.

So, physico – chemical parameter has significant relationship with black – fly population and its diversity.

CHAPTER VII

7. CONCLUSIONS

Six months (Oct, 2007- March, 2008) survey on *Simulium* fauna in Lalitpur District made following conclusions:

1. Excluding unidentified samples, the identified samples of various numbers were categorized into four different species:
    a. *Simulium (Simulium) variegatum gr.*
    b. *S. (S.) multistriatium gr.*
    c. *S. (S.) tuberosum gr.*
    d. *S.(S.) indicum* Becher

2. Monthly variation was observed and the highest peak of collections (larvae, pupae and adults) was recorded in January and the least in October in the sites I and II but no record of any stage in site –III.

3. Common species diversity was found in both sites ($\bar{H}$ =1.154 in site I and $\bar{H}$ = 1.150 in site II)

4. Very less capture of adults was made in the study sites but their successful rearing was done in the lab conditions.

Larval and pupal stages of black-fly species have intimate relation with the different aquatic parameters but present study revealed that species richness was not highly correlated with a single point of measurement of stream condition.

CHAPTER VIII

8. Recommendation:

The study of black-flies from different localities of Lalitpur reveals following recommendation:

1. Since black-flies are vector, their detail study on biology and species composition should be carried out.

2. The morphological study is strongly recommended for further identification of excluded species.

3. As flies are recorded as vector of filarial and protozoan, government should emphasis for the research on National level.

4. Government should focus on lunching awareness program among the people about black-flies.

CHAPTER- IX

9. REFERENCES

*Adler, P.H. et al. 2004 a. The black-flies (Simuliidae of North America. Cornell University: 941.

2004 b. Black-flies in the boreal biome. Key organism in both terrestrial and aquatic environment. Ecoscience. **11** (2):187-200.

*Azeveda, G. et al. 2005. Identification key to pupa of *simuliidae* (Diptera) recorded from South eastern of Brazil. Revista Brasileira de Zoologica. **22** (3): 742-752.

Baba, M. 1992 a. Development of immature stages in a black-flies *Simulium kawamure* (Diptera: Simuliidae) observed in the laboratory. Jpn. J. Saint. Zool. **43** (2): 81-82.

1992 b. Oviposition habits of *Simulium kawamure* (Diptera: Simuliidae), with reference to seasonal changes in body size and fecundity. J. Med. Entomol. **29** (4): 603-610.

1992 c. Oviposition site and preimaginal growth in a black-fly *Simulium quinquestraitum (Doptera: Simuliidae).* Jpn. J. Sanit. Zool. **44** (2): 85-90.

Baba, M. and Takaoka, H.1989 a. Oviposition sites and the number of larval insects of two mountain black-fly species, *Simulium joponicum* and *S.rufibasis* (Diptera: Simuliidae). Ibid. **40**: 307-313.

1990 b. Egg development, hatching and larval growth of a univoltine black-fly, *Prosimulium yezoense* (Diptera: Simuliidae), in Kyunshu, Ibid. **41** (4): 383-387.

1991 c. Oviposition habit of a univoltine black-fly, *Prosimulium kiotoense* (Diptera: Simuliidae), in Kyunshu, Japan. Medical and Veterinary Entamology. **5** (1): 351-357.

1991 d. Larval instars and growth pattern of univoltine black-fly, *Prosimulium kiotoense* (Diptera: Simuliidae) in Kyunshu, Japan. Entomology Society of America. **28** (2): 214-218.

1992 e. Dormacy and embryogenesis and hatching of eggs of *Prosimulium kiotoense* (Diptera: Simuliidae), under natural and laboratory conditions. Ibid. **29** (3): 430-435.

Choochote, W. et al. 2005. Seasonal abundance and daily flying activity of black-fly (Diptera:Simuliidae) attracted to human baits in Doi Inthanon National Park, Northern Thailand. Med. Entomol. Zool. **56** (2): 335-348.

*Coscavon, A. and Laura. 2003. Key of Simuliidae (Insecta, Diptera) of Ecuador. Folia Entomologica Mexicana. **42** (2):191-219.

*Creadie, Mc. et al. 2004. Spatial-temporal distribution of preimaginal black-flies in Neotropical streams. Hydrobiologica. **513** (1-3): 183-196.

*Credie, Mc. et al. 2005. Patterns of species richness for black-flies (Diptera: Simuliidea) in Nearctic and Neotropical regions. Ecological Entomology. **30** (2): 201-209.

Datta, M. 1973. New species of black-flies (Diptera: Simuliidae) of the sub genera *Eusimulium* Roub and *Gomphostilbia* Enderlein from the Darjeeling area, India. Oriental Ins. **7**: 363-403.

1974 a. Some black-flies (Diptera: Simuliidae) of the subgenus *Simulium* latreille from the Darjeeling area, India. Ibid. **8**:15-27.

1974 b. New species of black-flies (Diptera: Simuliidae) from Darjeeling area, India. Ibid. **8**:457-468.

1975 a. A new black-fly species (Simuliidae: Diptera) from Darjeeling area, India. Proc. Indian Acad, Sci. (B) **81**:67-74.

1975 b. Simuliidae (Diptera) from Assam foothills, India; Japanese. J. Sanit, Zool. **26**: 31-40.

Datta, M. and Pal, T.K.1975. Few rare black-fly species (Diptera: Simuliidae) of the subgenus *Simulium* latreille from the Darjeeling area, India. Proc. Indian Acad. Sic. (B) **81**: 154-161.

Ellis, M.M. 1937. Detection and measurement of stream pollution. Bull. U.S. Bar. Fish. **48**: 365-437.

Feld, C.K. et al. 2002. The indication of morphological degradation of streams and rivers using Simuliidae. Limnologica.**32**:273-288.

Hiroko et al. 2005. Revised the description of *Simulium (Nevermania) sasai*, a rare and poorly known species Japan (Diptera: Simuliidae): Med. Entamol. Zool. **56** (4): 299-307.

Jensen, F. 1997. Diptera Simullidae, Black flies. In: Nilsoon A. N. (ed.): Aquatic Insects of North Europe. **2**: 209 - 207

Kiel, E. and Fruitger, A. 1997. Behavioral responses of different black-fly species to short term oxygen depletion. Internationale Revue der gesamtem Hydrobiologie and Hydrographie. **82** (1): 107-120.

Kiel, E. and Roder, T. 2005. Geletrophoretic studies on labial glands secretions of immature black-flies (Diptera: Simuliidae). Limnologica - Ecology and Management of Inland waters. Elsevier GmbH. **32** (3): 201-205.

Lewis, D.J.1964. Diptera from Nepal Simuliidae. Bull. Brit. Nat. Hist.**15** (6-8): 294-297.

1972. *Simulium indicum* in Nepal (Diptera: Simuliidae). Senckenberg boil. **53** (5-6): 15-22.

1973. The Simuliidae (Diptera) of Pakistan. Bull. Ent. Res. **62**: 453-470.

*Malmqvist, B. et al. 2004. Testing hypothesis on egg number and size in black-flies (Diptera: Simuliidae). Journal of vector Ecology. **29** (2): 248-256.

Maskey, M. 1985. A new species of black-fly (Diptera: Simuliidae) from Nepal. Journ. Nat. Hist. Mus. **9** (1): 15-22.

1989. A new species of black-fly (Diptera: Simuliidae) from Nepal. Journ. Entomol. Sec. of Nepal. **1** (1): 37-45.

1998. One new O*dagmia vega* and two unnamed species under the Subgenus *Odagmia* Enderlein have been described for the first time. Journ. Nat. Hist. Mus. **17**: 7-22.

*Opara, K. N. and Fagbemi, 2005. Physico – chemical indices of breeding sites of *Simulium damnosum* in the lower cross River Basin, Nigeria. Journal of Environmental Science (China) **17** (3): 511-517.

*Pond, A. C. Werner, D. 2003. A new species of Xenomyia Mollach, 1921 (Diptera: musidae) from South Africe, a probable natural antagonist of black-flies (Diptera: Simuliidae). African invertebrate. **44** (2): 147-155.

*Reeves, W.K. et al. 2007. Molecular detection of Trypanosoma (Kinetoplastida: Trypanosomatidae) in black-flies (Diptera: Simuliidae). Comparative Parasitology. **74** (1): 171-175.

Rustov, I.A. 1956. Dipterous Insects. Black flies (Simullidae). In: E. N. Pavlosky, (ed.). The fauna of USSR. **6** (6): 1 – 853.

*Sirin, U. and Yalchin, S. 2005. New records of black-flies (Diptera: Simuliidae) for Turkish fauna. Zoology in Middle East. **36** (2): 87-98.

Takaoka, H. 1994. Natural vectors of three bovine *Onchocerca* species (Nematoda: Onchocercidae) and seasonal transmission by three black-fly species Diptera: Simullidae) in Central Kyushu, Japan. J. Med. Ent. **31**: 404-416.

2003. The black-flies (Diptera: Simuliidae) of Sulawesi, Maluku and Irian Jaya, Kyushu University Press, Fukuoka.

2004. Insecta: Diptera, Simuliidae: Freshwater invertibrates of Malaysian Region. Monash University. Akademi Sains Malaysia.

Takaoka et al. 1992. Natural infection of black-flies with larvae of zoonotic *Onchocerca spp.* In Northeast Japan. Jpn. J. Trop. Med. Hyg. **20**: 1-9.

Takaoka et al. 2003. Black-flies (Diptera: Simuliidae) attracted to man and water buffalos and natural infections with filarial larvae, probably *Onchocerca spp.* in Northern Thailand. Parasite. **10**: 3-8.

Takaoka, H. and Choochote, W. 2005 a. Two new species of black-flies i.e., (Diptera: Simuliidae) from Northern Thailand. Med. Entomol. Zool. **56** (4): 319-934.

2005 b. A new subgenus and new species of *Simulium* s.l. (Diptera: Simuliidae) from Thailand. Med. Entomol. Zool. **56** (1): 33-41.

2005 c. Discovery of two new black-fly species of *Simulium (Montisimulium)* (Diptera: Simuliidae) in Doi, Inthanon National Park, Chiang Mai, Thailand. The Japanese Society of Tropical Medicine and Health. **33** (4): 209-215.

2005 d. Two new species of *griseifrons* species of *Simulium (Simulium)*, (Diptera: Simuliidae) from Northern Thailand. Med. Entomol. Zool. **56** (3): 219-235.

2005 e. Two new species of *Simulium (Montisimulium),* (Diptera: Simuliidae) Northern Thailand. Med. Entomol. Zool. **56** (1): 21-31.

Takaoka, H. and Davies, D.M. 1996. The black-flies (Diptera: Simuliidae) of Java Indonesia. Bishop Museum Bulletin in Entomology 6. Bishop Museum Press, Honolulu: **81**.

Takaoka, H. and Saito, K. 2005. A new species of *Simulium (Nevermannia)* from Izu Islands. Tokyo, Japan (Diptera: Simuliidae). Med. Entomol. Zool. **56** (4): 309-317.

ANNEX – I

I. Monthly variation

SITE I: Godawari River

Table3: No. of larvae, pupae and adults collected in different month

Life Stages	Number (Month/Temp)						Total
	Oct	Nov	Dec	Jan	Feb	Mar	
	20˚c	16˚c	12˚c	14˚c	15˚c	18˚c	
Larva	6	52	79	136	98	49	**420**
Pupa	2	10	19	27	20	9	**87**
Adult	1	7	12	18	11	2	**51**
♂	1	3	5	9	5	1	**24**
♀	-	4	7	9	6	1	**27**

Table4: Variation in number of larval stage of the black-fly species at different temperature (îC)

Species Type	Number (Month/Temp)						Total	%
	Oct	Nov	Dec	Jan	Feb	Mar		
	20˚c	16˚c	12˚c	14˚c	15˚c	18˚c		
S.(S.) variegatum gr.	3	28	42	79	40	21	**213**	**51**
S. (S.) multistriatum gr.	3	11	19	30	28	17	**108**	**26**
S. (S.) tuberosum gr.	-	9	10	15	17	11	**62**	**15**
S. (S.)	-	4	8	12	13	-	**37**	**9**

indicum								

Table5: Variation in number of pupal stage of the black-fly species at different temperature (îC)

Species	Number (Month/Temp)						Total	%
	Oct	Nov	Dec	Jan	Feb	Mar		
	20˚c	16˚c	12˚c	14˚c	15˚c	18˚c		
S.(S.) variegatum gr.	2	7	10	14	10	5	**48**	**55**
S. (S.) multistriatum gr.	-	3	7	7	3	3	**23**	**26**
S. (S.) tuberosum gr.	-	-	2	6	7	1	**16**	**18**

Table 6: Variation in number of adult stage of black-fly species at different temperature (îC)

Species	Number (Month/Temp)						Total	%
	Oct	Nov	Dec	Jan	Feb	Mar		
	20˚c	16˚c	12˚c	14˚c	15˚c	18˚c		
S.(S.) variegatum gr.	1	3	9	11	5	-	**29**	**57**
S. (S.) multistriatum gr.	-	2	2	4	5	2	**15**	**29**
S. (S.) tuberosum gr.	-	2	1	3	1	-	**7**	**14**

SITE II: NAKKHU RIVER (Monthly variation)

Table 7: No. of larvae, pupae and adults collected in different month

Life Stages	Number (Month/Temp)						Total
	Oct	Nov	Dec	Jan	Feb	Mar	
	22˚c	18˚c	12˚c	14˚c	18˚c	23˚c	
Larva	2	10	25	47	18	22	**124**
Pupa	2	3	7	16	-	8	**36**
Adult	1	2	2	4	-	2	**11**
♂	-	2	-	2	-	2	**6**
♀	1	-	2	2	-	-	**5**

Table 8: Variation in number of larval stage of black-fly species at different temperature (îC)

S/N	Life Stage	Species	Number (Month/Temp)						Total	%
			Oct	Nov	Dec	Jan	Feb	Mar		
			22˚c	18˚c	12˚c	14˚c	18˚c	23˚c		
1.	**Larva**	*S. (S.) multistiatum gr.*	2	7	10	18	12	10	**59**	**48**
		S. (S.) variegatum gr.	-	2	8	15	4	10	**39**	**31**
		S. (S.) tuberosum gr.	-	1	4	12	2	2	**21**	**17**
		S. (S.) indicum	-	-	3	2	-	-	**5**	**4**

Table 9: Variation in number of pupal stage of black-fly species at different temperature (îC)

S/N	Life Stage	Species	Number (Month/Temp)						Total	%
			Oct	Nov	Dec	Jan	Feb	Mar		
			22˚c	18˚c	12˚c	14˚c	18˚c	23˚c		
1.	Pupa	*S. (S.) multistriatum gr.*	2	3	2	4	-	4	**15**	**42**
		S. (S.) variegatum gr.	-	-	4	8	-	3	**15**	**42**
		S. (S.) tuberosum gr.	-	-	1	4	-	1	**6**	**17**

Table 10: Variation in number of different adult stage of black-fly species at different temperature (îC)

S/N	Life Stage	Species	Number (Month/Temp)						Total	%
			Oct	Nov	Dec	Jan	Feb	Mar		
			22˚c	18˚c	12˚c	14˚c	18˚c	23˚c		
1.	Adult	*S. (S.) multistriatum gr.*	1	1	1	2	-	2	**7**	**64**
		S. (S.) variegatum gr.	-	-	1	2	-	-	**3**	**24**
		S. (S.) tuberosum gr.	-	-	-	1	-	-	**1**	**9**

SITE I

Table 11: Abundance and species diversity of black – fly (larva, pupa and adult)

Species	Month of collection						Total (n_i)	$p_i=n_i/N$	$Log_e n_i/N$	$-p_i log_e ni/N$
	Oct	Nov	Dec	Jan	Feb	Mar				
Simulium variegatum gr.	6	38	61	104	55	26	290	0.519	-0.655	0.339
Simulium multistriatum gr.	3	16	28	41	36	22	146	0.261	-1.343	0.350
Simulium tuberosum gr.	-	11	13	24	25	12	85	0.152	-1.883	0.286
Simulium indicum	-	4	8	12	13	-	37	0.066	-2.718	0.179
Total(N)							**558**			
$\bar{H}$= 1.154, e = 0.83										

SITE II

Table 12: Abundance and species diversity of black – fly (larva, pupa and adult)

Species	Month of collection						Total (n_i)	$p_i=n_i/N$	$Log_e n_i/N$	$-p_i log_e n_i/N$
	Oct	Nov	Dec	Jan	Feb	Mar				
Simulium variegatum gr.	-	2	13	25	4	13	57	0.333	-1.099	0.365
Simulium multistriatum gr.	5	11	13	24	12	16	81	0.473	-0.748	0.353
Simulium tuberosum gr.	-	1	5	17	2	13	28	0.163	-4.814	0.295
Simulium indicum	-	-	3	2	-	-	5	0.029	-3.540	0.102
Total(N)							**171**			
$\bar{H}$ = 1.115, e = 0.80										

ANNEX-II
PHOTO PLATES

1. Immature Larvae (Without histoblast gills)

2. Mature Larvae (With histoblast gills)

3. Gragarian Larvae

4. *Simulium variegatum* (larva)

5. *Simulium multistriatum* (larva)

***6. Simulium tuberosum* (larva)**

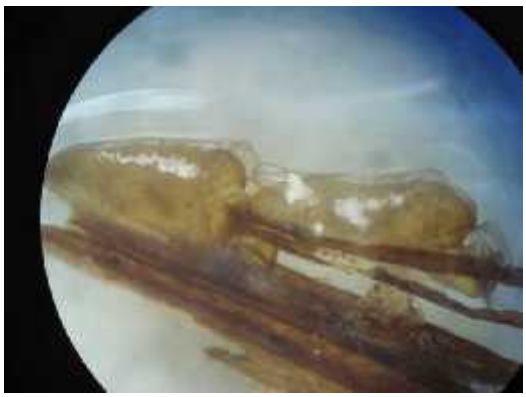
7. Gragarian Pupae

9. *Simulium multistriatum* (pupa)

11. *Simulium indicum* (pupa)

8. *Simulium variegatum* (pupa)

10. *Simulium tuberosum* (pupa)



12. Rearing of Pupae

13. *Simulium multistriatum* (Female)

15. Simulium tuberosum (Female)

17. Authur in the Field

14. *Simulium tuberosum* (Male)

16. *Simulium variegatum* (Male)

18. Authur at Lab

19. Godawari river (Site I)

21. Karmanasa river (SiteIII)

20. Nakkhu river (Site II)

22. Preserved larvae