## A STUDY OF THE LARVAL POPULATIONS AND COMPARISON OF LENGTHS OF PUPAL FILAMENTS OF SIMULIIDS

IN NORTH-CENTRAL OKLAHOMA

By

Robert Wayland Upham, Jr. Bachelor of Science University of Maine

Orono, Maine

1957

Submitted to the faculty of the Graduate School of the Oklahoma State University in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE August, 1964

UKLAHOMA STATE UNIVERSITY LIBRARY DEC31 1964

A STUDY OF THE LARVAL POPULATIONS AND COMPARISON OF

LENGTHS OF PUPAL FILAMENTS OF SIMULIIDS

IN NORTH-CENTRAL OKLAHOMA

Thesis Approved:

Settowell Thesis Advisor

Dean of the Graduate School

#### PREFACE

Reports of sporadic attacks of black flies (Simuliidae) on horses and turkeys in north-central Oklahoma have been received in recent years by the Department of Entomology, Oklahoma State University. During the Spring of 1962, an unusually large number of attack reports were received from animal owners who sought information on control of the pest. Before effective control methods could be recommended, the life cycle of the insect had to be determined. No information is available in the current literature on the biology of simuliids from north-central Oklahoma and little is known concerning the responses of the life cycle stages to environmental conditions. Because of the lack of biological data on this important insect, a series of experiments was undertaken to study the aquatic stage populations of <u>Simulium vittatum</u> Zetterstedt in this area.

Differences in the ecological conditions at the sites studied prompted the comparison of the lengths of the pupal respiratory filaments. It is hoped that this information will be of value to those workers engaged in studies of simuliid flies which occur in the area and those entomologists involved in simuliid assessment and control.

The author wishes to express appreciation to his major advisor, Dr. D. E. Howell, for encouragement and guidance throughout the thesis program. Special appreciation is expressed to Dr. R. A. Ward, Entomology Department, Walter Reed Army Institute of Research, for advice and aid in the analysis of data and preparation of this thesis. Sincere thanks

iii

are expressed to Drs. D. E. Bryan, Professor of Entomology, E. D. Besch, Assistant Professor of Parasitology, and M. T. Abdel-Razig, Graduate Student, for their constructive criticism of the thesis manuscript and to Mr. R. Baumgartner for his aid and advice in the analysis of water samples. Finally, I wish to acknowledge the assistance, encouragement and support throughout the experimentation and preparation of this thesis by my wife, Jimmie Upham.

## TABLE OF CONTENTS

Chapte	Page
I.	Introduction
II.	Review of Literature
	Larval Survey
	Comparison of Pupal Filaments
III.	Materials and Methods
	Description of Test Sites 6
	Topography
	Climate
	Vegetation
	Procedure
	Establishment of Counting Stations 8
	Counting
	Collecting Specimens
	Water Temperatures and Velocities 11
	Pupal Filament Measurements
	Water Analysis
IV.	Results and Discussion
	Spring Count
	Analysis of Spring Count
	Fall Count
	Comparison of Pupal Filaments
٧.	Summary and Conclusions
VI.	Literature Cited
APPEND	IX

## LIST OF TABLES

Table		Page
1.	Analysis of Variance of Larval Populations	24
2.	Comparison of Ecological Conditions at Sites A and B During January, 1964	24
3.	Measurements of Simulium Pupal Respiratory Filaments	25

## LIST OF FIGURES

Figur	e	Page
1.	Site A is a riffle located on Boomer Creek	26
2.	Site B is the fall located at the end of the Boomer Lake Power Plant effluent sluiceway	27
3.	Site C is a riffle located on Cow creek	28
4.	Site D is a riffle located on Stillwater Creek	29
5.	Numbers of simuliids and water temperature at Site A during the Spring of 1963	30
6.	Numbers of simuliids and water temperature at Site B during the Spring of 1963	31
7.	Numbers of simuliids and water temperature at Site C during the Spring of 1963	32
8.	Numbers of simuliids and water temperature at Site D during the Spring of 1963	33
9.	Site A. Graphs show larval counts and water temperature for Fall, 1963. The pupal counts were too low to graph	34
10.	Sites B and C. Water temperatures for the Fall, 1963. Larval and pupal counts were too low to graph	35
11.	Site D. Graphs show larval counts and water temperature for Fall, 1963. The pupal counts were too low to graph	36
12.	Distribution of number of pupal filaments in Sites A and B .	37
13.	The occurrence and relative abundance of the stages of simuliids observed in the test area. Stillwater, Oklahoma, 1962-64	38

#### CHAPTER I

#### INTRODUCTION

Although the attacks on livestock by simuliids are not widespread in north-central Oklahoma, the sudden appearance and viciousness of these insects is no less devastating to a victimized animal. Often damage is done to the animal before the owners are aware of the marauding insects. Simuliids are characterized by aquatic egg, larval, and pupal stages. The pupae and larvae require an environment of running water for development. The length of a life cycle from egg to adult varies from two weeks to more than one year. To determine the occurrence and abundance of the simuliid flies in this area, observations and counts were made of aquatic stage populations. Observations that showed constantly higher water temperatures and swifter currents at one study site were compared to observations taken from three other sites. To determine whether morphological differences in simuliids could be due to environmental causes, measurements were made of the lengths of pupal respiratory filaments and were compared to determine variations that may exist between different populations. Pupae were selected for this purpose for their position is stationary and they must rely solely on their aquatic environment for oxygen.

#### CHAPTER II

#### REVIEW OF LITERATURE

Larval Survey - In many early assessments of simuliid populations, emergence cages were constructed over the natural breeding sites (Emery, 1913), (Idle, 1940), (Twinn, 1950), and (Davies, 1950). While this method gives precise information on emergence and succession of species, it is not practical for assessment during control operations (Wolfe and Peterson, 1959).

Arnason et al. (1949) sampled known areas along transects to estimate larval infestation during treatment of the Saskachewan River. Thirty to fifty rocks were collected along a staked line and the black fly larvae were washed off and preserved. The Canadian workers developed the five-minute stone count method for estimation of larval populations. Three observers, using hand tallies, counted all larvae and pupae on preselected stations for a period of five minutes to obtain an index of the number of larvae present (Wolfe and Peterson, 1958). Peterson and Wolfe (1956) developed white hollow metal cones with a base diameter of 10 cm, a height of 20 cm, and an apex of 30° to determine larval populations. These cones were suspended in streams by a wire attached to the apex of the cones. Two cones were placed in the stream to be checked and the number of larvae that were attached to these cones were counted at intervals. One of the cones was cleaned following each count while larvae were permitted to accumulate on the second. The cones were considered

to be of value for the following purposes: Determining the level of infestation at a particular locality; estimating the growth rate of larvae; determining periods of peak abundance of larvae and pupae; indicating streams suitable for larval growth and evaluating larval control methods.

Wolfe and Peterson (1959) used pulp wood logs, four feet long and painted white to obtain data on larval populations. The logs provided a good surface for larval attachment when they were anchored at right angles to the stream flow and weighted so that they remained four to six inches beneath the surface of the water. However, a long time period was required to count all of the larvae. The logs frequently would become fouled with debris coming down stream or would break loose from the anchoring ropes.

<u>Comparison of Pupal Filaments</u> - Fox (1920) demonstrated simuliid pupal filaments as the site of oxygen uptake by using protozoans which formed aggregations in particular oxygen concentrations.

Dodds and Hisaw (1924) studied the relationship of the size and number of respiratory organs of mayflies and caddisflies to environmental conditions that were present in Colorado streams. The mayfly nymphs studied showed a definite correlation between gill area and the oxygen content of the water and a less distinct correlation with the velocity of the water of the stream. The case-bearing caddisfly larvae showed a striking correlation between the number of gill filaments and the size of the larvae. They did not show a definite relation between the number of filaments and the oxygen content of the water, though some facts observed indicated the existence of such a correlation. The caseless caddisfly larvae did not show a correlation between the number of gill filaments and the size of the larvae. Some of their observations showed a definite relationship between the number of gill filaments and the oxygen content of the water.

In his discussion of environmental resistance, Chapman (1931) used simuliids as an example, noting that the resistance of the environment begins to have its effect in the egg stage when the female introduces her egg into the swift water. This resistance continues until the adult insect emerges from the pupal case and escapes into the air above the water.

Grenier (1949) considered two environmental requirements as predominant in the larval ecology of simuliids: the amount of dissolved carbon dioxide in the water and the current speed. He considered the oxygen requirements to be peculiar to each species.

Field and laboratory studies by Phillipson (1956) on the effect of oxygen concentration and the speed of the current on the larvae of <u>Simulium ornatum</u> Mg. showed the current speed to be more important than oxygen concentration in governing distribution. The larvae seemed to prefer water with the velocity range of 1.7 - 3.9 ft/sec. with the greater numbers occurring at velocities between 2.6 - 2.9 ft/sec. Larvae move independently of oxygen content in concentration above the 50% saturation level.

Peterson and Wolfe (1956) reported a current velocity ranging from one-third to one foot per second was preferred by the larvae of most species. Pupae preferred to attach in more protected locations, provided there was constant turbulence. This situation existed on the downstream surfaces of logs, stones, and blades of grass. Turbulent

microcurrents provided a greater access to oxygen for the pupal respiratory filaments. Once average water temperatures exceeded  $40^{\circ}$  F, black fly development progressed rapidly. Optimum temperatures for development were  $55^{\circ} - 65^{\circ}$ F. Black fly streams are usually alkaline with a pH of 7.0 - 7.5 but development can occur in water having a pH range from 5.8 - 8.5. Current velocity rather than oxygen concentration was found to be the most important factor in determining the distribution of larvae. Heavy algal growth may cause diurnal fluctuations in the oxygen tensions of the water without affecting the larval distribution. In rearing black flies in the laboratory, Fredeen (1959) observed that temperature was a critical factor in survival and development.

In her analysis of the response of salamander gills to changes in the oxygen concentration of the medium, Bond (1960) found that the gills became much enlarged upon exposure to low concentrations, whereas the gills were reduced in size in those animals that were exposed to a medium of high oxygen concentration.

#### CHAPTER III

#### MATERIALS AND METHODS

Description of Test Sites - The four test sites were located in Payne County, Oklahoma, in the north-central part of the state. All were within Township 19 North, Range 2 East in the county. Site A was the riffle north of Redbud Drive Bridge over Boomer Creek. It was in the southeast one-fourth of Section 11. Figure 1 was taken from the bridge, facing north. Site B was the effluent falls of Boomer Lake Power Station. It was in the southwest one-fourth of Section 2. Figure 2 was taken from the head of the falls, looking toward the lake. Site C was the riffle south of the low-water bridge on Cow Creek. It was in the southwest one-fourth of Section 16. Figure 3 was taken from the east bank of the creek, facing north. Site D was the riffle on the south side of the low-water bridge on Stillwater Creek. It is in the northeast one-fourth of Section 21. Figure 4 was taken from the east bank of the creek, facing north.

<u>Topography</u> - The Stillwater test sites are about 888 feet above sea level and are a part of the Cimarron River water shed. Sites A, B, and D were located at man-made erosion-preventive structures. Site A, a concrete rubble-filled spillway for a small retaining dam, was three and one-half feet wide and thirty feet long. The water was one-half to four inches deep and moved at a surface velocity of approximately

0.7 feet per second. The west branch water source was seepage from Boomer Lake dam. The lake had not reached spillway level during the past eighteen months. The east branch drained grassland to the north and east. Site B was composed of wooden-building pilings with cement bases packed closely together in an upright position. The falls were 25 feet wide and 100 feet long. The water was 2 - 8 inches deep and had a surface velocity of two feet per second. The water source was Boomer Lake. Water was drawn from the lake to cool the power plant generators and was discharged via a cement sluiceway 200 yards in length. The falls were located between the end of the sluiceway and the lake. Site C was composed of red tile chips and small pieces of rubble that had been washed from the roadway. The flood-borne materials were deposited in the shallows below the low-water bridge. The site was four feet wide and 40 feet long. The water was 0.5 to 3 inches deep and moved with a surface velocity of 0.9 feet per second. The Cow Creek water source was grazing land and land under cultivation to the north. Site D was composed of cement slabs and small pieces of construction rubble. It was 68 feet wide and 35 feet long. The water was 0.5 - 6 inches deep and moved with a surface velocity of approximately 0.7 feet per second. Water sources for Stillwater Creek were seepage from Lake Carl Blackwell Dam, occasional discharges from the Oklahoma State University Hydraulic Research Laboratory testing area located at the base of the dam, and drainage of grazing and crop land to the west of Stillwater. All measurements were taken at winter mean low water. Sites A, C, and D were subject to flooding as a result of heavy rains and were observed at twelve feet above normal in 1958. Site B was not subject to the high water effects of rain. The infrequent flushing of

the cooling system of the power plant produces a rise of about 12 inches for a few hours. Silt content of the water at all sites depended on rainfall in the area. It was heaviest during flooding and lightest during dry periods during the winter. Water temperatures taken during the test period are recorded in Figures 5, 6, 7, 8, 9, 10, and 11.

<u>Climate</u> - The United States Weather Bureau Station for Stillwater, Oklahoma, was located on the Oklahoma State University Campus. Records for the period 1893 to 1960 showed a mean annual rainfall of 30.83 inches. Total annual rainfall in 1961 was 38.98 inches, in 1962 was 32.43 inches, and in 1963 was 27.32 inches. About 80 percent of the rain falls from March to November. The average mean temperature for the period 1893 to 1960 was  $60.9^{\circ}$  F. The 1961 mean temperature was  $59.2^{\circ}$  F. The 1962 mean temperature was  $60.6^{\circ}$  F. The 1963 mean temperature was  $61.8^{\circ}$  F.

<u>Vegetation</u> - The sites vary widely in the amount of shade received. Site A was located in a pecan grove. The trees were widely spaced, affording it only moderate shade. Tall grass and brush were cleared from the grove and creek banks each spring. Site B was completely devoid of tree shade, and brush shade was scant. Site C stream channel was completely shaded during the Spring test. Clearing brush, cutting trees, and straightening the stream channel during the month of August left the site with light to moderate shade. Site D received moderate shade from trees on both banks. It was too wide to be shaded by brush.

#### Procedure

Establishment of Counting Stations - The four sites in the area were chosen on the basis of observations of simuliid activity during the period October 1962 to April 1963. At each site, three counting stations were selected near the head, middle, and tail of each riffle. The stations were numbered 1, 2, and 3, starting with the upstream station. A part of a red-clay building brick, 3 and 3/4 inches long by 3 and 3/4 inches wide by 2 and 3/16 inches deep, was used for each station. The pieces of brick were spray-painted yellow on one surface which measured 3  $3/4 \ge 3/4$  inches. This surface was marked with the site designation and the station number. The painted surface was considered the top surface while the rough broken end was considered the rear surface. Thus each brick was in the same relative position, with top up and rear surface downstream, when in it was placed in the stream. Bricks, instead of cones as suggested by Wolfe and Peterson (1958), were used, not because of their shape, but because they will stay in place without anchors. If carefully placed, bricks are not affected by flash floods. Comparisons at the sites indicated showed that the larvae and pupae attached to the introduced brick stations as readily as to the natural surfaces.

<u>Counting</u> - Larval population data were obtained by periodically counting the larvae on the front, bottom, and sides of each station brick. The larvae attached to top and rear surfaces of each brick were not included in the population counts. Apparently the front, bottom and sides of the brick were preferred attachment sites, for only a few larvae were observed on the top and rear surfaces of the bricks examined. Fifty larvae per counted surface was set as the maximum population count. This figure was selected arbitrarily to allow the counting of all stations in the time available, and especially during peak population periods. Pupae were counted on the front, bottom, sides and rear

surfaces of the bricks to which they attached readily. All pupae observed were counted and no number limit was set for any collection period. After counting larvae and pupae at a station the empty pupal cases were removed from the brick surfaces and the station was replaced in the water with as little disturbance to the adherent population as possible. Seven days after the initiation of the yellow series, a second series was begun. Bricks used in this series were marked with white paint and were placed a few feet to the rear of the bricks of the yellow stations, but not directly downstream from them. Similar counting techniques were used at these stations with the exception that after counting the larvae and pupae on the white stations, all stages were removed from all surfaces of the brick, and the cleaned station was returned to the water.

Count records were kept on three by five data cards. Population counts included those for larvae observed on each brick surface designated as a station and for the total number of pupae observed per station. Water temperatures at each site during each observation period were also recorded. During autumn, counts of egg masses per station were included in the data collected. A total of 30 counts was made during the spring test period and 20 counts were made during the fall test period.

<u>Collecting Specimens</u> - Representative specimens of all aquatic stages and some adult females that were caught in egg masses during oviposition were collected from each test site. The specimens were placed in vials of 70 percent ethyl alcohol. Adult specimens netted during this same period were killed with HCN and were pinned on minuten nadelns for identification.

Water Temperatures and Velocities - Water temperatures obtained during the spring observation periods were taken with a Taylor alcohol Fahrenheit water thermometer. Fall water temperatures were obtained with a thermister thermometer using a calibrated surface-type probe. Water velocities were measured by surface float method recommended by King (1954). Results were calculated from the formula: distance divided by time in seconds equals feet per second. A stop watch was used for timing. The courses used in these determinations were measured with a one-hundred-foot steel tape. Two different materials were used as floats in these studies: 0.5- by 0.5- by 0.12-inch paraffin squares and spray paint samples that were applied to the surface of the water. The paraffin squares worked well at Site B for there the turbulence of the water caused the spray paint sample to dissipate before the marked course distance was traversed. The spray paint worked successfully in the very shallow water at Sites A, C, and D. All of the available surface courses at each site had a number of obstructions. Thus multiple floats had to be used so that a small percentage of the paraffin squares or paint samples would reach the end of the known distance without interruption. A series of three runs was made at each site on three different occasions to obtain water velocity determinations.

<u>Pupal Filament Measurements</u> - Random samples of pupae were collected from Sites A and B and were brought to the laboratory in vials of stream water. Pupal filament trees were removed from the specimen while it was in water. This operation was performed with honed teasing needles while observing through a dissecting microscope. Six to ten trees were placed on a one- by three-inch slide mounted in a layer of Hoyer's medium and covered with a 22 mm square cover slip. A Victor 16 mm Magnascope was used to cast the image of the filaments of the respiratory trees on paper. The magnification of this instrument was calibrated with a stage micrometer and a 300 mm ruler. All images of the gill filaments sampled were traced with a pencil at 100 magnifications on paper. The tracings were measured using a planimeter and the distance this instrument traveled was calibrated against the 300 mm ruler. Gill filament lengths were recorded at the distal end of each filament tracing. The sums of the measured lengths of the filaments and the number of filaments measured were recorded on each tracing.

<u>Water Analysis</u> - The dissolved oxygen content of the water at Sites A and B was determined by the Alsterburg (Azide) modification of the Winkler Method (A.P.H.A., 1960). Hydrogen-ion concentration and bicarbonate alkalinity were determined following a procedure recommended by Welch (1948).

#### CHAPTER IV

#### RESULTS AND DISCUSSION

<u>Spring Count</u> - The spring counts showed the highest numbers of larvae and pupae in late April and early May. Stations showed dwindling numbers of larvae and pupae when the water temperature was  $75^{\circ}$  F or above for a period of several days. This indicates the inability of the simuliids to survive extended periods of hot weather in the test areas. Populations of the yellow series, on which the larvae and pupae were allowed to accumulate, and the white series, from which the larvae and pupae were removed following each count, showed varied reactions as a result of heavy rains and high water from 25 to 30 April. Only Site B was counted during this period.

Comparison of the white and yellow series counts at each site showed a constantly higher number of pupae and larvae on the yellow series. This indicates a tendency for the larvae to remain at their point of attachment under normal conditions.

At Site A, the yellow and white series increased in numbers of larvae and pupae during the prolonged high water. Losses, if incurred during flooding, were offset by continuing development (Figure 5). A sharp increase in the larval count, in the white series, indicates movement of larvae during the high water period. All white stations were cleaned the day before the site was inundated. The larvae at this site reached their highest number on the first count following high water, indicating that the increased volume and velocity of the water was

timely and beneficial. The disappearance of the population was abnormal in both series. The number of pupae is usually equal to or surpasses the larval population as they both gradually disappear. The abrupt disappearance of the larval and pupal population on the same day coincided with the spraying of the pecan trees for the pecan nut case bearer. The site was located in the midst of a pecan grove, and it is assumed that the spray received by the stream during control operations caused the eradication of the population.

The location designated Site B had unique conditions of water temperature and velocity. Water temperatures average  $10^{\circ}$  F higher than those of the other sites because of heat absorbed from the power station generators where the water served as a coolant. It was the only site which had a relatively constant water velocity, two feet per second, throughout the test and observation period. The resident population disappeared when water temperatures rose above  $80^{\circ}$  F for forty-eight hours. High numbers of larvae and pupae in the white series (Figure 6) as compared to the yellow series indicate a high rate of movement by larvae in waters with a temperature of  $70^{\circ}$  F or above.

At Site C, the yellow series showed that a single generation developed during the period of high water in late April, (Figure 7). The interval between the larval peak and the pupal peak was nine days. The low white series numbers indicate little movement of larvae during the high water period (Figure 7). Following the large single generation on the yellow series, both series showed low numbers of pupae and larvae well into June. As water temperatures increased and stream velocity decreased, the counts on the white stations increased in proportion to the yellow counts, indicating migration of larvae as conditions became

more unfavorable. During the spring counts, the site was heavily shaded and water temperatures remained below  $75^{\circ}$  F until mid-June.

Yellow and white counts at Site D demonstrated the destructive effect of high water on the large larval population present in late April (Figure 8). The pupal population does not show a proportional decrease as the attachment sites chosen for pupation are more protected than the larval attachment sites and less subject to the deleterious effects of high water. The yellow series reflects two peaks of larval population in May. The tenacity of the population in late May and early June differs from the other sites although the weather and water conditions were similar. The site has a great number of good attachment sites for the population and a steeper stream gradient, thus creating a favorable habitat until high temperatures caused unfavorable conditions.

<u>Analysis of Spring Count</u> - An analysis was made to answer the following questions about the spring larval populations: Was there a difference between the populations of sites A, B, C, and C? Was there a difference between the populations of the yellow and white stations of each site? Was there an interaction between the individual stations of each site?

The analysis was based on data from selected days when all stations were counted. Periods when it was impossible to get counts because of high water were not considered. For the analysis, the number one was added to each observation and the logarithm of the resulting number was used. The results of the analyses were indicated in Table 1.

The <u>F</u> value for locations was significant at the 0.01 level. However, the <u>F</u> value for treatments (yellow vs. white stations) and locations

x treatments showed no significance. This indicated that the differences observed in the larval populations at the various sites are real and were not due to sampling errors. The lack of significance for the treatments indicated that the removal of a population from a sample station did not affect the repopulation of that location. No interaction was apparent between locations and treatments. Hence the removal of larvae from the white stations had no apparent effect on the population of that site.

<u>Fall Count</u> - The fall count showed very low numbers of simuliids present at Sites A, B, and D in December. No simuliids were found at Site C during the fall count. Record high temperatures and dry weather prevailed during September, October, and November, 1963. Station counts are here discussed on a per count basis to emphasize lack of population during the count. Because of the very low population figures analyses of the fall counts were not made. Larval counts of yellow and white series combined, totaled twenty-one larvae per count from 30 September to 30 November and 394 larvae per count from 30 November to 4 January, as compared to the spring average of 409.5 larvae per count. The fall pupal count for the combined series was two per count as compared with 124.7 per count in the spring. With the onset of cold weather and rain in late November, simuliid activity increased slightly. A continuing small increase was apparent at all sites except Site C through early January.

On the two upstream stations at Site A, a total of fifteen egg masses was counted the first two weeks in November. The combined count showed thirty-four larvae per count from 30 September to 30 November and 113.6 larvae per count from 30 November to 4 January (Figure 9).

Numbers of pupae found were too low to be significant and results were not graphed.

The water temperature at Site B averaged  $67.5^{\circ}$  F in November and  $51^{\circ}$  F in December. The combined larval count showed 0.3 larvae per count from 30 September to 30 November and 5.4 per count from 30 November to 4 January. There were no pupae present from 30 September to 30 November and 0.6 per count from 30 November to 4 January. Only the water temperature was graphed for Site B (Figure 10).

Site C was cleared of brush and most of the trees and the creek bed was graded in August. After renovation, the site contained only standing water which dried up in early October and remained dry until 20 November. On 7 December, five egg masses were counted on the stations, indicating reinfestation by flies from outside sources. The eggs were laid at the water line but did not hatch as a slight drop in the water level allowed the eggs to dry out. No other black fly activity was observed during the fall count. Only the water temperature was graphed for Site C (Figure 10).

The combined larval count at Site D showed 3.1 larvae per count from 30 September to 30 November to 4 January (Figure 11). The combined pupal count showed one pupa per count from 30 September to 30 November and three pupae per count from 30 November to 4 January. The pupal population was not graphed. Observations made in the fall of 1962 indicated the height of simuliid activity was about 15 November.

<u>Comparison of Pupal Filaments</u> - The paired respiratory organs of the pupa are filamentous in form. They are multiple, hollow, thin-walled evaginations of the integument containing finely-branched tracheae and

abundant tracheoles. The organs, also called gills or trees, arise anteriorly from the thorax. The trees show great variability in the number of filaments and the method of their branching, but usually these characters are quite constant for a given species. A respiratory gill of Simulium vittatum Zetterstedt has a single base which divides in two. From each of these, there arise four branches; these again are divided into two, making sixteen tracheal filaments for each gill (Emery, 1913). Oklahoma species were collected in December, 1963 and January, 1964 from sites A and B. Site B presented unique conditions as it had water temperature 10° F higher and a surface water velocity twice that of the other sites. Table 2 shows a comparison of ecological conditions at Sites A and B. Site A presented typical area conditions but was located near enough to Site B that the weather conditions were very similar. Specimens showed a range of thirteen to twenty filaments per tree (Figure 12) which were normally distributed. The mean number of filaments for Site A was 15.70  $\pm$  0.25 and for Site B was 15.78  $\pm$  0.24 (Table 3). A "t" test indicated that the means did not differ significantly, P = 0.80. The mean length of filaments from Site A was 2.00  $\pm$ .04 mm and from Site B was  $1.42 \pm .03$  mm. These showed a highly significant difference in mean length, P = 0.001.

An explanation for the differences in the filament length cannot be made without extensive laboratory studies. A consideration of the various environmental factors indicates that the most important ones appear to be stream velocity and dissolved oxygen content. Larvae in water having a slower velocity and lower oxygen content require more filament surface to come in contact with the required amount of oxygen during a given period of time.

#### CHAPTER V

#### SUMMARY AND CONCLUSION

An aquatic population study was conducted to gain information on the biology of black flies of north-central Oklahoma.

Bricks were found to be suitable larval habitats for aquatic simuliid counting stations. Station counts at selected sites showed a significant difference between populations. High water caused changes in the populations of sites affected by it. Movement of larvae was evident when unfavorable environmental conditions prevailed. Populations failed to maintain themselves when water temperatures rose above 75° F for several consecutive days. No summer population was observed in the area. The hot, dry weather conditions delayed and suppressed black fly populations in the fall.

Conclusions drawn from the results of the spring station count analysis showed significant difference between the sites in population. High water had a stimulating effect on the population of Sites A and C and a deleterious effect on the population of Site D. Site B was not affected by high water. The effect of high water is dependent on the topography of the site and the degree of development of the population. Populations of the sites could not maintain themselves when water temperatures rose above 75° F for prolonged periods in June. The accidental application of DDT to Site A eradicated the population in mid May.

During the fall of 1963, hot, dry weather conditions severely

limited the growth of simuliids. Site C, which was rendered uninhabitable by lack of water and stream velocity, was repopulated by ovipositing females from other sources.

A comparison of the lengths of pupal filaments of specimens collected from Sites A and B showed a range of thirteen to twenty normally distributed filaments with no significant difference in the number of filaments collected at different sites. The mean length of filaments showed a highly significant difference between the two selected sites.

The occurrence and relative abundance of the black fly population of north-central Oklahoma was dependent on stream conditions. The stream conditions were in turn governed by the weather of the area. Observations of the simuliid habitats were made from August, 1962 to January, 1964. All stages were observed in March, April, May, October, November, and December. No stages of black flies were found during July, August, and most of September. A search for oversummering eggs was unsuccessful. These observations differ from those of Emery (1913) who found spring, summer, and fall broods in Kansas. <u>Simulium vittatum</u> Zetterstedt was the only species found in the area.

#### LITERATURE CITED

- American Public Health Association. 1960. Standard methods for examination of water and waste water. A.P.H.A. 11th Ed. 626 pp.
- Arnason, A. P., A. W. A. Brown, F. J. H. Fredeen, W. W. Hopewell, and J. G. Rempel. 1949. Experiment in control of <u>Simulium</u> arcticum Malloch by means of DDT in the Saskachewan River. Sci. Agr. 29: 527-35.
- Bond, A. N. 1960. An analysis of the response of salamander gills to changes in the oxygen concentration of the medium. Developmental Biology. 2: 1-20.
- Chapman, R. N. 1931. Animal ecology. McGraw-Hill Col, New York. 464 pp.
- Davies, D. M. 1950. A study of the black fly population of the streams of Algonquin Park, Ontario. Trans. Roy. Can. Inst. 28: 121-26.
- Dodds, G. S. and F. L. Hisaw. 1924. Ecological studies of aquatic insects. 2. Size of respiratory organs in relation to environmental conditions. Ecology. 5: 262-71.
- Emery, W. T. 1913. Morphology and biology of <u>Simulium vittatum</u> and and its distribution in Kansas. Kansas Univ. Sci. Bul. 8: 323-62.
- Fredeen, F. J. H. 1959. Rearing black flies in the laboratory. Diptera:Simuliidae. Can. Ent. 91: 73-82.
- Fox, H. M. 1920. Protozoa as site of oxygen uptake. J. Gen. Physiol. 3: 565-74.
- Grenier, P. 1949. Facteurs ecologiques conditionnant la repartition des larves <u>Simulium</u> (Dipt). Variations morphologiques specifiques en rapport avec la vetesse du courand. Physiol. Comparata et Oecol. 1: 165-330.
- Idle, F. R. 1940. Quantitative determination of the insect fauna of rapid water. Bio. Ser. 27. Pub. Ont. Fish Res. Lab. 59: 1-27.
- King, H. W. 1954. Handbook of hydraulics for the solution of hydraulics problems. McGraw-Hill Col, New York. 523 pp.

- Peterson, D. G. and L. S. Wolfe. 1956. The biology and control of black flies (Diptera:Simuliidae) in Canada. Proc. Intern. Congr. Ent. 10th Congr. Montreal. 3: 551-64.
- Phillipson, J. 1956. A study of factors determining the distribution of the larvae of the black fly <u>Simulium</u> <u>ornatum</u> Mg. Bul. Ent. Research. 47: 227-38.
- Twinn, C. R. 1950. Studies of biology and control of biting flies in northern Canada. Arctic. 3: 14-26.
- United States Weather Bureau. 1963. Climatological data for Oklahoma. Washington, D. C. p.1-12.
- Weiss, P. 1939. Principles of development. Henry Holt and Co., New York. 601 pp.
- Welch, P. S. 1948. Limnological methods. Philadelphia, Penn. 381 pp.
- Wolfe, L. S. and D. G. Peterson. 1958. A new method to estimate levels of infestation of black fly larvae (Diptera: Simuliidae). Can. J. Zool. 36: 863-67.
- Wolfe, L. S. and D. G. Peterson. 1959. Black flies (Diptera: Simuliidae) of the forests of Quebec. Can. J. Zool. 37: 137-59.

. .

Source of Variability	Degrees of Freedom	Sum of Squares	Mean Squares
Locations	3	6.5014	2.1671
Treatments	1	1.1813	1.1813
Locations x Treatment	s 3	1,1367	0.3789
Within Cells	64	31.5418	0.4928
Totals	71	40.3612	

Table 1. Analysis of Variance of Larval Populations.

Table 2. Comparison of the Ecological Conditions at Sites A and B During January, 1964.

Factors	Site A	Site B
Mean Stream Velocity*	0.8 ft/sec	2.0 ft/sec
Mean Water Temperature*	44 <sup>0</sup> F	56 <sup>0</sup> F
Mean Air Temperature*	62.6° F	63.7° F
Shade Factor*	Light	None
Dissolved Oxygen Content**	84%	108%
Alkalinity of Water**	330 ppm	135 ppm
Hydrogen-ion Concentration**	7.8	7.2

\*For growth period of simullids. \*\*Determined at end of test period. Table 3. Measurements of <u>Simulium</u> Pupal Respiratory Filaments.

	Location	
	Site A	Site B
No. of Filaments	362	426
Mean Length <u>+</u> S. E.	2.00 <u>+</u> 0.04	1.42 <u>+</u> 0.03

Number of Filaments on Pupal Respiratory Trees

	Location	
	Site A	Site B
No. of Trees	23	27
Mean No. of Filaments <u>+</u> S. E.	15.70 <u>+</u> 0.25	15.78 <u>+</u> 0.24



Figure 1. Site A is a riffle located on Boomer Creek



Figure 2. Site B is the falls located at the end of the Boomer Lake Power Plant effluent sluiceway.



Figure 3. Site C is a riffle located on Cow Creek



Figure 4. Site D is a riffle located on Stillwater Creek.



Figure 5. - Numbers of simuliids and water temperature at Site A during the Spring of 1963.



at Site B during the Spring of 1963.



Figure 7. - Numbers of simuliids and water temperature at Site C during the Spring of 1963.



Figure 8. - Numbers of simuliids and water temperature at Site D during the Spring of 1963.







Figure 10. - Sites B and C. Water temperatures for the Fall, 1963. Larval and pupal counts were too low to graph.











# Robert Wayland Upham, Junior Candidate for the Degree of

Master of Science

#### Thesis: A STUDY OF THE LARVAL POPULATIONS AND COMPARISON OF LENGTHS OF PUPAL FILAMENTS OF SIMULIIDS IN NORTH CENTRAL OKLAHOMA

Major Field: Entomology

Biographical:

- Personal Data: Born in Providence, Rhode Island, 29 April 1933, the son of Robert Wayland and Villette White Upham.
- Education: Graduated from Providence Country Day School, East Providence, Rhode Island in 1952; received the Bachelor of Science Degree from the University of Maine with a major in Entomology, June, 1957.
- Experience: Restaurant counter man, Summers, 1951 and 1952; experimental farm assistant, Summer 1953; construction worker Summers 1954 and 1956; Lieutenant, United States Army Medical Corps, MOS 3315, Section Officer, 37th Medical Company (PM) (Sep) Korea, 1957-1959; Section Officer, Executive Officer, and Commanding Officer, 714th Medical Company (PM) (Sep) Fort Stewart, Georgia, 1959-1962.
- Organizations: Beta Theta Pi Social Fraternity, Phi Sigma, Sanborn Entomology Club, Association of the United States Army, Loyal Order of the Boar.

#### VITA