SEASONAL ABUNDANCE AND HOST PREFERENCE OF *CULICOIDES* IN VIRGINIA: WITH EMPHASIS ON THE ECOLOGY OF *CULICOIDES VARIIPENNIS* (DIPTERA: CERATOPOGONIDAE).

by

ROBERT HENRY ZIMMERMAN

Dissertation submitted to the Graduate Faculty of the
Virginia Polytechnic Institute and State University in
partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY
in
Entomology

E. C. Turner, Jr., Chairman

R. Andrews

D. E. Mullins

R. L. Pienkowski

J. R. Voshell

November, 1981

Blacksburg, Virginia

ACKNOWLEDGEMENTS

The writer wishes to thank his major advisor, Dr E.C. Turner Jr., for his advice and support. Also, the constructive criticism of his committee members, Dr. R. Andrews, Dr. D.E. Mullins, Dr. R.L.Pienkowski and Dr. J.R. Voshell was extremely valuable.

The technical assistance of was appreciated. The help and discussion of fellow graduate students including contributed to the quality of the reasearch.

Partial financial support was from USDA, Aphis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter I

INTRODUCTION

Biting midges of the genus *Culicoides* are mainly pests of warm blooded mammals, including man and livestock. They have been implicated in the transmission of several diseases, Bluetongue virus (BTV), of livestock is the most important disease transmitted by *Culicoides* in North America (Kettle 1965).

The transmission of arbovirus (viruses transmitted by arthropods) involves three major aspects of vector host interaction: (1) affinity of the vector for a vertebrate species that can circulate the pathogen in its blood in an infective dose for the vector, (2) numerical abundance of the vector and (3) longevity of the vector at temperatures that favor completion of extrinsic incubation of a pathogen in the vector (Reeves 1971).

It is well known that cattle and sheep are capable of carrying an infective dose of BTV in their blood for a vector (Metcalf 1976), but very little is known about which *Culicoides* species have an affinity for domestic livestock. Also, knowledge on the abundance and longevity of *Culicoides* species is lacking. Therefore, it is important to examine the biologies of *Culicoides* with emphasis on the disease transmission variables listed above before implicating them as vectors.

Due to the lack of a virus laboratory in Virginia, vector-host interactions cannot be entirely examined. I will examine the potential vector-host affinities as well as the abundance and longevity of potential vectors. I have assumed that the potential vectors are those species that are more abundant on cattle and sheep and have greater longevities than other species collected on the host animals.

The purpose of this study is to examine the *Culicoides* of Virginia in relation to:

1. The numerical abundance of *Culicoides* and their affinity for sheep and cattle.

2. Examine the longevity of *Culicoides* individuals collected in the field.

3. Single out the most important potential vector(s) and examine the relation between dispersal, sex, female age and height of flight.

4. Relate these results to potential BTV transmission.

This study is divided into two sections: (1) The seasonal distribution and host preference of *Culicoides* in Virginia and (2) A detailed examination of the ecology of *Culicoides variipennis* (Coquillett) in Saltville, Virginia.

Chapter II

LITERATURE REVIEW

BLUETONGUE VIRUS

Bluetongue (BTV) is in the family Reoviridae; group orbovirus. There are 16 serotypes with four occurring in the United States. It is an infectious, non-contageous viral disease of ruminants and is characterized by inflammation and congestion of the mucous membranes leading to cyanosis, endema and ulceration (Metcalf 1976). Death usually results from secondary pneumonia. In the United States, it was first confirmed in sheep in 1952 and in cattle in 1959 (Bowne 1973). Because of the presence of BTV in North America, Australia and New Zealand refuse to accept U.S. cattle, semen or embryos, representing a 10-15 million dollar loss to U.S. cattlemen alone.

The disease is transmitted by midges of the genus Culicoides. In the United States, C. variipennis is the only known vector (Leudke et al. 1976). This vector must ingest the virus from a reservoir because transovarial transmission is not known to occur in this species (Jones & Foster 1971). BTV transmission to cattle and sheep reaches a peak 10-14 days after virus ingestion under laboratory conditions (Foster & Jones 1973). Congenital infection of lambs and calves by BTV has been demonstrated and outbreaks of BTV occur from mid to late summer (Metcalf 1976).

Since midges do not overwinter as adults and no transovarial transmission occurs, the vector cannot be a reservoir of BTV. Wild vertebrates have been shown to have BTV antibodies, but are unlikely reservoirs (Metcalf 1976). The overwintering reservoir appears to be cattle (Luedke et al. 1977). Also, it was shown by Luedke et al. (1977) that bites from noninfected _C. variipennis_ caused the appearance of BTV in the bloodstream of a bull that previously had BTV, but after several years the bull was not serologically positive for BTV. This vector-mediated viral recovery mechanism caused noninfected midges at the next biting interval to become infected with BTV and transmit BTV to sheep. Not only does this point out the importance of cattle as the reservoir of the disease, but it suggests that other noninfected midge species, which may or may not be able biologically to transmit BTV, could stimulate BTV to appear in the peripheral blood of cattle and thereby promote vector transmission.

HOST PREFERENCE

Three genera of Ceratopogonidae (_Culicoides_, _Leptoconops_ & _Forcipomyia_) bite warm-blooded vertebrates (Kettle 1962). Only _Culicoides_ species are implicated in the transmission of BTV to sheep and cattle. Bloodfeeding occurs only in females as is the case in all Nematocera. It appears that most species are opportunistic feeders, though several are selective for specific hosts and feeding sites on the host (Kettle 1977).

Humphreys (1969) and Tanner (1971) have extensively reviewed the host preference of *Culicoides* and studied host preference and feeding activity of *Culicoides* in southwestern Virginia. These studies were not specifically designed for the study of midges attracted to livestock nor to examine host preference in other parts of the state. Most studies concerning the attraction of midges to livestock in North America have been conducted following an epizootic or in endemic regions in the western states (Jones 1961a, Jones et al. 1972, Jones & Akey 1977, Jones et al. 1977). The most abundant species collected was *Culicoides variipennis* (Coq.). In central New York State *Culicoides biguttatus* (Coq.), *Culicoides obsoletus* (Meigen) and *Culicoides stellifer* (Coq.) were the most abundant species attracted to pastured livestock (Schmidtmann et al. 1980a). All four species occur in Virginia (Battle & Turner 1971).

Generally it is assumed that if a biting fly is collected from a host or host trap the insect is seeking blood from the host. It is necessary to either collect the insect in the act of feeding or examine bloodmeals serologically to be positive about the source of the bloodmeal, but for practical reasons bait traps indicate which species have a particular affinity for a host.

PAROUS RATES

Potential disease transmission has been described as the epidemiological risk and is generally assessed by the parous rates of the females (Detinova 1962). The reproductive stages are defined as nulliparous (females that have not yet oviposited), engorged, gravid (have eggs) and parous (females that have oviposted at least once). Because females can be engorged or gravid and still be nulliparous or parous, and determining parity in engorged or gravid females is difficult, it is necessary to redefine nulliparous and parous excluding engorged and gravid females. I consider engorged and gravid females as separate stages, because at the time of collection neither stage is actually part of the population seeking a host and would not be considered potential vectors at that time. Therefore, I define parous rate (PR) as the number of empty females that have layed eggs at least once (parous females (P)) divided by the sum of parous females plus the number of empty females that have not oviposited (nulliparous females (NP)) times 100.

$$PR = (100)(\# P / \# P + \# NP)$$

The sequence of reproductive events from nulliparous through engorgement and egg maturation (gravid) to parous has been characterized as physiological aging. Recently, Klowden and Lea (1980) have argued that being physiologically old is not the same as being reproductively old. This means that a nulliparous female may be physiologically quite old, but may not be old with regard to ovarian development. They suggest that gonotrophic aging be used to distinquish female reproductive changes. I will use gonotrophic aging in this study to distinquish the above sequence of ovarian development.

To examine the epidemiological risk (or vector potential) parous rates are observed. The parous condition in Culicoides is usually discernable by: (1) Examination of the ovaries and associated uncoiling of the tracheoles after oviposition (Detinova 1962), (2) Observation of the abdominal tergite color change from clear (nulliparous) to burgundy colored (parous) (Dyce 1969). The latter method is easier to use in the field and therefore is used here. For C. variipennis, a combination of color change and a change in trilobed abdominal tergites (nulliparous) (see Figures 8 & 9), to unlobed abdominal tergites (parous) is used (Potter & Akey 1978). Other midges such as Culicoides venustus Hoffman do not show a color change with aging and can only be examined by Detinova's method. Therefore, specimens must either be collected live or in saline to prevent the drying of the ovarian tracheoles.

Though more precise methods of determining adult longevity are used such as examination of ovarial dilatations (number of egg layings) or following a cohort through time, parous rates have been conveniently applied to the study of adult longevity. Parous rates provide an estimate of the average longevity per average oviposition cycle, providing that recruitment rate is somewhat constant (Birley and Rajagopalan 1981). Estimations of longevity using parous rates are limited by short trap times and rapidly fluctuating environmental conditions. Because of these limitations parous rates in this study will be assessed in a broad sense. For example, if a species has high parous rates and is multivoltine, it will be considered longlived compared to a multivoltine or univoltine species which has low parous rates.

A species which has a high parous rate does not necessarily reflect an epidemiological risk. Characteristics such as host preference other than livestock, a seasonal cycle out of rhythm with BTV transmission, or a low total number of parous females would tend to make a species epidemiologically less important.

DISPERSAL

Numerical abundance is a dynamic characteristic of a population and is not only affected by the emergence of adults and adult longevity, but also by the movement of individuals. The movement of insects has been reviewed by Johnson (1969) and for Culicoides by Johnson (1969) and Kettle (1977). Maximum distance traveled varies with species. Some species travel only short distances (74 m) and others 50 Km aided by the wind. It has been suggested that outbreaks of BTV in Spain and Cypress are caused by the windborne movement of midges from other countries (Sellers et al. 1978, Sellers et al. 1979). Also, Glick (1939) collected Culicoides as high as 4,000 m above the ground. Therefore, vertical movement must also be considered an important attribute of BTV transmission.

The term migration has been generally defined as "the act of moving from one spatial unit to another" (Barker 1978). This broad definition has been used in an attempt to better understand the evolution of animal migration and is not applicable to this study. The general definition is dependent on a clear understanding of spatial unit which is not an objective of this study. Also, I make no distinction between appetential flight (host-seeking) and undisturbed movement from place to place in an adaptive or evolutionary sense. Barker (1978) further defines dispersal as an increase in the mean distance between individuals. In this study, I use dispersal to describe the movement of midges, not because it fits the above definition, but because it follows the general trend in entomology to define general movement away from a source as dispersal.

The relationship between the age of an insect and when it disperses is important when studying vector dynamics. Extensive literature exists on ovarian development in biting Nematocera, especially mosquitoes, but very little information is avaliable on the movement of different gonotrophic ages. Nulliparous *Aedes taeniorhynchus* (Wiedemann) migrate as soon as they emerge (Provost 1952). Crans et al. (1976) found *Aedes sollicitans* (Walker) to have a reduced tendency to migrate after oviposition. Parous *Culiseta melanura* (Coq.) were found to be farther away from the breeding site than nulliparous females in the latter part of the season when eastern Equine Encephalomyelitis virus was transmitted (Morris et al. 1980). Nulliparous *Prosimulium mixtum* Syme & Davies were found farther away from the breeding site than were parous females (Johnson 1969).

Studies on the movement of *Culicoides* at different gonotrophic ages are limited. Nicholas (1953) studied the dispersal of nulliparous *Culicoides grahamii* (Austen) and *Culicoides austeri* C.,I.&M. from a breeding site and collected both species at the greatest distance trapped (368m). No other information on the movement of midges of different gonotrophic ages is known. Though, it has been suggested that parous *Culicoides impunctatus* Goet. move relatively little (Johnson 1969).

Since we base the epidemiological risk of a disease on the number of parous individuals it is important that we subdivide the total females into gonotrophic ages. Using this approach the movement of all gonotrophic ages can be examined separately to get a better understanding of the epidemiology, dispersal and evolution of life history tactics for a given species.

Chapter III

SEASONAL DISTRIBUTION, ABUNDANCE AND HOST PREFERENCE OF CULICOIDES

INTRODUCTION

The *Culicoides* of Virginia and their geographical distribution has been summarized by Battle & Turner (1971), but studies on their seasonal occurrence, abundance and host preference has been limited primarily to those species inhabiting the forested regions of western Virginia (Murray 1957, Humphreys & Turner 1973, Tanner & Turner 1974, 1975). Other studies on species known to be present in Virginia have been summarized by Blanton and Wirth (1979).

Because there is a lack of knowledge of the species present in other areas of the state and because *Culicoides* are important vectors of livestock diseases (Kettle 1965), a study of these midges was conducted in three of the five major physiographic provinces of Virginia. The purpose of this study was also to examine the attractiveness of *Culicoides* to cattle and sheep and to associate those species attracted to these animals with the total number of species collected in blacklight traps. Then to relate seasonal abundance, host preference and age to vector potential.

MATERIALS AND METHODS

Study Sites

One site was selected in three of the five physiographic provinces of Virginia (Ridge & Valley Province (RVP), Piedmont Plateau (PP) and Coastal Plain (CP) (excluding the tidewater area) (Hoffman 1969). Forest types for each province were oak-hickory, oak-hickory with secondary pine and southeastern evergreen, respectively. In this study, I assume that the RVP trap site is typical of the livestock raising areas of the other two provinces in western Virginia (Blue Ridge and Appalachian Plateaus).

The RVP site was located on the VPI & SU farm in Blacksburg, VA (41° 12' N 80° 26' W) at an elevation of 677 m. The trap site was located between the VPI & SU Beef and Sheep Centers on Co. Rte. 314 in a pasture with two small oak-hickory woodlots approximately 20 m^2 each. The nearest woodlot was 60 m from the trap site. Two small first order streams as well as one impounded and polluted first order stream were located 500 m from the trap site. One six acre pond was located 750 m from the trap site and the entire area was well drained.

On the Piedmont, in Campbell Co., two sites were used in 1978 and one in 1979. The Bennett farm (41° 12' N 73° 51' W), (used both years) and the Farris farm (37° 16' N 79° 1' W) were at an elevation of 244 m. Both trap sites were off Co. Rte 663. The Bennett trap site was located in a pasture directly behind the main house in a pasture with a 40 m^2 oak-hickory woodlot 33 m from the trap site. Six impounded first order streams ranging from 2-10 acres were located within 500 m of the trap site. This area was well drained as was the Farris farm. The Farris farm was located 11 Km west of the Bennett farm and contained a continuous mixture of oak-hickory woods throughout and two first order streams 150 m from the trap site. The trap site was 20 m from the barn. The Farris farm had less pasture.

The CP trap site was located on the Redd farm in Suffolk Co. (36° 48' N 76° 41' W) at an elevation of 11 m. This farm was off State Rte. 360, three miles west of Suffolk. The traps were placed directly behind the main house in a pasture with the nearest mixed deciduous woodlot 250 m from the trap site. Two large brackish lakes were situated 500 m north and south of the trap site. Both lakes were affected by tidal fluctuations. Twelve 1-3 acre ponds were also located within 330 m of the site. Drainage was poor.

Trap Type

The first trap used was a New Jersey light trap modified with a 4.7 cm circular blacklight and 64 mesh plastic screening (placed around the opening to inhibit the collection of large insects). The blacklight trap was operated from 30 min pre-sunset to 90 min post-sunset once a week at all sites (SS trap period). At the CP trap site an additional light trap was operated from 90 min post-sunset to 0800 the next day (RN). On occasion, additional traps were also run at the other two sites. When the SS trap time and the RN trap time were combined the trapping period was designated AN. Also, on eight nights the blacklight trap was operated during the same four trap times as the bait traps (SS-30, SS, SS+30, SS+60, see below). Collections were made in half-pint mason jars 2/3 filled with 80% ethyl alcohol. Collections were poured through 50 mesh sieves which effectively removed the large insects, but not the midges. Samples were placed in 3.5 x 4.3 cm white enamel pans which had been subdivided into 45 equal squares by raised beads of silicone rubber caulking. Squares were selected at random and a minimum of 200 midges were identified. This separation and identification method was similar to that used by Jamnback and Watthews (1963). Identifications to species were made using a binocular microscope (60x), and when necessary the midges were dissected and mounted on slides in Hoyers media for identification using a Zeiss phase contrast microscope. Battle and Turner (1971) and a revised key (Turner and Knausenberger, unpub.) were used for all identifications.

Bait animals at all sites were cattle and sheep. At the RVP site a 382 Kg Angus steer and a 70 Kg Suffolk ewe were placed 500 m apart in 1978 and 30 m apart in 1979. The steer was placed in the same location both year, but the ewe was moved closer to the steer in 1979 to decrease possible differences in catch due to site. In 1978 the blacklight trap was operated 100 m from the ewe and 400 m from the steer, while in 1979 the light trap was operated 45 m from the steer and 70 m from the ewe.

At the PP site, during 1978, a 55 Kg Suffolk ewe was used as the bait animal on the Bennett farm . It was placed 30 m from the blacklight trap. A 80 Kg Angus calf was used on the Farris farm and positioned 30 m from the blacklight trap. In 1979, both animals were located on the Bennett farm, but a 100 Kg Holstein calf was used instead of the calf. The distance between the bait animals in 1979 was 33 m and both were placed approximately 100 m from the light trap. The blacklight trap was put in the same location both years.

The bait animals used at the CP site were a 365 Kg Jersey cow and a 68 Kg Suffolk ewe. In both years the animals were placed 36 m apart, the heifer was located 30 m and the sheep 20 m from the blacklight trap.

In 1978, a 12 volt portable vacuum cleaner modified to trap small midges was used to collect Culicoides from the bait animals. However, on several occasions more midges were observed on the hosts than trapped in the vacuum cleaner, so in 1979 the collection procedure was changed to a drop trap over the animals (Figure 1). The drop trap (2.4 x 1.8 x 1.2m) for the cow was made from 5.8 x 5.8 cm framing boards. Each side was constructed separately to provide for portability and sides were bolted together when in use. The top, front and back were covered with monofilament Saran screening (44 x 44 mesh). On the sides the upper 30 cm were covered with superclear Butyrate which overlapped the screening by 5 cm. This provided visibility as well as a slot to allow the use of a

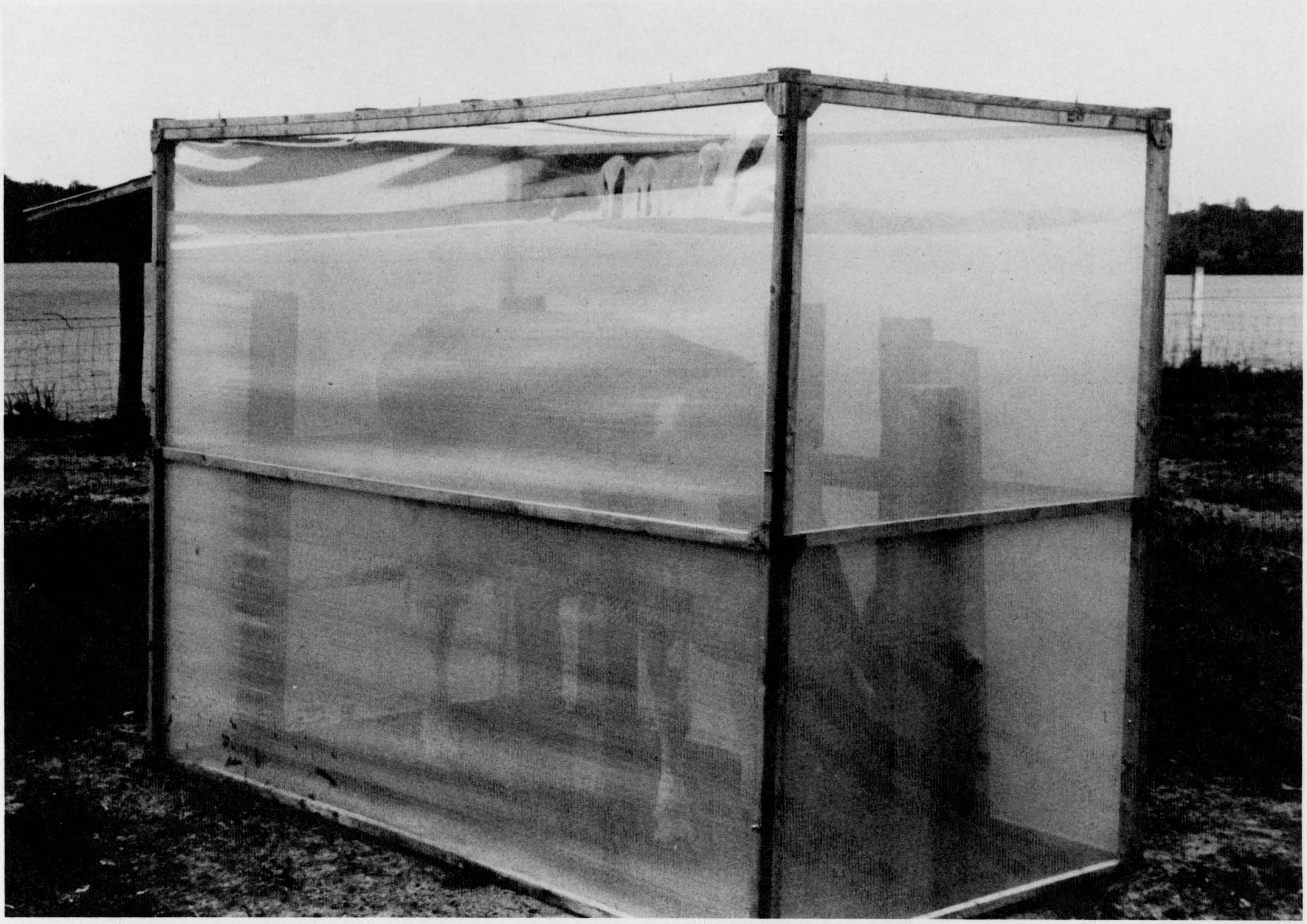

Figure 1. Cattle drop trap.

hand aspirator in collecting the midges from the trap. Midges are positively phototaxic and could be easily collected from the inside top and upper sides of the trap. The cow was restricted from damaging the trap by placing it in a stall consisting of four corner posts (10.2 x 10.2cm) with one hitching post (10.2 x 10.2cm). Two boards (2.5 x 10.2cm) connected the posts to prevent sideward movement of the cow. Calf and sheep traps were similar to the cattle trap , but differed in dimensions. The sheep were restricted from movement by being placed in a wire cage (2.5 x 2.5cm mesh) before the drop trap was used.

Collections from the bait animals were made during the same time period as the SS blacklight traps. Bait animals were covered until trapping began at 30 min before sunset (SS-30). They were exposed to midge attack for 15 min, the drop trap was then placed over the bait animals and midges collected for 15 min. This 15 min interval was long enough for two people to collect all midges. This procedure was carried out four times. Time periods were designated SS-30, SS, SS+30 and SS+60. After sunset, battery operated head lamps were used to aid in the collection of midges. Midges were transferred into vials containing 80% ethyl alcohol and preserved for later identification.

The gonotrophic ages of all Culicoides were classified as either nulliparous (NP), engorged (E), gravid (G) or parous (P). Parity for all species except C. variipennis (Coquillett) and C. venustus Hoffman was determined by the pigmentation method described by Dyce (1969), where abdominal color changes from a clear white to a burgundy red as the female becomes parous. This method is not accurate for C. variipennis nor C. venustus, but it has been shown by Potter & Akey (1978) that the abdominal tergites of C. variipennis change from a nulliparous condition of a dark trilobed pattern to a completely dark appearence when parous. Though engorged females were not classified as NP or P using Dyce's method because of possible inaccuracy, C. variipennis could be further classified as engorged NP (ENP) or engorged P (EP) using the tergite method which does not rely on a color change similar to the color of blood. Neither method could be used for C. venustus, because information on pigmentation changes in this species is lacking. The classical tracheolation technique (Detinova 1962) could not be substituted, because collections were made in alcohol which dries the ovarian tracheoles. C. venustus were classfied as nonengorged (NE) or engorged (E).

Data on the most abundant species collected in blacklight traps were analyzed in two ways: (1) results were presented in numbers per trap time so comparisions between sites and species could be made (Service 1976) and (2) weekly collections were transformed to Log (N+1) to examine trends in population abundance (Williams 1937).

Because bait animals varied in size, their weights were converted to surface area using the formulas of Brody (1945), and number of midges per square dec. was compared between bait animals.

Climatological data was obtained from the nearest National weather station to the trap site (RVP in Blacksburg, PP in Appomatox and CP at Lake Kilby).

RESULTS AND DISCUSSION

Blacklight Traps

Of the 47 species of *Culicoides* recorded in Virginia, I collected 30 in blacklight traps. All species collected are listed by year, site, trap time and reproductive status in Appendix A. Table 1 shows the five most common species collected and average catch by site in 1978 and 1979. Of the remaining 25 species collected, *C. mulrennani* Beck (n=9) from the CP site and *C. ousairani* Khalaf (n=1) were collected only from the VP site (Bennett farm) when additional traps were operated all night. Both species have been previously reported from Virginia (Battle & Turner 1971) and would be expected to occur in all regions.

Tables 2,3 and 4 show the dates and numbers caught of the remaining 23 uncommon species. The eleven species collected at all sites were *C. alexanderi* Wirth & Hubert, *C. arboricola* Root & Hoffman, *C. bickleyi* Wirth & Hubert, *C. crepuscularis* Malloch, *C. guttipennis* (Coquillett)(collected at the RVP site only when an additional light trap was operated at night). *C. haematopotus* Malloch, *C. piliferus* Root & Hoffman, *C. sanguisuga* (Coquillett), *C. spinousus* Root & Hoffman, *C. testudinalis* Wirth & Hubert and *C. travisi* Vargas. The other 12 species were absent from at least one site in both years. The presence or absence of three species can be attributed to their seasonal distribution and larval habitat. *C. furens* (Poey) is a saltmarsh species and would only be

Table 1. Average number of most common *Culicoides* collected per trap time in blacklight traps during bait trap time periods, 1978 and 1979, in Virginia.

	1978			1979		
Species	Coastal Plain (12)*	Piedmont Plateau (15)	Ridge and Valley (11)	Coastal Plain (18)	Piedmont Plateau (12)	Ridge and Valley (9)
C. biguttatus	10.33	2.13	0.18	9.33	17.33	2.78
C. obsoletus	1.83	7.67	16.27	0.08	18.25	5.55
C. stellifer	116.58	58.47	8.18	108.22	121.67	8.33
C. variipennis	43.25	1.40	0.18	105.22	6.33	8.11
C. venustus	13.33	3.33	1.27	5.61	4.25	0.67
All *species*	189.08	76.67	31.55	232.17	180.17	65.33

* Number of trap times.

expected to occur along the coast (Battle & Turner 1971). *C. niger* Root & Hoffman is a coastal treehole species (Blanton & Wirth 1979) and was not trapped at the RVP site and only trapped once at the PP site (Farris farm). The third species *C. jamnbacki* Wirth & Hubert has a northern distribution and would not be expected on the Coastal Plain (Battle & Turner 1971).

The remaining nine species captured were *C. bergi* Cochrane, *C. baueri* Hoffman, *C. debilipalpis* Lutz, *C. hinmani* Khalaf, *C. husseyi* Wirth & Blanton, *C. nanus* Root & Hoffman, *C. parapiliferus* Wirth & Blanton, *C. villosipennis* Root & Hoffman (only at night at the Bennett farm), and *C. scanloni* Wirth & Hubert. They have been previously collected in these three provinces, but in low numbers (Battle & Turner 1971).

Trapping in the three provinces showed that the total abundance of all species decreased from the CP site to the RVP site (Table 1). This can partially be attributed to different habitat and climate. Moving inland, the aquatic and semi-aquatic larval habitats of *Culicoides* decreased as did the numbers captured. This was primarily due to drainage, trap location and amount of soil water interface. The CP trap locality had the poorest drainage and also contained several ponds and marshy lakes in close proximity. The PP trap locality (Bennett farm) had good drainage but was surrounded by several impounded streams which created excellent larval habitats for certain *Culicoides* species, especially *C. stellifer*. The RVP site had the least favorable larval habitats and the lowest numbers caught.

TABLE 2. Dates of catch and(numbers caught)of *Culicoides* species which were uncommon in blacklight traps on the Coastal Plain during 1978 and 1979, in Virginia.

Species	Year	May	June	July	August	September
C.alexanderi	1978				21(1)	
	1979					
C.arboricola	1978		19(1)		15(1)	
	1979		10(1)	17(1)	20(1)	10(1)
C.bergi	1978					
	1979			17(1)		
C.bickleyi	1978		19(4)			
	1979					
C.crepuscularis	1978		26(2)	10(4)	6(1)	
	1979			16(1),17(5)	6(1),13(1) 20(1)	4(1)
C.debilipalpis	1978			10(2),17(2)		
	1979					17(2)
C.furens	1978		26(1)	10(4),24(1)	15(4),21(1)	
	1979		10(1),17(4)	8(2),16(1)	20(2)	10(4)
C.guttipennis	1978			17(1)		
	1979			1(5)		
C.haematopotus	1978		19(1)	10(2)		
	1979		17(2)		20(1)	
C. niger	1978					
	1979	27(1)				

TABLE 2. cont.

Species	Year	May	June	July	August	September
C. piliferus	1978					
	1979			1(5)		
C. sanguisuga	1978		19(1)		21(2)	
	1979		17(2)			
C. spinosus	1978		19(4)	10(2)		
	1979	27(1)	17(2)	1(5),17(2)		
C. testudinalis	1978					
	1979		10(3)			
C. travisi	1978		19(1),26(2)			
	1979	27(3)				

TABLE 3. Dates of catch and (numbers caught) of *Culicoides* species which were uncommon in blacklight traps on the Piedmont Plateau during 1978 and 1979, in Virginia.

Species	Year	May	June	July	August	September
C.alexanderi	1978	16(1)				
	1979					
C.aroricola	1978			18(2)		
	1979			9(3)	7(6),27(4)	
C.bickleyi	1978	16(6)				
	1979					
C.crepuscularis	1978			18(2),25(6)	16(1)	
	1979		18(3)	9(3)	7(1)	11(1)
C.debilipalpis	1978					
	1979				7(1)	
C.guttipennis	1978			18(4),25(2)	16(2)	
	1979			9(3)		3(1)
C.haematopotus	1978		20(1)	11(1),25(4)		5(1),12(1)
	1979		12(2),18(3)	2(3),9(3)	14(1)	3(4),11(1)
C.husseyi	1978					
	1979		18(3)	9(3)		
C.jamnbacki	1978					
	1979		18(6),26(2)			
C.piliferus	1978					
	1979		18(3)	2(3),9(6),18(1)		

TABLE 3. cont.

Species	Year	May	June	July	August	September
C. sanguisuga	1978			25(8)		12(2)
	1979		18(3),26(2)		27(4)	
C. scanloni	1978					
	1979		18(3)			
C. spinosus	1978	23(3)	20(1)	11(7)		
	1979		12(5),18(16) 26(6)	9(3),18(1)		
C. testudinalis	1978					
	1979		18(5),26(2)	2(3),18(3)		
C. travisi	1978					
	1979		12(7),18(13)	9(7)		

TABLE 4. Dates of catch and (numbers caught) of *Culicoides* species which were uncommon in blacklight traps at the Ridge and Valley Province site, during 1978 and 1979, in Virginia.

Species	Year	May	June	July	August	September
C. alexanderi	1978	18(11)				
	1979	31(1)				
C. arboricola	1978					
	1979	31(5)	6(3),19(1)	12(2)		
C. baueri	1978					
	1979		6(16)			
C. bergi	1978					14(2)
	1979	31(1)				
C. bickleyi	1978	18(11)				
	1979	31(12)	6(15)			
C. crepuscularis	1978	18(7)		6(4)	10(1),31(1)	7(1),14(1)
	1979		2(2),6(1) 19(19)	12(43)	2(3),10(1)	
C. haematopotus	1978			6(2)		
	1979	31(3)	6(2)	12(2)		
C. hinmani	1978			2(1)		
	1979					
C. jamnbacki	1978	18(1)				
	1979					

TABLE 4. cont.

Species	Year	May	June	July	August	September
C. parapiliferus	1978	18(4)				
	1979					
C. piliferus	1978	18(1)				
	1979	31(1)	6(5)			
C. sanguisuga	1978	18(1)		6(1)		27(1)
	1979					
C. spinosus	1978			6(1)		
	1979	31(13)	2(2),6(36)	12(1)		
C. testudinalis	1978					
	1979	31(1)			2(1)	
C. travisi	1978			6(7)		
	1979	31(34)	6(121),19(4)	12(7)		

Potential larval habitats can be abundant, but rainfall is necessary for larval survival. Rainfall has been shown to result in increased larval and adult Culicoides abundance (Kettle 1956, Davies & Giglioli 1977) and in both years the amount of rainfall decreased from the CP site to the RVP site (Table 5). Also, for all sites, there was a greater amount of rainfall in 1979 than in 1978, except during the first week of August in 1978 at the CP site when 2.59 cm of rain fell.

Temperature can also cause increased population abundance by decreasing the time necessary to complete larval development, hence the generation time. In both study years the mean monthly temperatures were lower in the higher elevations (Table 6), resulting in lower numbers collected at the inland sites.

The importance of trap location can be seen by examining the data from the two PP sites in 1978. At the Farris farm 33.4 midges were collected per trap time compared to 76.6 at the Bennett farm, but they were located only 11 Km apart. The Bennett farm contained several impounded streams while the Farris farm did not. This could have accounted for the low numbers collected at the latter farm.

The largest number of species was collected at the Farris farm (24) (see Appendix A). This appears to be due to its location next to a more or less continuous woodlot where several Culicoides species breed and may not move out into open pastures. For example, five treehole species were collected at the Farris farm compared to three from the other sites during SS. If I included the night trap catches, five treehole species

TABLE 5. Total rainfall (cm) recorded at nearest weather station to trap sites from May 15 to Sept. 15, 1978 and 1979.

Year	Site		
	Coastal Plain	Piedmont Plateau	Ridge and Valley Province
1978	8.57*	4.69	4.26
1979	7.20	6.61	5.13

* first week of August contributed 2.59cm.

TABLE 6. Mean temperature (C^0) recorded at nearest weather station to trap sites during months trapped, 1978 and 1979.

Site	Year									
	1978					1979				
	MAY	JUNE	JULY	AUG.	SEPT.	MAY	JUNE	JULY	AUG.	SEPT.
Coastal[1] Plain	18.0	23.2	24.5	26.5	23.0	19.3	21.2	24.5	25.5	21.9
Piedmont[2] Plateau	17.0	22.4	23.5	25.0	21.5	17.1	20.3	23.0	23.6	19.5
Ridge and[3] Valley Province	14.0	17.9	20.2	20.9	20.0	14.0	19.8	21.0	22.0	18.5

[1] Lake Kilby

[2] Appomattox

[3] Blacksburg

were collected also at the Bennett farm site. On the Bennett farm, a woodlot was located closer to the trap site than at the coastal plain site or mountain site. This could have influenced midge movement if night conditions became favorable enough to cause outward movement of the forest breeding species of midges into the adjacent pastures. Movement between habitats has been examined in mosquitoes (Bidlingmayer, 1971, Bidlingmayer & Hems 1981). They suggested that certain species favored woods over pastures and many species may have restricted nocturnal flight in the open to the vicinity of their resting places. Woodland mosquitoes did not venture far from the woods and perhaps used the woodland edge silhouette as a visual marker. This also could have been the case for midges.

Examination of the five most common species provides further biological information (Table 1). C. biguttatus was more abundant at the coastal plain site in 1978, more abundant at the PP site in 1979 and least abundant at the RVP site (Table 1). This can be directly attributed to its preferred larval habitats which include lake margins, pools, stream edges, poorly drained mud, sand and decaying leaf matter (Hair et al. 1966, Blanton & Wirth 1979). These habitats decrease in abundance from the CP site to the RVP site.

Figure 2 shows the seasonal abundance of C. bigutattus at the PP site (SS) and at the CP site (AN) I did not present the results from the RVP site because seasonal patterns were similar to the PP site. Data from the CP site provided information on seasonal occurrence and emergence of this species that is supported by the data from the other two sites. This species occurred from early May to mid-July, and its occurrence was similar to that observed in Virginia by Murray (1957) and Messersmith (1966). At the CP site parous rates remained above 55% from early June until they disappeared in July. There were no inverse fluctuations in parity with numbers except perhaps on June 15 and July 10, 1978 and July 1, 1979. This suggests that fluctuations in numbers are mainly due to trap catch variability and not to emerging nulliparous females. If discrete emergences did occur, then parity would probably drop when the population suddenly increased. Also, if emergences overlapped, the parous rates would fluctuate like those for C. stellifer (Figure 4) and C. variipennis (Figure 5), without a consistant increase in parous rate. Because of the consistantly high parous rates, this species is considered univoltine in Virginia with a possible minor emergence in early summer at the CP site. A univoltine phenology for C. biguttatus also has been suggested by Schmidtmann et al. (1980a) in New York State.

Culicoides obsoletus was most abundant at the RVP site in 1978 and the PP site in 1979 (Table 1). The larval habitats of this species are moist terrestrial habitats such as straw-manure, corn composting stalks, and moist leaf litter (Murray 1957, Jones 1961b, Jamnback & Wirth 1963). This type of habitat related to moisture would suggest a greater abundance at the CP site, but the range of this species does not extend further south than Tennesse (Jamnback 1965), and its absence from south-

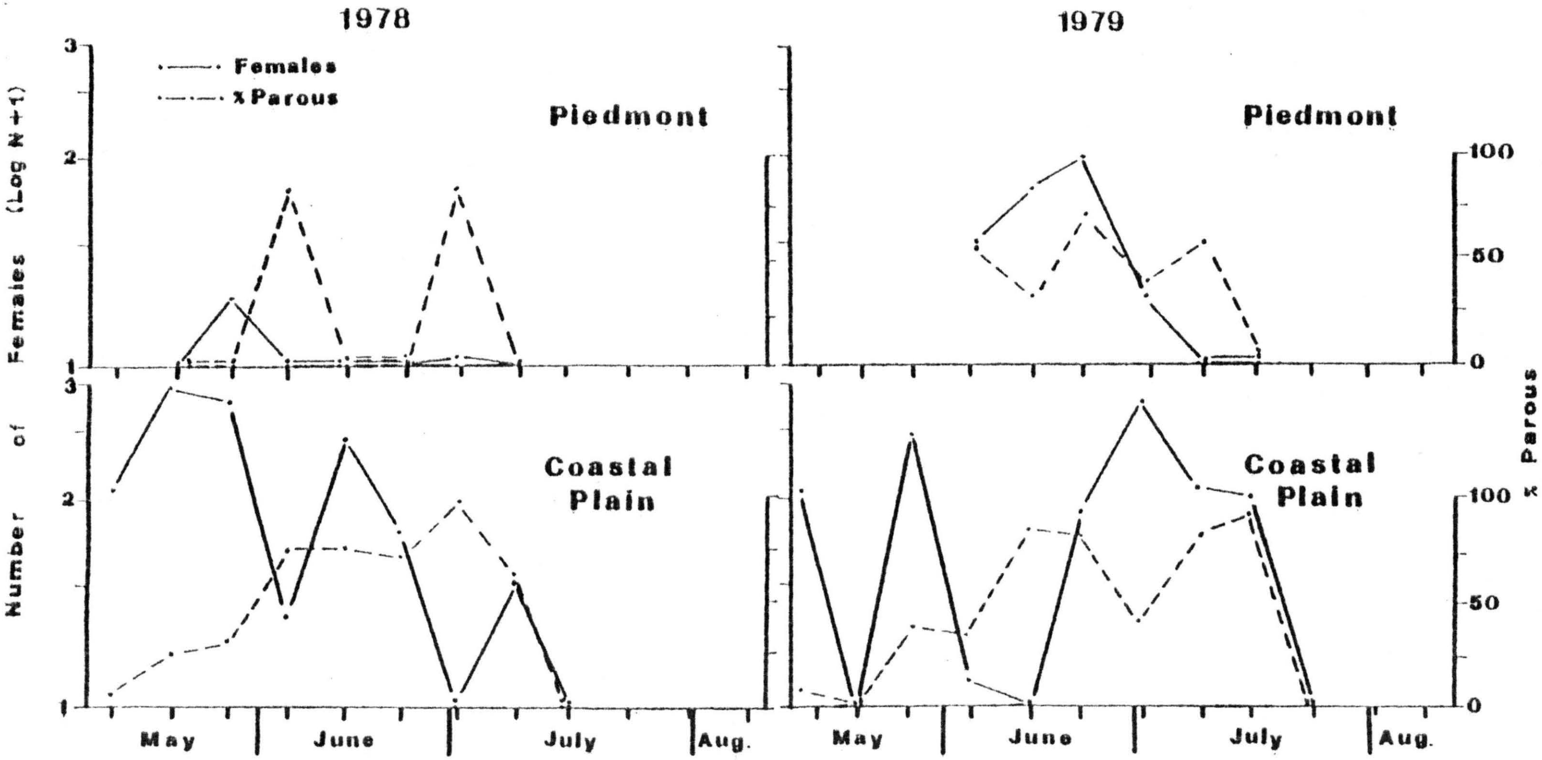

Figure 2. Seasonal abundance and parous rates of Culicoides biguttatus collected in blacklight traps once a week at the Piedmont Plateau (Bennett farm) (30 min. pre sunset to 90 min. post sunset) and at the Coastal Plain (30 min. pre sunset to 60 min. post sunrise) in 1978 and 1979.

ern coastal areas of Florida (Blanton & Wirth 1979) suggest an affinity for more northern type climates. Therefore, low numbers would be expected at the CP site. This species occurred from May to at least mid-September in Virginia (Figure 3) and its temporal distribution is similar to observations made in New York (Jamnback 1961).

Sixty-two percent of the total females collected were gravid (n=660). Similar catches occurred in three other species, _C_. _crepuscularis_, 83% gravid (n=189) _C_. _travisi_, 81% gravid (n=242) and _C_. _variipennis_, 37% gravid (n=2599). In England, Service (1969a) found 21.2% of the _C_. _obsoletus_ to be gravid and similar results in other _Culicoides_ species. In Washington State, Jorgensen (1969) found that most of the _C_. _crepuscularis_ collected in light traps were also gravid. The exact reason for the high percentage of gravid females may be their distraction to the light source while flying to an oviposition site. Nevertheless, the high gravid rate made analysis of phenology difficult because numbers caught to assess parity were too low. The use of gravid females has not been used to assess seasonal phenology, and perhaps its potential use should be explored.

During 1978, a light trap was run at the RVP site all night (AN) for 11 weeks. Five hundred and fifty-eight female _C_. _obsoletus_ were collected of which 328 were gravid. This left only 230 females to assess seasonal phenology. Figure 2 shows the number collected and the parous rates during this trap time. Numbers fluctuated over trap time and increased concurrently with parous rates. Therefore, peak numbers were not due to nulliparous females.

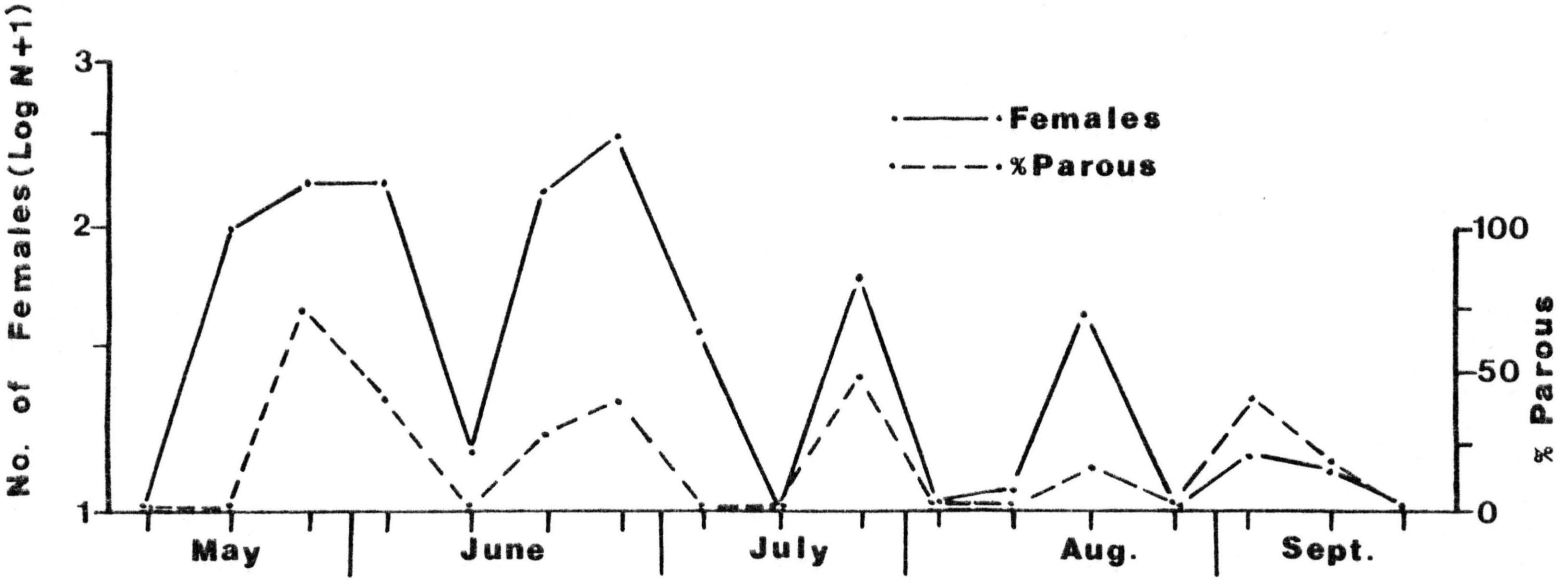

Figure 3. Seasonal abundance and parous rates of Culicoides obsoletus collected in blacklight traps once a week at the Ridge and Valley Province (30 min pre sunset to 60 min post sunrise) in 1978.

In order to assess the phenology of C. obsoletus I looked also at the seasonal parous rates of females collected at all sites. Only once on May 25, 1978 did the parous rate exceed 41%. The parous rates for 1978 ranged from 8-41% and in 1979 from 8-31%. This suggests that this species is continuously emerging in low numbers throughout the season and possibly has low survivorship. The possibility of continuous emergence is supported by the findings from New York State where C. obsoletus was observed to emerge continuously throughout the season and was considered multivoltine (Jamnback 1965, Schmidtmann et al. 1980a).

Culicoides stellifer was the most abundant species collected in the blacklight traps in both years at all sites except in 1978 at the RVP site where C. obsoletus was the most abundant (Table 1). The large numbers of C. stellifer collected at the CP site and the PP site reflect the proximity of their preferred larval habitat. These consist of diverse freshwater soil types including ponds, streams and poorly drained areas of mud and leaf litter (Murray 1957, Jones 1961b, Hair et al. 1966, Blanton & Wirth 1979). The PP site had several impounded and wooded streams which were near the trap site. The CP site had a high water table which left water standing throughout the site much of the time, while the RVP site had good drainage and few freshwater habitats, hence fewer breeding sites.

Culicoides stellifer occurred from May until at least mid-September. This is in agreement with Pickard and Snow (1955) in Tennesse and most other studies (Blanton & Wirth 1979). Figure 4 shows the seasonal abundance and parous rates of this species at the PP site (SS) and the CP site (AN) for 1978 and 1979. Continuous fluctuations in abundance were observed as well as low fluctuating parous rates. Mean seasonal parous rates ranged from 19-34% in 1978 and from 4-30% in 1979. These results indicated that C. stellifer was multivoltine in Virginia. It also suggests that for a continually emerging species in high numbers with low parity that there may be low survivorship. During 1979, I attempted to send live adult female C. stellifer to Denver for virus isolation studies, but in all attempts following established techniques for shipping Culicoides (Jones & Schmidtmann 1980), the adults did not survive over two days. The low survivorship suggested that the high numbers collected may be explained by continuously large adult emergences and the low parity rates by low survivorship and continuous emergence.

Culicoides variipennis was the second most abundant species collected in the blacklight traps at the CP site in 1978 and 1979, but at the PP site and at the RVP site this species was the third and fourth most abundant species, respectively (Table 1). These rankings can again be attributed to preferred larval habitat. This species breeds in a diversity of freshwater, saltwater and alkaline habitats, but most abundantly in streams and ponds polluted with livestock excrement (Jones 1961, Hair et al. 1966, Blanton & Wirth 1979). Its larval abundance in polluted areas accounts for the large numbers of adults collected at the CP site. The area was not well drained and wherever pasture depressions and hoofprints were found there was a potential mixing of livestock

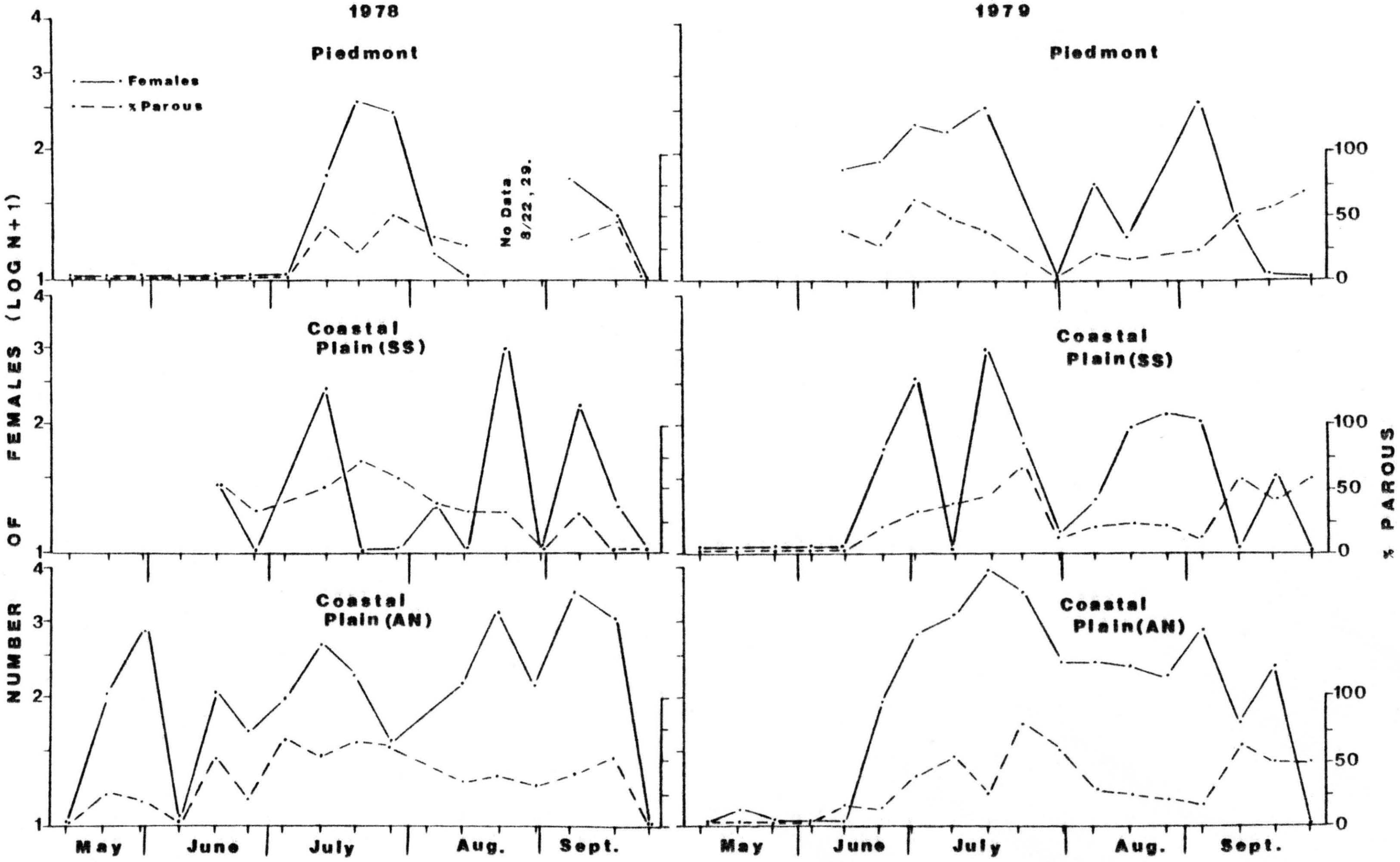

Figure 4. Seasonal abundance and parous rates of Culicoides stellifer collected in blacklight traps once a week at the Piedmont Plateau (30 min pre sunset to 60 min post sunset), at the Coastal Plain (30 min pre sunset to 60 min post sunset) and at the Coastal Plain (30 min pre sunset to 60 min post sunrise) in 1978 and 1979.

excrement with standing water. At the PP sites and RVP site the pastures were well drained which did not result in much mixing of manure and water.

This species was collected from May until at least mid-September and has been recorded in Colorado from April to November (Barnard & Jones 1980a). Blacklight trap data and parity results from the CP site indicated that this species is long-lived and multivoltine in Virginia (Figure 5). I did not present the results from the other sites because the numbers caught were too small to be meaningful. The number of females collected fluctuated over time and parous rates decreased as numbers increased. The fluctuations in females collected were similar to C. stellifer, but C. variipennis had consistantly higher parous rates. Mean seasonal parous rates ranged from 73-83% in 1978 and from 53-80% in 1979. These high parous rates persisted throughout the season and were seldom recorded below 30%, suggesting that C. variipennis is a long-lived continuously emerging species. Contrasted to C. stellifer, C. variipennis adults survived in the laboratory up to 14 days. Jones (1957) observed adult survivorship up to 37 days in his laboratory.

Culicoides venustus was most abundant at the CP site and least abundant at the RVP site (Table 1). It breeds in wet pastures, muddy hoofprints of livestock, stream edges and swamps (Jamnback 1965, Hair et al. 1966, Blanton & Wirth 1979). These habitats were most prevalent at the CP site thereby accounting for an increase in numbers collected there.

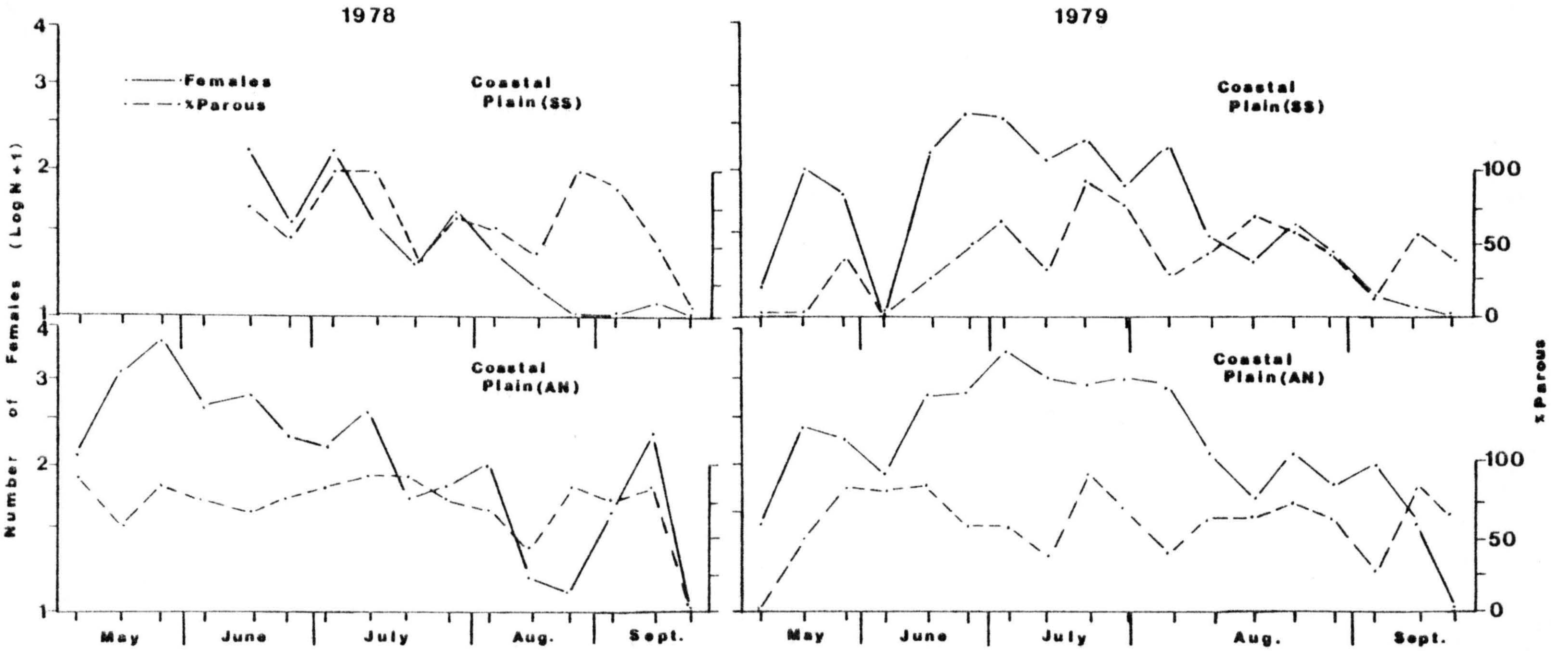

Figure 5. Seasonal abundance and parous rates of Culicoides variipennis collected in blacklight traps once a week at the Coastal Plain (30 min pre sunset to 90 min post sunset and 30 min pre sunset to 60 min post sunrise in 1978 and 1979.

Figure 6 shows the seasonal occurrence and abundance of this species at the CP site (AN) in 1978 and 1979. C. venustus occurred from May to at least mid-September and agreed with the seasonal occurrence results of Murray (1957) and Messersmith (1966) in Virginia. Because I did not determine parity for this species and used a once a week collection schedule it was difficult to accurately assess the longevity or voltinism of this species. This midge persisted throughout the season in Virginia and had been reported to do so in Tennesse (Snow et al. 1957), New York (Jamnback 1965), Missouri (Chiders & Wingo 1968) and Florida (Blanton & Wirth 1979), therefore it is probably multivoltine. Its survivorship and potential bi-(or multi)-parity cannot be determined until further studies are undertaken.

Host Preference

A total of 17 species and 3,661 individuals were collected from all animal bait traps with 92% being taken at the CP site (Tables 7 & 8). All species collected are listed by site, year, bait, and trap time in Appendix B. Eight species, C. biguttatus, C. crepuscularis, C. furens, C. obsoletus, C. sanguisuga, C. spinosus, C. stellifer, and C. variipennis were collected in both years.

Eight times more Culicoides were collected from bait animals in 1979 than in 1978. Because no comparison was done between the vacuum trap in 1978 and the drop trap in 1979, I indirectly compared the trap

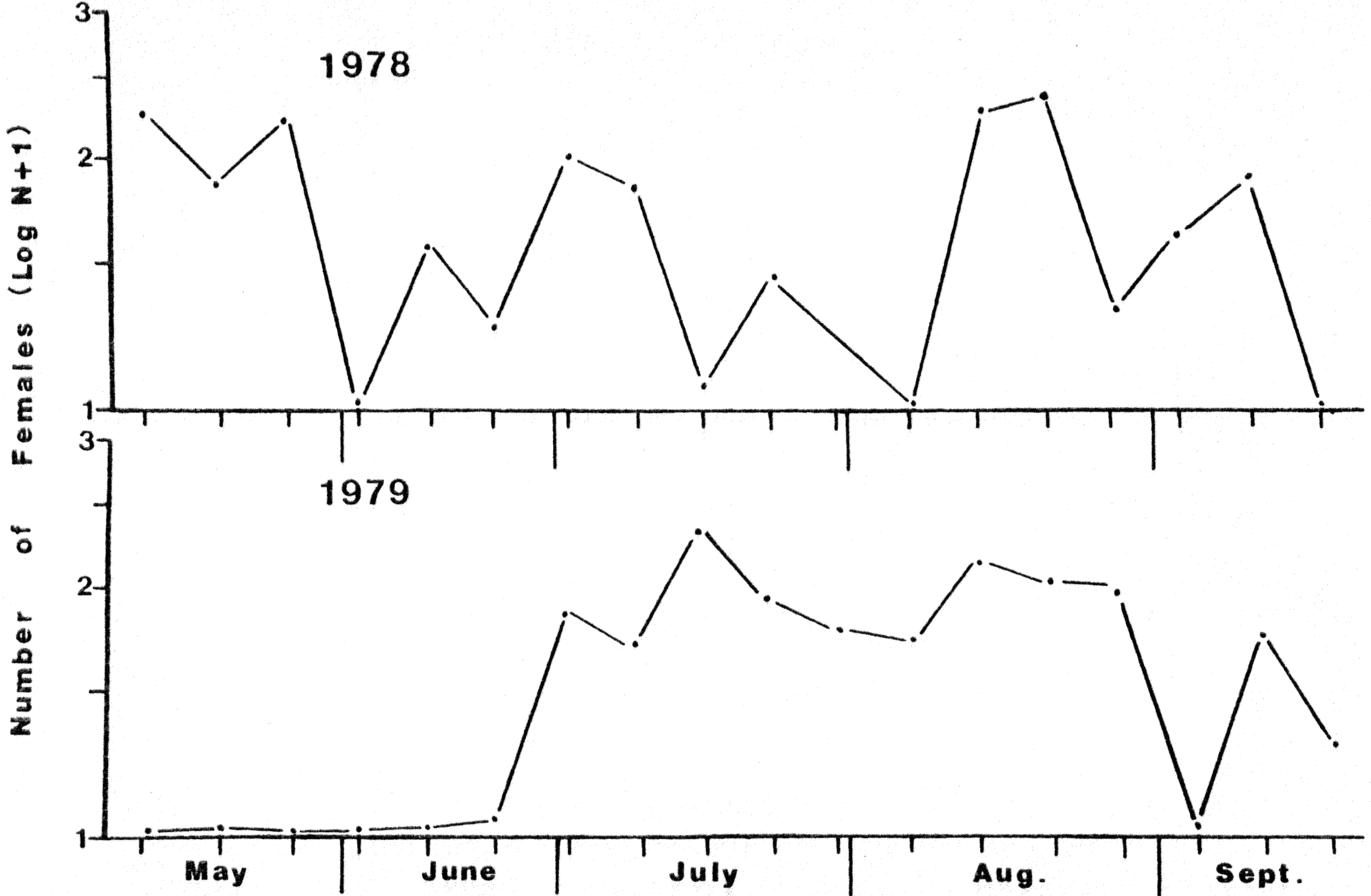

Figure 6. Seasonal abundance of Culicoides venustus collected in blacklight traps once a week at the Coastal Plain (30 min pre sunset to 60 min post sunrise) in 1978 and 1979.

TABLE 7. Number of *Culicoides* species collected by vacuum sampler from bait animals in Virginia during 1978.

	Coastal Plain		Piedmont Plateau		Ridge and Valley Province		
Species	Cow	Sheep	Cow	Sheep	Cow	Sheep	Total
C. arboricola	1						1
C. bickleyi						1	1
C. biguttatus	15	5		1		9	30
C. crepuscularis	2	1					3
C. debilipalpis				2			2
C. furens	1						1
C. guttipennis			1				1
C. hollensis	1						1
C. niger	1	1					2
C. obsoletus		1	2	2		1	6
C. sanguisuga	2					1	3
C. spinosus					1		1
C. stellifer	22	29	2	49	2	28	132
C. variipennis	200	28	1			1	230
Totals	245	65	6	54	3	41	414

TABLE 8. Number of *Culicoides* species collected from bait drop traps in Virginia during 1979.

	Coastal Plain		Piedmont Plateau		Ridge and Valley Province		
Species	Cow	Sheep	Cow	Sheep	Cow	Sheep	Total
C. biguttatus	165	33	11	5	1		215
C. crepuscularis	1						1
C. furens	15	2					17
C. niger	2	1					3
C. obsoletus			3	1			4
C. piliferus	1				1		2
C. sanguisuga	1		1				2
C. spinosus	4	4	2		2	1	13
C. stellifer	723	141	81	58	7		1010
C. travisi	11	3			1		15
C. variipennis	1721	182	1	8	1		1913
C. venustus	44	6	2		1		53
Totals	2688	372	101	72	14	1	3248

efficiency of each bait trap with the blacklight trap results during the trap time periods using the the three major species collected in bait traps. I used the CP site for the comparison. C. biguttatus and C. stellifer were slightly less abundant in the blacklight traps in 1979 but 7.2 and 7.7 times more abundant in the bait traps, respectively. C. variipennis was 3.7 times more abundant in the blacklight traps in 1979 and 8.3 times more abundant in the bait traps. These increases in catch in the bait traps without a comparable increase in catch in the blacklight traps indirectly indicated that the drop trap was more efficient.

A major factor which affects host attractiveness is physical size (Downes 1962, Humphreys & Turner 1973, Koch & Axtell 1979a) with the larger animal attracting the greatest number of insects. At all times in this study when bait animals were in close proximity, the cow attracted more midges than did the sheep. Because surface area is a function of weight, this relationship has been used to equalize size and compare numbers attracted with surface area (Humpherys & Turner 1973). But surface area-weight relationships can be compounded by factors such as age, temperature and activity of the host (Brody 1945). Thus, I tested various formulas presented by Brody to see if there was indeed a direct relationship between numbers caught and surface area. If so, then most of the difference in catch would have been attributed to size. The formulas used were:

1. 10 (weight of the animal $(W)^{2/3}$ = surface area (S)

2. $0.12W^{.60} = S$

3. $0.15W^{.56} = S$

The ratios between cattle and sheep were: 2.8 midges/dec^2 vs 3.4/ midges dec^2, for the first formula, 3.07 midges/dec^2 vs 3.08 midges/dec^2 for the second formula, 3.31 midges/dec^2 vs 2.87 midges/dec^2 for the third formula. The first and third ratios imply that the sheep attracted more midges per unit surface area, and the second ratio suggested a direct relationship between numbers and surface area. As pointed out by Brody (1945), one needs actual results to make any conclusions on whether or not attractiveness is affected by weight alone (surface area). In this study I showed that one should use caution in relating components of attraction to surface area formulas.

It has been suggested that by placing cattle with sheep one decreases the possibility of bluetongue virus transmission to sheep because of a greater attractiveness to cattle (Nevill 1978). Cattle did attract more midges in this study and suggests that Nevill's idea should be investigated.

Species Collected

One *C. arboricola* (1NP, 5/29) was collected from the cow at the CP site in 1978. This is the first record of this species taken from cattle, and though it is considered to be primarily ornithophilic (Blanton & Wirth 1979), it also has been observed on man (Smith & Varnell 1967) and rabbits (Humphreys & Turner 1973). Its absence from livestock and blacklight traps (Appendix A) may be due to its arboreal habit rather than ornithophilic behavior as pointed out by Tanner & Turner (1974).

Culicoides bickleyi is considered to be mammalophilic, feeding on man and goats (Wirth & Hubert 1962), but it also was collected once from a turkey baited trap in Virginia (Humphreys & Turner 1973). One C. bickleyi (1NP, 8/10) was collected from the sheep at the RVP site in 1978.

Culicoides biguttatus was the third most abundant species collected from bait animals in Virginia. It is considered to be a general feeder (Hair & Turner 1968) and has been collected from livestock in New York (Schmidtmann et al. 1980a). In 1978 at the RVP site, nine females (4NP, 5E, 6/1, 6/22) were collected from the sheep, and one (1E, 7/18) was collected from the sheep at the PP site. At the CP site five females (3NP, 2E, 5/22-6/26) were collected from the sheep and five (5NP, 5/15-5/29) from the cow. In 1979, at the RVP site, one female (1E, 5/31) was collected from the cow. At the PP site 11 females (2NP, 2E, 7P, 6/12-7/9) were collected from the cow and five (2NP, 2P, 1E, 6/12-7/9) were collected from the sheep.

Culicoides crepuscularis was collected only at the CP site. In 1979, two females (2NP, 5/22) were collected from the cow and one from the sheep (1P, 6/26). One female (1NP, 6/17) was collected from the cow in 1979. This is the first record from cattle or sheep. Previously, it had been collected from man but is considered to be primarily ornithophilic (Blanton & Wirth 1979). It has been shown in Tennessee to prefer the canopy at the forest edge (Snow 1955) and in Canada to prefer boreal forests over lakeshores (Bennett 1960). This preference for forested area is supported by the results obtained in Virginia. At the Farris farm, which is partially wooded, _C_. _crepuscularis_ was collected in large numbers in blacklight traps (Appendix A). At the other sites which contained fewer woods, it was not collected in large numbers. This could be an important reason for its not being reported attacking pastured livestock.

Two _C_. _debilipalpis_ females (1NP, 1E, 7/18, 8/8) were collected from sheep at the PP site in 1978. This species has been recorded from chicken houses in Virginia and also reported to bite man and horses in South America (Battle & Turner 1971). This is the first record of _C_. _debilipalpis_ biting sheep.

Culicoides _furens_, being halophilic, would be expected to be collected only from the CP site. In 1978, only one female (1NP, 5/29) was collected from the cow. Fifteen females (6NP, 9E, 5/13-7/17) were collected from the cow in 1979 and tw from the sheep (2NP, 5/13, 6/17). It is considered to be a general feeder (Hair & Turner 1968, Koch & Axtell 1979a) and is recorded here for the first time from domestic livestock.

Blanton and Wirth (1979) reported that _C_. _guttipennis_ is a general feeder, and in this study cattle are added to the list of hosts. One female (1NP, 5/23) was collected from the Angus calf at the PP site in 1978. This species breeds in treeholes and also feeds in forested areas. It has been collected primarily in forest situations in Virginia (Humphreys & Turner 1973). This could explain its lack of abundance at pastured sites.

One C. hollenis female (1NP, 5/22), a halophilic species, was collected from the cow at the CP site in 1978. Although it is an important man biting species in salt marshes, it is considered to be more general in host preference (Koch & Axtell 1979a). This study adds cattle to the list of hosts.

Culicoides niger was collected only at the CP site. In 1978, one female (1NP, 5/15) was collected from the cow and one (1NP, 5/15) from the sheep. In 1979, two females (2E, 7/1) were colleted from the cow and one (1NP, 6/17) from the sheep. This midge is considered to be orthinophilic (Jamnback 1965), but it has been observed biting man in South Carolina (Snow et al. 1958) and is recorded here from cattle and sheep.

Culicoides obsoletus is known to attack domestic livestock (Jamnback 1965, Schmidtmann et al. 1980a) and was collected in low numbers in all three provinces in 1978 but only at the PP site in 1979. In 1978, at the RVP site one female (1E, 6/1) was collected from the sheep. Two females (2NP, 7/25) were collected from the sheep and two (1NP, 1P, 6/13) from the cow at the PP site. At the CP site one female (1E, 6/26) was collected from the sheep. In 1979, three females (1NP, 2P, 7/2, 9/3) were collected from the cow and one (1E, 7/12) from the sheep.

Culicoides piliferus appears to be a general feeder (Blanton & Wirth 1979), but this is the first time C. piliferus has been recorded from cattle. are added to the list of hosts. In 1979, one female (1E, 5/31) was collected at the RVP site and one (1NP, 7/1) from the CP site.

Culicoides sanguisuga appears to be a general feeder (Humhpreys & Turner 1973) and has recently been reported from livestock (Schmidtmann et al. 1980a). It was collected in low numbers at all sites. In 1978, one female (1NP, 6/1) was collected from the cow at the RVP site and two (1NP, 1E, 6/29) from the cow at the CP site. One female (1E, 7/1) was collected from the cow at the CP site and one (1E, 8/27) from the cow at the PP site in 1979.

Culicoides spinosus is considered to be mammalophilic, but it has been collected from galliform birds in Virginia (Blanton & Wirth 1979). One female (1NP, 6/15) was collected from the cow at the RVP site in 1978. In 1979, low numbers were collected at all sites. At the RVP site two females (1NP, 1E, 5/31, 6/6) were collected from the cow and one (1NP, 6/31) from the sheep. Two females (2NP, 6/12) were collected from the cow at the PP site and four (1NP, 2E, 1P, 6/17-7/30) from the cow at CP site. The sheep trap at the CP site collected three females (1NP, 2E, 5/27-7/1).

Culicoides stellifer is considered to be mammalophilic (Schmidtmann et al. 1980a) but has been recorded once in a turkey baited drop trap (Humphreys & Turner 1973). In this study it was the second most abundant species collected from livestock. At the RVP site in 1978, more females were collected from the sheep than from the cow. Two females (1NP, 1G, 7/16) were collected from the steer and 28 (6NP, 2P, 18E, 6/1-8/17) from the sheep. In 1979, seven females (1NP, 3P, 3E, 5/31-6/6) were collected from the steer and none from the sheep. This difference in host preference between the two years could have been due to the movement of the sheep closer to the cow in 1979 and away from the midges nearest potential breeding site. A difference in host preference also occurred at the PP site. When the cow was moved to the sheep site in 1979, where extensive breeding existed for C. stellifer, the cow trap collected more. Two female C. stellifer (2NP, 6/13) were collected from the cow in 1978 and 49 (31NP, 4P, 10E, 3G, 6/4-9/9). were taken from the sheep. In 1979, 81 females (30NP, 24P, 27E, 6/12-9/3) were collected from the cow and 58 (19NP, 1P, 38E, 6/12-9/3) from the sheep.

At the CP site in 1978, 22 females (19NP, 1P, 2E, 5/27-9/18) were collected from the cow and 29 (6NP, 1P, 22E, 5/27-9/18) from the sheep. In 1979, the numbers caught were much larger with 723 females (72NP, 23P, 1G, 627E, 5/21-9/17) collected from the cow and 141 (24NP, 8P, 109E, 5/21-9/17) from the sheep.

Culicoides travisi appears to be a general feeder (Blanton & Wirth 1979) and was recorded from sheep and cattle in 1979. One female (1G, 6/6) was collected at the RVP site and 11 (1N, 10E, 5/13-7/8) at the CP site from the cow. Three females (1NP, 1E, 1P, 5/15-6/17) were collected from the sheep at the CP site.

Culicoides variipennis was the most abundant species collected from the bait traps (Tables 7 & 8) and was frequently reported from livestock (Blanton & Wirth 1979). At the RVP site in 1978, one female (1P, 7/6) was collected from the sheep and none from the steer. In 1979, one female (1EP, 6/6) was collected from the steer, but none were collected from the sheep. At the PP site in 1978, one female (ENP, 6/10) was collected from the cow. In 1979, one female (EP, 7/18) was collected from the cow and eight (1NP, 1ENP, 6EP, 6/12-8/27) from the sheep. At the CP site in 1978, 200 females (53NP, 4ENP, 115P, 26EP, 2G, 5/15-8/6) were collected from the cow and 28 (6NP, 6ENP, 5P, 10EP, 1E, 5/15-8/21) from the sheep. In 1979 1,721 females (158NP, 519ENP, 215P, 716EP, 113G, 5/13-9/17) were collected from the cow and 182 (8NP, 95ENP, 11P, 68EP, 5/21-9/10) from the sheep. One male was collected from the cow.

Culicoides venustus was collected only from cattle and sheep in 1979. At the RVP site one female (NE, 7/19) was collected from the cow. At the PP site two females (2E, 7/2, 7/18) were collected from the cow. Forty-four females (32NE, 12E, 6/17-8/20) were collected from the cow and six (5NE, 1E, 7/1-9/10) were collected from the sheep at the CP site. The host preference of this species was unknown until Schmidtmann et al. (1980b) collected them in bait traps after dusk. It appeared that their absence from bait traps at dusk was due to their nocturnal feeding behavior. The lack of catch in vacuum traps in 1978, but not in drop traps in 1979 suggests that differences in trap efficiency may have influenced catch in Virginia.

Thirteen fewer species of *Culicoides* were collected in bait traps than in blacklight traps. All 13 species collected in the blacklight traps but not in the bait traps were collected in low numbers for two years (140 total). The host preference of four of the species collected is unknown. They are: *C. husseyi*, *C. jamnbacki*, *C. parapiliferus* and *C. nanus*. Three species are only known to bite man. They are: *C. alexanderi*, *C. bergi* and *C. baueri*. Two are ornithophilic: *C. scanloni* and *C. ousarini*. One has been collected previously from a turtle, *C. testudinalis*, and three, *C. haematopotus* *C. mulrennani* and *C. villosipennis*, are general feeders but not recorded from livestock. The lack of preference for sheep and cattle may account for their absence in bait traps, but it may also be due to low populations in the area of trap placement.

Activity Period

Most species of biting midges are considered to be crepuscular, although some are nocturnal (Kettle 1977). Activity patterns can be dependent on environmental conditions such as temperature, wind and humidity. The degree of influence of these factors can vary with the species. Table 9 shows the activity of the four most abundant species collected in bait traps. Activity of both *C. biguttatus* and *C. variipennis* peaked at SS+30 (Table 9). This was also the case in a comparison of bait traps with the blacklight traps (Table 10). The number of *C. stellifer* collected was similar for the first three bait trap times and decreased at SS+60 (Table 9). During the comparison between blacklight traps and bait traps this species was more abundant at SS+30 (Table 10). C. venustus was most abundant after sunset in all traps (Tables 9 & 10).

Only C. venustus, which was nocturnal in host activity, had an increase in catch at SS+60 for both bait traps and light traps (Table 10). At the CP site, 87-90% of the total seasonal catch of this species in blacklight traps was taken from SS+90 to 0800 (RN). This is in agreement with a nocturnal activity pattern; assuming no large morning peak. Based on bait trap collections at the CP site, the activity of C. biguttatus peaked at SS+30, but light traps collected 86-94% of the total catch from SS+90 to 0800. Nocturnal activity was also recorded by Williams (1955). He also used light traps and found C. biguttatus to be most abundant after midnight. In this study, I observed a continuation of nocturnal activity for C. stellifer with 79-87% of the total catch occurring during the RN trap time.

The activity of C. variipennis has been examined using sheep and man as bait (Jones 1961, Foulk 1969). Both investigators found C. variipennis to be most active at dusk. I observed similar results in this study. CO_2 traps are considered attractant traps, and Nelson and Bellamy found them also to collect more C. variipennis at sunset. Nelson and Bellamy (1971) and Barnard and Jones (1980b), using truck traps, concluded that there was a crepuscular peak in activity, but there was also an increase in nocturnal catch in mid-summer. Barnard and Jones (1980b) attributed the increase in night catches to delayed activity due

TABLE 9. Most common *Culicoides* collected from bait animals at sunset minus 30 minutes (SS-30), sunset (SS), sunset plus 30 minutes (SS+30), and sunset plus 60 minutes (SS+60), in Virginia, 1978 and 1979.

Species	SS-30	SS	SS+30	SS+60
C. biguttatus	26	72	104	32
C. stellifer	368	366	343	66
C. variipennis	53	354	1403	333
C. venustus	1	1	26	25

TABLE 10. Common *Culicoides* collected from bait animals compared to blacklight traps at sunset minus 30 minutes (SS-30), sunset (SS), sunset plus 30 minutes (SS+30), and sunset plus 60 minutes (SS+60) during the comparison test on the Coastal Plain in Virginia, 1979.

Species	Trap	SS-30	SS	SS+30	SS+60
C. biguttatus	BLT	0	0	30	1
	Cow	0	0	17	2
	Sheep	0	0	2	1
C. stellifer	BLT	31	119	1172	355
	Cow	8	33	91	29
	Sheep	4	6	6	3
C. variipennis	BLT	4	30	183	164
	Cow	2	49	198	71
	Sheep	0	1	4	7
C. venustus	BLT	1	5	28	37
	Cow	0	1	14	17
	Sheep	0	0	2	2

to high temperatures. This may have been a determining factor in the continuation of activity after dark of this species in Virginia. Light traps collected from 76-92% of the total catch of C. variipennis during the RN trap period. The factors that influence a continuation of flight activity as indicated by the blacklight traps are not known, though they probably involve more than light intensity.

Differences in catch between trap types are frequently reported for Culicoides (Hill 1947, Bidlingmayer 1961, Service 1969b, Linley et al. 1970, Tanner & Turner 1975, Kline & Axtell 1976). Attractiveness to shape (Bidlingmayer & Hems 1979) or color (Hill 1947) could be inhibited by darkness when the host animal is not visible. The light trap would be attractive after dark (less attractive before this period) and continue to collect active species, thereby explaining the differences between trap catches. The nonattractant truck trap used in other studies, would be seeking the insect rather than visa versa. This could be another important difference. If visual cues are important, a nocturnal species like C. venustus would have to possess host-seeking senses different from other species. Perhaps C. venustus is better able to detect a component of host odor or exhaled CO_2 from the host for an adaptive nocturnal host-seeking activity. It would be of interest to examine the sensory receptors of day-flying, crepuscular and nocturnal Culicoides to see if a morphological adaptation exists that is related to host-seeking activity.

Other environmental parameters could be important in the flight activity of Culicoides. For example, temperature has been shown to influence flight patterns of many species of biting midges (Fontaine et al. 1957, Kettle & Linley 1967, Kettle 1969) but not for C. impunctatus (Reuben 1963). Kettle (1969) presented evidence suggesting that the effects of temperature on biting rates of Culicoides may depend on its magnitude. He showed a positive correlation between temperature below 21°C and biting rates, independent correlation between 21-24°C and a negative correlation above 24°C.

In this study, C. stellifer appeared to show similar trends in relation to temperature. When I examined the results of the comparison test, a larger catch occurred at SS+30 (Table 10) rather than similar catches at SS-30, SS and SS+30 as occurred when the the seasonal results were examined (Table 9). The comparison was run between July 16 and September 5. On four of the eight trap nights when the temperature was >25°C at sunset the catch was much higher at SS+30 than at SS-30 or SS. On two nights the temperature at sunset was <22°C and results were similar for the first three trap times and showed a decrease at SS+60. On the other two nights temperatures were <22°C, but numbers were too low for comparision. This suggests that flight was inhibited when temperatures were >25°C, but as the evening progressed and temperatures decreased, flight behavior was initiated. This behavior is similar to that suggested for C. variipennis by Barnard and Jones (1980b). Although this trend was not observed for other species in this study, the effect of temperature and other climatic conditions are not mutually exclusive and trends may be hidden by such factors as rainfall, humidity, illumination etc.. Also, effects may be species dependent as in mosquitoes (Haufe 1966), and climatic factors may not completely explain the activity patterns of insects at night (Haddow 1966, Reuben 1963).

Comparison between Traps, Trap Time and Gonotrophic Age

The first comparison I made was between blacklight trap time periods at the CP site for the three major species collected in 1978 and 1979 (C. biguttatus, C. stellifer & C. variipennis). Trap times were SS and RN. Results showed no difference in gonotrophic age between trap times for C. biguttatus and C. variipennis, but a significantly younger portion of the C. stellifer population was collected during the SS trap period (Table 11). Bait traps were subdivided into four time periods, and in 1979, light traps were subdivided into the same four periods on eight occasions. Therefore, the 1979 catches could be used in support of the above results. The four bait trap periods showed an increase in parity for C. stellifer from 13%-45%. The parity of females collected at SS+60 was significantly different from SS-30 ($p < 0.019$), barely nonsignificant from SS ($p < 0.058$) and nonsignificant from SS+30 ($p < 0.105$). The parity of females collected during the other trap time periods were not significantly different from each other. Combined results from all sites showed no significant differences in parity between the four bait trap time periods, but parous rates were 24.0%, 20.0%, 20.0%, 36.0%, respectively. Subdivided blacklight trap catches from the comparision test showed a progressive increase in parity on seven of the eight trap dates. These results further suggest that the flight time for C. stellifer differed with age.

The second comparison was made between cattle and sheep. I only used data from those nights when both bait traps collected midges (Table 12). C. biguttatus and C. stellifer showed no difference in age of catch at the CP site , but C. stellifer did show a difference in age of catch at the PP site. A significantly higher parous rate was observed for C.stellifer from the cow trap ($p < 0.001$). I could only analyze the collections of C. variipennis at the CP site because of low numbers collected at the other two sites. In 1978 and 1979, the cow attracted an older population ($p < 0.087$ & $p < 0.001$, respectively).

The final comparison was made between bait animals traps and light traps which were run during the entire SS trap time. It was done for C. variipennis at the CP site (1978 & 1979) and for C. stellifer at the CP site (1979). In all tests there were no significant differences in age of midges collected from the bait animals.

It has been shown in other biting flies that the gonotrophic age of the catch can differ depending on trap type, trap location and trap time (Duke 1960, Davies 1963, Service 1976). For biting midges in Africa, Walker (1977) found no difference in age of catch using suction traps,

Table 11. Gonotrophic condition of midges collected in blacklight traps run at sunset (SS) and the remainder of the night (RN) in Virginia, 1978 and 1979.

	1978			1979		
Species	SS	RN	p**	SS	RN	p
C. biguttautus	117(68.4)*	277(70.0)	0.743	149(61.7)	998(56.4)	0.219
C. stellifer	1378(31.1)	5446(38.1)	0.001	1934(29.4)	13,716(36.1)	0.001
C. variipennis	298(73.2)	1191(57.2)	0.559	1217(53.1)	4039(55.5)	0.139

* Number collected and (parous rate).

** P value of Chi Square test for differences in parous rates

Table 12. Gonotrophic condition of midges collected in traps baited with a sheep or cow in Virginia, 1978 and 1979.

	1978			1979		
Species	Cow	Sheep	p**	Cow	Sheep	p
C.biguttatus	5(0.0)*	3(0.0)	n.s.	9(78.0)	6(83.0)	n.s.
C.stellifer (Coastal Plain)	20(5.0)	7(14.0)	n.s.	95(25.0)	32(24.2)	n.s.
C.stellifer (Piedmont Plateau)	-	-	-	50(44.4)	20(5.0)	0.001
C.variipennis	201(71.6)	27(55.6)	0.087	1721(57.9)	182(42.9)	0.001

* Number collected and (parous rate).

** P value of Chi Square test of differences in parous rates.

CDC traps and Monks Woods traps for *Culicoides pallidipennis* C.,I.& M. and *Culicoides schultzei* (End.). However, Duval (1971) found nulliparous *Leptoconops spinosifrons* (Carter) more active during the middle of the day, and Dyce and Standfast (1972) found *Culicoides marksi* Lee & Reye flying earlier in the evening. Recently, Nathan (1981) observed older female *Culicoides phlebotomus* (Will.) flying later in the evening. Results here indicate that the flight behavior of *C. stellifer* is similar to that of *C. marksi* and *C. phlebotomus*. Also, because an older portion of the *C. stellifer* population was attracted to the cow at the PP site and not at the CP site, I suggest that trap location can also influence the gonotrophic age of the population collected. The difference in gonotrophic age of midges collected from the cow and the sheep for *C. variipennis* is unusual and could play an important role in the control of BTV (see page 75).

Vector Potential

The characteristics of an insect that make it an effective vector include pathogen receptibility, host preference, longevity, frequency of feeding, mobility, numbers and physiological and behavioral plasticity (James & Harwood 1969). In this study, I examined host preference, numbers collected and longevity. Seventeen species of *Culicoides* were collected from the bait animals, but only four were collected in large enough numbers to obtain data on seasonal abundance and parity to allow speculation on their potential involvement in BTV virus transmission. C. venustus was collected throughout the season. It was present during the time of year when BTV virus outbreaks have occurred. But, no records of its previous involvement are available, and Jones (pers. comm.) has shown, in his laboratory, that C. venustus would not successfully transmit BTV. Therefore the potential for BTV transmission in C. venustus would appear to be low.

Culicoides biguttatus was an early summer species occurring in high numbers and with high parous rates. Its potential disease transmission is limited because of its absence in late summer and early fall when BTV outbreaks occur. But, it may be involved in a spring amplification of BTV or perhaps it promotes the reentry of the virus into the peripheral blood stream of cattle and sheep.

The second most abundant species collected on host animals, C. stellifer, occurred in high numbers throughout the year, but it had low parous rates and appeared to have low adult longevity. This species should be examined further in relation to pathogen receptibility to see if it can transmit BTV.

The vector potential of C. variipennis is highly dependent on the biting behavior of the parous females since transovarial transmission is unlikely. Nevill (1978) suggested that cattle, because of their high level of attractiveness to Culicoides, can be used to prevent BTV transmission to sheep is supported in this study by the fact that a larger portion of the parous females are attracted to cattle rather than to sheep. *C. variipennis* was abundant throughout the year, had high parous rates and was longlived. These results and its known involvement in BTV transmission make it the prime candidate for further studies on vector efficiency in Virginia.

SUMMARY

Blacklight Traps

Culicoides collected in Virginia during this study were affected by trap site, trap location and geographical region. Thirty species were collected in blacklight traps with *C. biguttatus*, *C. obsoletus*, *C. stellifer*, *C. variipennis* and *C. venustus* being most abundant. *C. biguttatus* was univoltine with a possible second emergence in early to mid-summer at the CP site. *C. obsoletus* appeared to be multivoltine with parous rates ranging from 8-41%. Low numbers were collected throughout the year, and 66% of the total females collected were gravid. The most abundant species collected in blacklight traps was *C. stellifer*. Mean seasonal parous rates ranged from 4-30%. Continuously high populations showed that this species was multivoltine. Also, *C. stellifer* adults appeared to have a short longevity.

The second most abundant species in blacklight traps was *C. variipennis*. It was multivoltine, had high mean seasonal parous rates ranging from 53-83% and adults appeared to have a long survivorship.

Culicoides venustus was present during the entire season(s), but since parity was not determined, I can only assume that it is multivoltine.

Host Preference

Seventeen *Culicoides* species totaling 3,661 adult *Culicoides* were collected from sheep and cattle bait traps. The major species collected were *C. biguttatus*, *C. stellifer*, *C. variipennis* and *C. venustus*. The drop trap was more efficient than the vacuum trap and cattle traps collected more midges than did sheep traps. Physical size appeared to be the major factor in numbers caught.

Activity Period

In bait trap collections, *Culicoides biguttatus* and *C. variipennis* were more active at SS+30, but light trap collections indicated that a portion of the population was active all night. Blacklight trap collections indicated that *C. stellifer* was also active at night, but showed similar attractiveness to bait animals for the first three trap times (SS-30, SS, SS+30) with a sharp decline at SS+60. However, during the comparison test when temperatures were above 25°C, flight activity peaked at SS+30. *Culicoides venustus* was nocturnal and more abundant in bait traps at SS+60.

Comparison Tests

Blacklight trap and bait trap results indicated that a younger portion of the *C. stellifer* population began flight activity earlier in the evening. This was not the case for the other major species. An older portion of the *C. variipennis* females was attracted to the cow at the CP site. For *C. stellifer*, older females were attracted to the cow at the PP site. This suggests that site influenced the gonotrophic age of catch. There was no differences in relation to gonotrophic age between bait traps and blacklight traps operated during the same trap period.

Vector Potential

Because *C. variipennis* was collected in large numbers, had high parous rates, had a longevity greater than other midge species collected and had been previously reported to be involved in BTV transmission, I decided that this species was the most important species for continued studies in Virginia. But, I recommend that more work be done on the biologies of *C. biguttatus* and *C. stellifer* in order to assess their potential involvement in BTV transmission.

Chapter IV

A MORPHOMETRIC STUDY OF CULICOIDES VARIIPENNIS

Introduction

Culicoides variipennis (Coq.) is a proven vector of Bluetongue virus to sheep and cattle in the U.S.A.(Luedke et al. 1967), and it has been shown to be the most abundant species collected from livestock in Virginia. C. variipennis was the most abundant Culicoides species collected on a dairy farm where several cattle were serologically positive for BTV antibodies (unpub. report, Virginia Dept. Health). These results, coupled with the lack of biological information on other aspects of its vector potential, led to population studies at an isolated breeding site in Saltville, VA (see Figure 12).

The population of C. varipennis in Saltville may not be the same species that I have designated C. variipennis in the rest of the state. Originally there were considered to be five subspecies in the C. variipennis complex. Two subspecies occurred in Virginia, C. var. australis in Saltville and C. var. variipennis in the rest of the state. (Wirth & Jones 1957). Other authors have suggested that the character, adult size, used to separate the subspecies varied with habitat (Rowley 1965, Atchley 1967, Childers & Wingo 1968). The unreliability of adult size for taxonomic separation of C. variipennis into subspecies was further examined by Hensleigh and Atchley (1977). They concluded that the subspecies classification be dropped until further taxonomic work was conducted. More recently, Downes (1978) proposed that there were actually two distinct species, C. occidentalis in Saltville and C. variipennis in the rest of the state. A major reason for the two species hypothesis was that no intermediates existed between the two species.

To determine whether I was dealing with another species in Saltville, I examined the potential variability of C. variipennis using a morphometric analysis of adult size.

Materials and Methods

Mud samples containing pupae of C. variipennis were collected from six sites in Saltville, Virginia (36° 52' N, 81° 52' W) and one site (site 7) 540 Km away in eastern Virginia (36° 48' N, 76° 41' W). Sites are described in Table 13. Four collections were made during 1979: April 18, May 30, July 27 and August 31 in Saltville and April 16, May 28, July 25 and August 29 in eastern Virginia. Samples were placed in clear plastic bags which were placed in half gallon size cardboard ice cream cartons. A one pint cardboard ice cream carton containing an inverted funnel was attached to the top which served as an emergence cage. Mud samples were kept at 22°C. Emerging adults were collected from 12-48 hrs post mud sample collection and placed in 80% ethanol. Twenty-five females from each collection were dissected for measurement by removal of the head, one wing, and the abdomen. Parts were arranged on microscope slides in order of decreasing part size with four glass chips placed on four sides to decrease distortion (unpub. method 1959, W. Wirth). Insect parts were cleared and mounted in Hoyers medium.

TABLE 13. Habitat description and salinity range for sites of *Culicoides variipennis* collections used in morphometric analysis in Virginia, 1979.

Site	Habitat	Salinity (0/00)
1	5 acre pond.	5-10
2	Along 1m wide channel 0.3m deep.	3-8
3	5-6 acre pond fed by a cold mountain spring.	0-0.5
4	2x3m pool; site of old brine well with no active flow.	10-22
5	End of a long drainage ditch; width at this point 33m. Back flow from freshwater creek and seepage from old brine well.	12-33
6	30-60cm pool; old well head with active underground seepage.	10-12
7	Temporary mud-manure pool (4x3m).	0

Twelve adult characters were measured (Table 14). In previous variability studies by Hensleigh and Atchley (1977) all 12 characters were significant. They all have been used previously in Culicoides taxonomy (Battle & Turner 1971). Measurements were made with the aid of a Zeiss phase contrast microscope equipped with a calibrated ocular micrometer.

Four environmental parameters were monitored over a three week period prior to each sample collection. Maximum and minimum air temperatures and rainfall were measured daily. Salinity was measured with a YSI Model 33 salinometer once a week. A three week developmental period for larvae and pupae was assumed (Jones 1957, 1964). Weather data at the eastern Virginia site were taken from Lake Kilby National Weather Station, located approximately 3.2 Km from the study site. In Saltville, the weather data were collected 1.6 Km from the study site at the town sewage treatment plant. It should be noted that the first collections of the year were remnants of the overwintering populations.

Factor analysis was used to analyze data (Atchley 1971, Hensleigh & Atchely 1977). Factor analysis "attempts to isolate and identify the morphogenetic forces that interact to produce the organism phenotype" and creates smaller subsets of correlated variables (Atchley 1971). These subsets are called factors and are made up of all the original characters. The original characters explain a certain amount of the variance of each factor and the amount that is explained is called the loading. Raw data could potentially increase the explanation of varia-

Table 14. Morphological characters measured in taxonomic analysis of *Culicoides variipennis* populations in Virginia, 1979.

1. Wing length (WL)
2. Wing width (WW)
3. Costal length (CL)
4. Length of the first 8 antennal segments (FA)
5. Length of the last 5 antennal segments (LA)
6. Length of the third maxillary palp (LM)
7. Width of the third maxillary palp (WM)
8. Head length (HL)
9. Proboscis length (PL)
10. Length of the 15th (distal) antennal segment (LD)
11. Width of the 15th (distal) antennal segment (WD)
12. Length of the hind femur (LF)

bility by adding common variances from different variables. The use of factor analysis eliminates this potential problem and examines more complex variability. Orthogonal rotation of factor scores was used because it assumes no correlation between variables (Atchley 1971). Factor analysis was also performed on the environmental variables.

Stepwise multiple regression was performed on measured characters and environmental parameters.

Posterior probability of membership in each site was determined by discriminate analysis (Hensleigh & Atchley 1977). It was used to examine whether an insect would have come from its original site of collection, if the site was not known. Measured characters from midges within a site are used to create a linear function for each site. Then all midges (measurements of characters) are fitted to all linear models. If a midge fit the linear function of its original site the best then it was a positive posterior identification and if not it was a negative posterior identification. This is called the determination of posterior probability of membership in sites.

All statistical procedures were carried out at the VPI & SU Computer Center using SAS (Helwig & Council 1979).

Results and Discussion

Five hundred and seventy-five midges were measured. No mud samples were collected from sites 2 and 3 on April 18 and no midges emerged from samples collected in late August at sites 3 and 7. Three factors

(I, II, III) were extracted from the 12 morphological characters measured (Table 15). Factor I was highly loaded by all characters except the length and width of the distal antennal segment. Factor II was highly loaded by the length of the last five antennal segments and the length of the distal antennal segment. Factor III was highly loaded by the width of the distal segment. The proportional contributions of common variance (eigenvalues) were 5.94, 1.92 and 1.04 respectively and explained 49.51% of the total variance for factor I, 16.01% for factor 2 and 8.69% for factor III.

I then used factor scores as new variables and preformed an analysis of variance (ANOVA) with date, site and date-site interaction as the main effects. In all ANOVAs the three main effects were highly significant ($p < 0.001$). This association is further shown in Figures 7 and 8, for the four sites from which four successful collections were made. Characters changed at each site over time and varied between sites on the same date. There was a general decrease in size over the entire time period, but some characters increased in size on August 29 in Saltville. This was the time period when temperature was the highest and smaller adult size would be expected (Linley & Hinds 1976, Hensliegh & Atchley 1977). However, it has been shown for other organisms that maximum size can be acheived both at high and low temperatures (Precht et al. 1973).

Environmental parameters (rainfall, minimum temp., maximum temp. and salinity) generated two factors (Table 16). The first factor was

TABLE 15. Varimax rotated factor matrix and final communality estimates (hx) of 12 morphological characters for *Culicoides variipennis*.

Variable	Factor I	Factor II	Factor III	(h_X)
1. WL	0.9425	0.1269	-0.0992	0.9143
2. WW	0.8360	0.1298	0.0375	0.7171
3. CL	0.9320	0.1314	-0.1081	0.8976
4. FA	0.8849	0.1371	-0.0705	0.8068
5. LA	0.3728	0.7928	-0.0724	0.7728
6. LM	0.8145	0.1839	0.0512	0.6998
7 WM	0.4620	0.2283	0.2394	0.3229
8. HL	0.6426	0.3524	0.0229	0.5376
9. PL	0.7015	0.3866	0.1046	0.6525
10. LD	0.0451	0.9227	0.0450	0.8554
11. WD	-0.0393	-0.0174	0.9677	0.9381
12. LF	0.8821	0.1088	0.0115	0.7901
Eigenvalues	5.9414	1.9207	1.0430	
% variance explained	49.51	16.01	8.69	

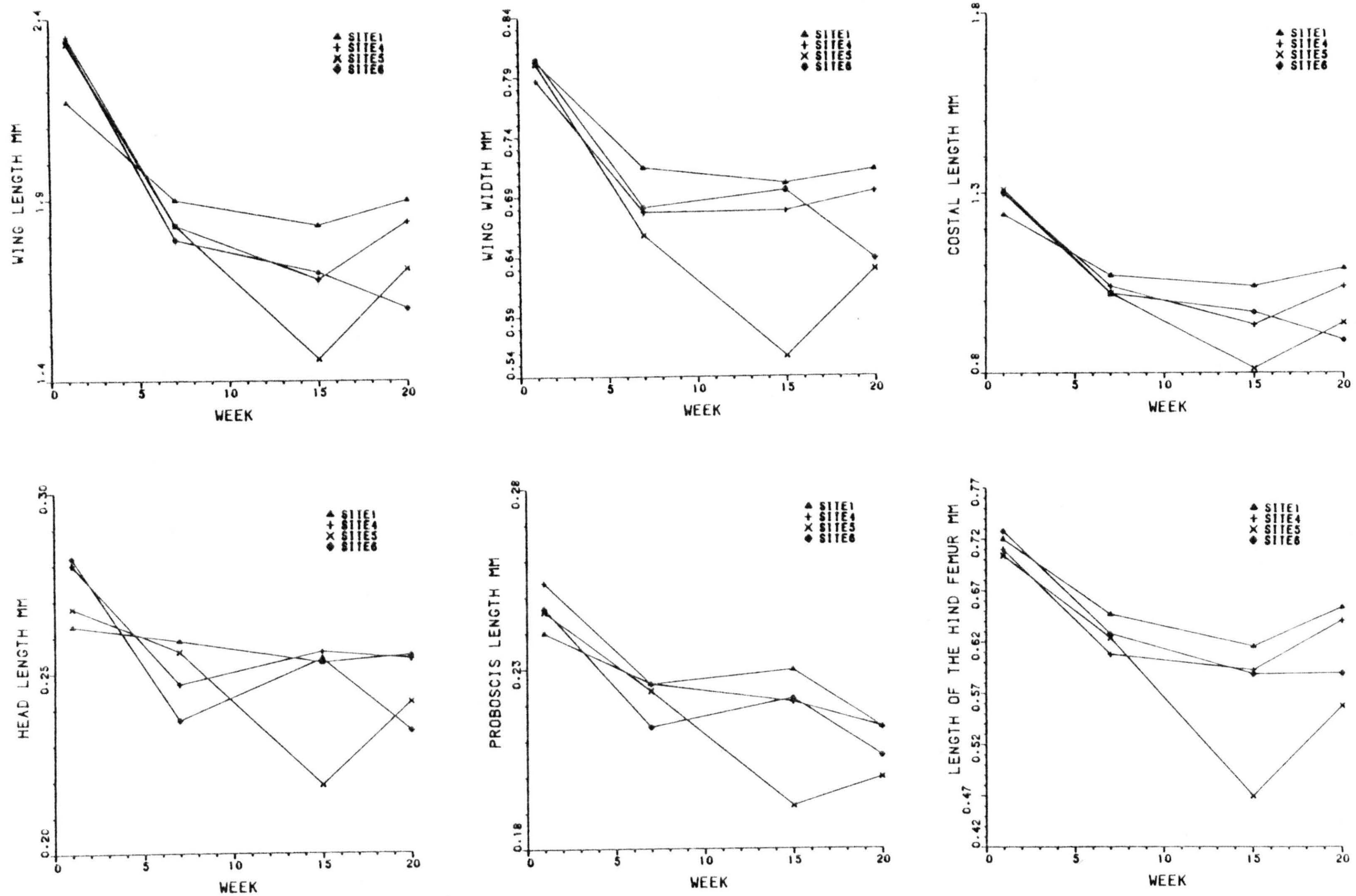

Figure 7. Seasonal changes in morphometric characters of Culicoides variipennis.

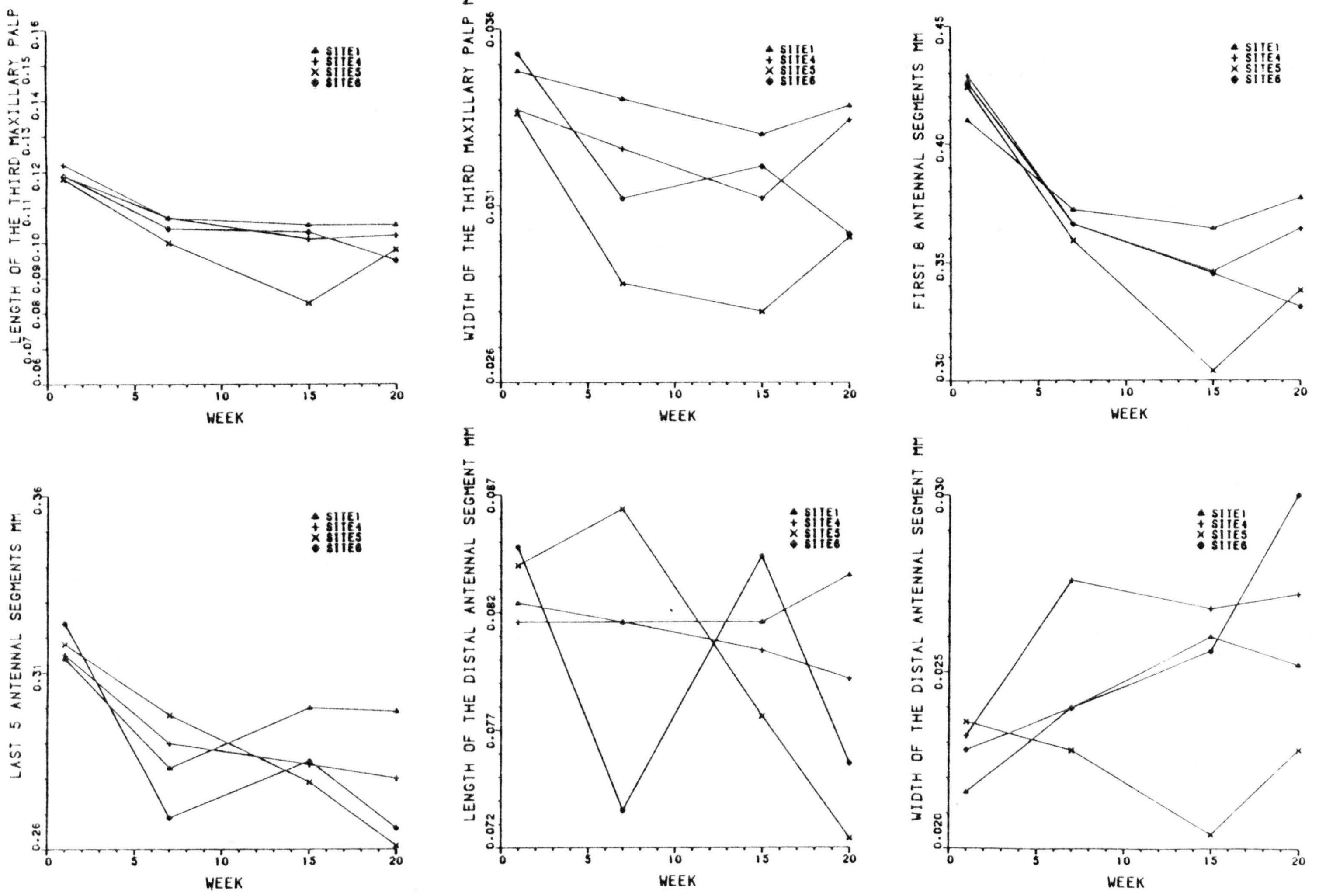

Figure 8. Seasonal changes in morphometric characters of *Culicoides variipennis*.

highly loaded by the temperature variables and the second by rainfall and salinity. The proportional contributions to common variance were 2.04 for factor I and 1.36 for factor II, explaining 51.2% and 34.0% of the total variance respectively.

Results of the stepwise regression for the morphometric size factor I are presented in Table 17. Factors II and III are not presented because all r^2 values were lower than 0.26 ($\bar{X}$=0.06). The explanation of variance for size factor I was highly correlated with the temperature factor(I), and r^2 varied with site. Only sites 4 and 5 showed a significant contribution to the variance explanation by the rainfall and salinity factor (II). These two sites had the highest salinity, and this may have contributed the significance of factor II.

The possible role of salinity in determining size has been alluded to but not tested (Wirth & Jones 1957, Hensleigh & Atchley 1977). I examined the role of salinity in this study, as well as the association of size change with one or the other temperature parameter, by stepwise regression using the morphometric factor scores and the four environmental parameters without the generation of factors (Table 18). The use of maximum or minimum temperature in all models appeared to be due to the presence or absence of a cold water source. Models for sites 1, 4, 6 and 7 were related to maximum temperature. The first three sites were small , and site 7 was located in a backwash of a pond. All four sites could have been influenced more by maximum air temperature because smaller pools heat up more rapidly. Minimum temperature was used in the

TABLE 16.Varimax rotated factor matrix and communality estimates (hx) of four environmental parameters.

Variable	Factor I	Factor II	(h_x)
1. Rainfall	0.3130	0.7692	0.6896
2. Minimum temperature	0.9856	0.1622	0.9977
3. Maximum temperature	0.9806	-0.0142	0.9617
4. Salinity	0.1254	-0.8614	0.7578
Eigenvalues	2.0466	1.3603	
% variance explained	51.12	34.01	

Table 17. Stepwise regression of size factor I with temperature factor(I) and rainfall-salinity factor(II) for *C. variipennis*.

Site	F_s	r^2	Model
1	113.0(I)***	.54	Y = 0.313 - 0.469(I)
2	10.80(I)**	.13	Y = 0.268 - 0.17(I)
3	134.17(I)***	.73	Y = -0.256 - 0.922(I)
4	59.6(I)*** 49.3(II)***	.68	Y = -0.255 - 0.498(I) - 0.592(II)
5	556.18(I)*** 49.3(II)***	.89	Y = -0.739 - 1.04(I) - 0.949(II)
6	195.3(I)***	.66	Y = 0.103 - 0.870(I)
7	15.79(I)***	.25	Y = 0.188 + 0.415(I)

** $p < 0.001$, *** $p < 0.0001$.

models for sites 3 and 5. Site 3 was influenced by a cold mountain stream, and site 5 was located in a wide backwash of an open ditch inundated by cool, underground seepage from old brine wells and a freshwater stream. Site 2 was located at an open ditch connecting sites 3 and 1, which contained continuously flowing water and was only correlated with salinity (r^2=0.16). At this site, the low variance explanation (r^2) and low r^2 from the factor-factor regression (Table 17) suggest that other unexplained parameters influenced changes in size of the adult insect. Salinity, when present, significantly contributed to regressions (Table 18). Results implied that salinity contributed to adult size. Laboratory studies are now necessary to prove this conclusively.

To determine if the C. variipennis populations could be taxonomically separated as species, even with this large amount of variability, I utilized posterior identification procedures between all sites and between the pooled midge population at Saltville and the eastern Virginia population. Results for the first test showed that only at sites 2 and 7 had over 70% of the midges been classified as coming from their site of collection (Table 19). The diagonal numbers indicate positive identification and the off-diagonal numbers represent misidentification. The second posterior identification (Table 20) showed that 69% of the midges collected from Saltville would have been classified as coming from Saltville, and 80% of those from eastern Virginia would have been classified as coming from eastern Virginia (Table 20). A high amount of misidentification has been previously observed between C. variipennis

Table 18. Stepwise regression of size factor I with maximum temperature (T_{max}), minimum temperature (T_{min}), salinity (SL) and rainfall (RF) for *Culicoides variipennis*.

Site	Parameter	F_s	r^2	Model
1	RF	57.06***	.69	$Y = 1.41-0.39(RF)-0.41(SL)+0.04(T_{max})$
	T_{max}	5.20*		
	SL	43.59***		
2	SL	14.24***	.16	$Y = 0.78-0.1(SL)$
3	T_{min}	134.17**	.73	$Y = 4.6-0.09(T_{min})$
4	T_{max}	10.45**	.83	$Y = -2.37+0.08(RF)-0.02(T_{max})+0.16(SL)$
	SL	180.22***		
5	T_{min}	971.51***	.92	$Y = 2.24-0.12(T_{min})+0.22(SL)$
	SL	204.71***		
6	RF	16.08***	.82	$Y = 6.67+0.22(RF)-0.17(T_{max})+0.27(SL)$
	T_{max}	389.28***		
	SL	65.00***		
7	T_{max}	15.79***	.25	$Y = -6.10+0.08(T_{max})$

* $p < 0.01$, ** $p < 0.001$, *** $p < 0.0001$.

populations in a microspatial comparison study ((Hensleigh & Atchley 1977). They attributed misidentification to large numbers collected and and closeness of sites which created stricter comparisions. The high amount of misidentification in this study may have also been caused by closeness of site and numbers collected, especially in Saltville.

Morphological characters were highly dependent on temporal and spatial differences, and a large amount of variance was explained by the environmental parameters. C. variipennis is considered the most stable subspecies (Ross 1962), but even in eastern Virginia there were intermediates. The second posterior identification suggests that Mayr's 75% rule of subspeciation may apply (Mayr 1969). But, the high amount of morphometric variability indicates that the presence of subspecies in Virginia is unlikely.

Therefore, I conclude that in Virgina, using the present taxonomic method, C. variipennis should be considered a single species with considerable ecotypic variability. This does not preclude the possibility that other taxonomic tools could confirm or deny this, but it emphasizes that the use of such tools as chromosome analysis and behavioral taxonomy should be employed to further examine this possibilty.

TABLE 19. Posterior probability matrix of *Culicoides variipennis* membership in each site, 1979.

Site	1	2	3	4	5	6	7
1	24	33	10	7	5	2	19
2	7	73	4	3	1	0	12
3	10	32	24	8	8	6	12
4	14	21	18	15	3	5	24
5	10	6	8	11	36	2	27
6	10	5	16	13	7	21	28
7	4	14	4	0	2	6	70

TABLE 20. Posterior probability matrix of *Culicoides variipennis* in Saltville and eastern Virginia, 1979.

Site	Saltville	Eastern Virginia
Saltville	69	31
Eastern Virginia	20	80

Chapter V

SWARMING AND MATING BEHAVIOR OF CULICOIDES VARIIPENNIS

INTRODUCTION

This Chapter describes the swarming behavior of C. variipennis in Saltville, VA and relates the results to the function of the swarm. Swarms of C. variipennis have been observed in nature but not examined in detail (Rowley 1965, Downes 1978). Downes (1978) has suggested that there may be differences in mating behavior between the western species (C. occidentalis) and the eastern species (C. variipennis) He suggested that the western "species" is a facultative swarmer and the eastern "species" an obligate swarmer (based on the successful laboratory colonization of the western species but not the eastern species). Jones and Schmidtmann (1980) have recently colonized the eastern species, but other behavioral evidence suggest that there may be other possible differences in mating behavior (Downes 1978, pers. obs.). Therefore, a closer examination of the mating behavior of this species complex is needed.

Culicoides variipennis (Wirth & Jones 1957) from Saltville would be called C. occidentalis by Downes (1978). I will use the name C. variipennis in this section. Evidence related to the taxonomy of this complex is presented.

MATERIALS AND METHODS

Swarms of C. variipennis were observed in the brine fields of Saltville, Va. during June, July and August 1980. The ponds in the brine fields range in salinity from 0-33 parts per thousand and all the ponds provide extensive breeding areas for this midge. Observations were made at dusk and dawn. For comparative purposes the swarms were described in a format similar to that used by Reisen et al. (1977) for mosquitoes. The criteria included: type of swarm, color preference, size, shape, time, flight path, pairing, time in copula, and parity.

Females from the swarms were aspirated by hand as they fell on a white sheet (1.2 x 1.2 m) placed directly downwind of the swarm. Parity was determined using the tergite method described by Potter and Akey (1978). Nulliparous females have three darkened lobes on the abdominal tergites (Figures 9 & 10). These tergites on parous females are completely darkened. Age of pairing females was compared with that of females collected in New Jersey light traps (modified with a circular blacklight) and a black circular sticky trap (30.5 cm, 8 cm dia). Both traps were placed 1.8 m above the ground and located adjacent to the breeding site.

Females that fell on the sheet in copula were divided into two groups. One group was immediately decoupled; the other group was allowed to complete copulation. After holding all females for 12 hrs they were then tested for the presence of sperm. They were dissected in 1.0 N saline and the sperm were stained using Noland's method for cilia and flagella (Pantin 1959).

Figure 9. Mating pair of <u>Culicoides variipennis</u> showing the tri-lobed darkening of the female abdominal tergites indicative of the nulliparous condition. Female on right.

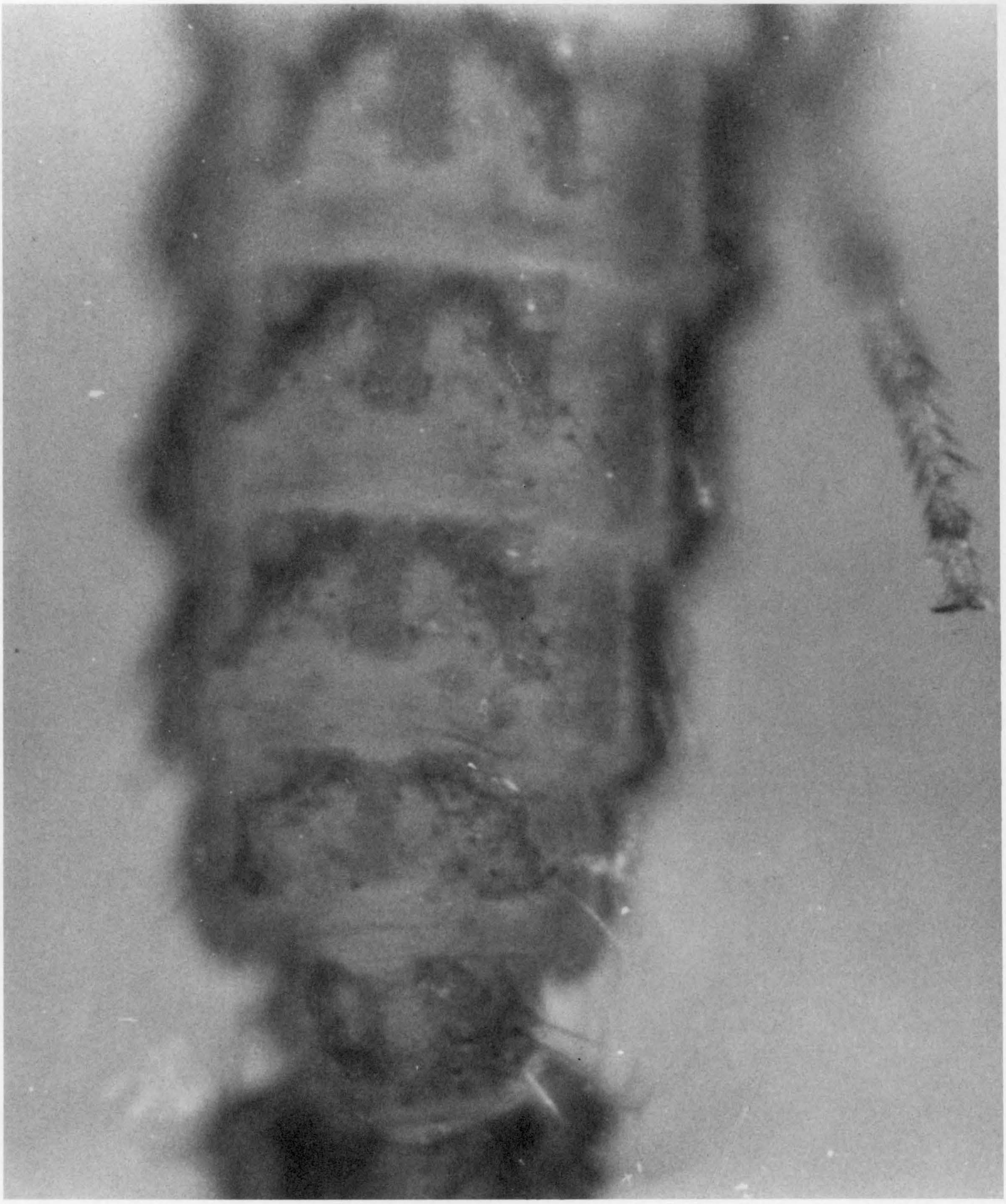

Figure 10. Closeup of nulliparous <u>Culicoides</u> <u>variipennis</u> with trilobed abdominal tergites.

Two swarm sites were visited on each of five nights and females were classified as C. occidentalis, C. variipennis, or an intermediate using the character of size and shape of the sensory pit on the third segment of the maxillary palp (Downes 1978). Sweepnets were used to collect additional swarms for species confirmation and swarm sex ratio.

RESULTS

Description of swarms

Location. Sixty-one swarms were observed 1-540 m from the breeding sites. Fifty-five were mostly "top swarms" as defined by Nielsen & Greve (1950) and modified by Reisen et al. (1977). They formed over grass clumps 10 to 90 cm high that contrasted with light areas of barren soil or roadways. Roadways in the area were of dirt with grass median strips. Six swarms were "marker swarms" marker swarms are areas of more or less horizontally contrasted light and dark patches (Nielsen & Greve 1950) such as those swarming over road edges and dark soil spots surrounded by vegetation. Defining the type of marker was difficult and appeared to be more directly related to contrast rather than to any vertical component.

Color preference. Dark substrate was preferred; when a white sheet was placed over a natural marker, the swarm moved to the upwind edge of the sheet, swarming over the light/dark interface. However, when a black poster board was placed over the natural marker, the swarm would disperse and reform over the artificial marker.

Size and Shape. Swarm size ranged between 3 - 3000+ males. Swarms over small markers (12-25 cm diam) were compact and columnar. Swarms over large markers were less compact and closely resembled the shape of the marker. Distance between midges ranged from 2-10 cm, decreasing as numbers of midges increased. On certain occasions when the swarms were large, they appeared funnel-shaped from a distance; at other times they would extend upwind and upward similar to that described for *Aedes hexodontus* Dyar (Downes 1958).

Height. The lower edge of the swarm varied from 30 cm to 1.8 m above the ground, changing as the midges reoriented over people or other objects placed over natural markers. Heights of the top of the swarm ranged from 1.5-3 m. Above 3 m it was hard to accurately see the swarms.

Orientation. Males faced into the wind with antennal setae erect. On calm nights when observers could not detect any wind, individuals oriented as if facing into the prevailing wind except on two occasions when they oriented in the opposite direction.

Time. Swarms formed 15-20 min before sunset and lasted up to one hr. Occasionally they could be observed with a flashlight after twilight. Swarms were also observed at dawn but were smaller (<50 males).

Flight path. Typically, a zig-zag pattern was observed with a traverse of 5-15 cm. Figure-eight movements were also observed, and some males flew at an upward angle toward the periphery of the swarm.

Pairing. Pairing took place at the periphery of the swarm as the females approached the swarm marker. Pairs fell to the ground and scattered over a large area. The number of pairs falling to the ground could not be determined accurately, but a conservative estimate from swarms of greater than 500 males was one pair every 10 sec. The maximum rate of pairing occurred from 15-25 min after swarm formation, and pairs were not observed on the white sheet after twilight. Pairs usually consisted of a female and a male (Figure 9), but in 34 of 279 observations, a third individual (always male) was present (Figure 11). At no time did both males appear to be copulating with the female. One was copulating while the other held onto the female's abdomen with his claspers. Clasping by the second male usually stopped within 10-50 secs after falling to the ground; and second males did not attempt copulation after the first male decoupled.

Time in copula. Mated pairs fell to the ground and remained *in copula* from 30-120 secs ($\bar{X} = 110$ s). On several occasions, the female would pull the male behind her for a few seconds until she became quiescent. Male antennae were always erect while the male was *in copula* on the ground.

Parity. Over 500 females from mated pairs were examined for parity (see Figures 9 & 10) and showed a parous rate of less than 1.0% (Table 21). In addition, 14 gravid females were also observed *in copula*. .

Other species. Frequently, *Aedes sollicitans* (Walker) males and females would exhibit swarming movements within the swarm of *C. variip-*

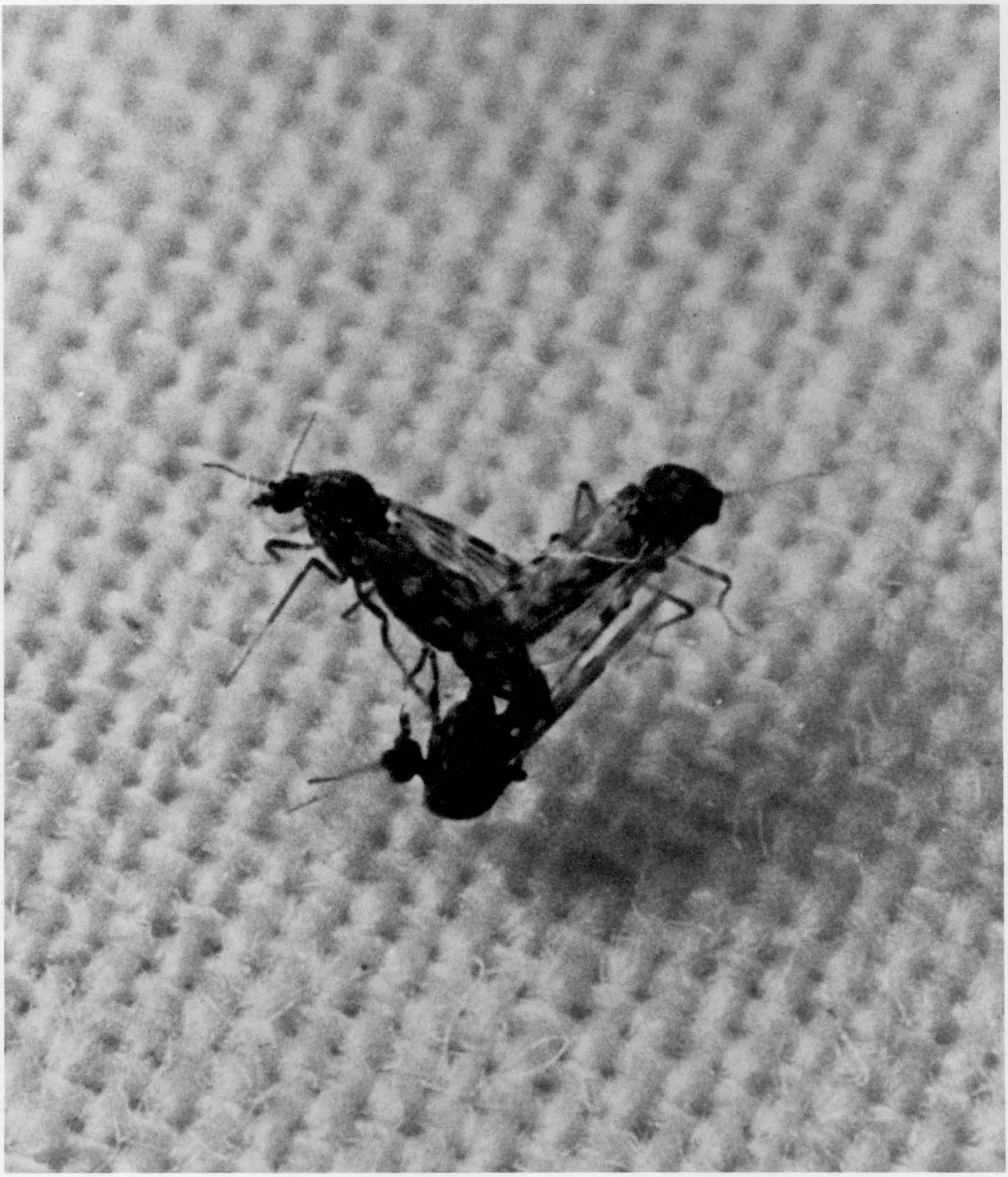

Figure 11. Triad of one female (upper left) and two male <u>Culicoides variipennis</u>. The upper right male in normal copulatory position and the lower male with claspers attached to the eighth abdominal segment of female.

TABLE 21. Relation between time *in copula* and successful insemination in *Culicoides variipennis*.

Copulation	Inseminated	Not inseminated	Total
Forced decoupling*	2	19	21
Natural	13	16	29
Total	15	35	50

---

[*] significant dependence on time *in copula* using Fisher's exact test ($p < 0.01$).

ennis. Also, male Chironomus attenuatus Walker swarmed over the white sheet while observations on C. variipennis were being made. On 20 July, 500 m from the nearest breeding site, two female Culicoides venustus Hoffman were collected when they fell out of a swarm of C. variipennis. Males immediately decoupled and could not be collected for identification to species. Males of C. venustus were never captured by sweepnet or collected from the white sheet.

Function of the Swarm

Although the function of the swarm in the Nematocera has been debated by several authors (Nielsen & Greve 1950, Downes 1958), swarms of Culicoides are generally considered to be assemblages that serve to assist mating. The present observations support this hypothesis. Much of the controversy over the function of the swarm in Nematocera is related to the lack of observed mated pairs and the low female:male sex ratio of swarms sampled by sweepnets. Recently, Campbell and Kettle (1979) collected swarms of Culicoides brevitarsis Kieffer in Australia using a sweepnet and observed a sex ratio (female:male) of 1:77. A lower sex ratio was obtained for C. variipennis (1:167) in 13 sweeps of 11 swarms (496 males and 3 females). However, observed mated pairs in swarms of 500+ suggested that a pairing occurred every 10-15 secs. These are more than enough matings over a 30-60 min period to confirm that the swarm is a mating assemblage (120-360 matings/ swarm).

The apparent discrepancy between sweepnet collections and observations in C. variipennis can be explained by examining two aspects of the techniques used. First, if a sweep is made of a whole swarm, very few females will be collected. Since a mated pair was observed every 10-15 secs, a single sweep only taking one second would not accurately represent the number of matings. Second, mating occurs on the periphery of the swarm, which decreases the probability of female capture (or observation), especially if a vertical sweep of the swarm is made. The influence of method on estimates of frequency of females within the swarm has been recorded also by Quraishi (1965) in Anopheles stephensi mysorensis Sweet & Rao. He found by using sweepnets that only males were captured, but females were observed to enter swarms once every 15-20 secs ($N > 500+$ males). The true rate of entry of females into swarms of C. variipennis could have gone unnoticed if only sweepnet captures were used.

The parous rate of less than 1.0% ($N > 500$ females) for females captured in mated pairs suggested that it was primarily nulliparous females that were coupled. A comparison in age was made among females collected by light traps, sticky traps and swarm pairs to see if there were any older (parous) females in the area. Results showed significant differences in parity from the three methods (Table 22). The parous rates from the swarm pairs were significantly lower than those from either the light trap or the sticky trap ($P < 0.001$).

TABLE 22. Comparison of percentages of parous females from swarm pairs, light traps, and sticky traps.*

% parous (no. examined)

Light trap	Swarm pairs	Sticky traps
83 (58)	4 (24)	39 (23)
63 (344)	0 (25)	89 (10)
50 (210)	0 (146)	25 (25)
42 (69)	0 (30)	21 (42)
73 (345)	0 (22)	25 (20)

*Differences in parity between swarm pairs vs. light traps and swarm pairs vs. sticky traps were significantly different ($p < 0.001$).

Previous authors working with Nematocera (Roth 1948, Quraishi 1965, Anderson 1974) indicated that pairing can take place regardless of the gonotrophic condition of the female. This would suggest indiscriminate mating. Others have observed that mating takes place only once in young females (Anderson 1974). In *C. variipennis* the bias towards nullipars suggests that mating takes place only in young females and is sufficient for the fertilization of all future egg batches.

Swarming formation ranges from obligate, facultative, to nonexistent (Downes 1969). Though *C. variipennis* will mate in the laboratory without swarming, a facultative ranking can only be truly resolved when one observes ground mating in nature without the presence of a swarm. Ground matings have not been observed in the field by previous investigators nor were they observed in this study. *C. variipennis* swarming behavior is similar to that of *Culicoides nubeculosus* (Downes 1955). Both species couple in the air, fall to the ground and remain *in copula* for up to two min. This time *in copula* on the ground raises the question of its function. In *Culicoides melleus* (Coquillett), a nonswarming species, spermatophore formation and sperm transfer takes 1.5 min (Linley & Adams 1972). The time spent *in copula* on the ground by *C. variipennis* could also be to allow for spermatophore formation and sperm transfer. To examine this possibility a comparison was made between time *in copula* and insemination rate.

Twenty-one midge pairs were forced to decouple as soon as they fell to the ground, and 29 pairs were allowed to remain *in copula* until natural decoupling occurred and then were captured (Table 21). Results showed a significant dependence ($P < 0.01$) of successful insemination on time *in copula*. Only 9.5% of the nulliparous females forced to decouple had sperm in their spermathecae, while 44.5% of the females had sperm present in their spermathecae after natural decoupling. It can therefore be concluded that the time spent *in copula* on the ground by *C. variipennis* promoted successful insemination. It is not known if the noninseminated females return to the swarm, but it would appear to be advantageous for the individuals to do so.

Dissection of all gravid and parous females collected either as soon as they fell out of the swarm or after copulation revealed 100% insemination in these groups.

The 44.5% insemination rate of natural decoupled females appeared low. It may have been caused by a combination of male competition for females and impotency of dominant males. Male impotency has been shown to increase with increased mating in *C. melleus* (Linley & Hinds 1974) and could be occurring here. Also, one cannot discount the possibility of mechanical isolation between undetected sympatric species which use the same swarm site.

TABLE 23. Classification of female _C_. _variipennis_ from mated pairs based on the size and shape of the sensory pit on the 3rd segment of the maxillary palp.

Date	_C_. _occidentalis_	_C_. _variipennis_	Intermediate	Total
July 7	15	3	3	21
20	1	1	2	4
21	2	2	0	4
28	39	2	20	61
Aug. 5	17	12	3	32
Total	74	20	28	122

Taxonomic Character of the Swarm

One hundred twenty-two females from mated pairs were collected; 74 were classified as *C. occidentalis*, 20 as *C. variipennis*, and 28 as intermediate (Table 23). The two swarm sites were combined because no difference in site variability was observed. These results indicate that either the morphological character relied upon by Downes (1978) to support the two species hypothesis is too variable in this area or, as suggested by Ross (1962), *C. var. australis* is a mixed hybrid population. If the Saltville area is a zone of hybridization, we would expect more intermediates and fewer of the other two forms or at least greater "kaleidoscopic" variation. This did not occur in the Saltville area and though it does not disprove hybridization, it does not support it. To examine further the variability of this species, a large number of *C. variipennis* larvae were collected and reared from mud-manure samples from eastern Virginia in 1979. According to Wirth and Jones (1957), they would have been expected to be *C. var. variipennis* and, therefore, would be classified as *C. variipennis* by Downes (1978). Following Downes classification, the results were 45 *C. occidentalis*, 25 *C. variipennis*, and 45 intermediate, indicating that even in eastern Virginia a clear-cut species distinction was impossible. Either the whole of Virginia is located in a zone of hybridization or, as seems much more likely, new taxonomic characters are necessary to define the taxa.

So far, insufficient variation in swarming behavior has been observed in this area to suggest swarming behavior as an isolating mechanism. Further field observations are necessary in other areas to determine if differences in mating behavior do exist.

SUMMARY

Swarming of Culicoides variipennis frequently occurred up to 540 m from the nearest breeding site. Male behavior and matings were similar to C. nubeculosus. The swarms were confirmed as pairing sites with observed matings every 10-15 secs. This frequency occurred concurrently with a sex ratio of 1:67.

Examination of the mated pairs showed that females were 99.0% nulliparous, suggesting that they mate only when nulliparous.

Further examination of mated pairs showed a dependence of successful insemination on time in copula. The time spent in copula on the ground by this species appears to promote an increase in successful sperm transfer.

The use of the size and shape of the third segment of the maxillary palp sensory pit in classifying females from coupled pairs as either C. variipennis or C. occidentalis was inadequate in Virginia. Further observations on mating behavior of C. variipennis from other areas are necessary to determine if mating behavior can be an isolating mechanism in this species complex.

Chapter VI

DISPERSAL, GONOTROPHIC AGE AND POPULATION TRENDS OF CULICOIDES VARIIPENNIS

INTRODUCTION

The numerical abundance of the vector is another important factor to be considered once it has been demonstrated that an insect has an affinity for a particular host. Two factors directly related to numerical abundance are gonotrophic age and dispersal. The interrelation of these two factors helps provide an evaluation of the epidemiological risk in a particular area.

The purpose of this chapter is to examine the dispersal of _C. variipennis_ in relation to density, sex and gonotrophic age. The isolated population of _C. variipennis_ in Saltville provides an excellent opportunity to study these relationships because immigration and emigration is minimal.

STUDY SITE

The brine fields within the town limits of Saltville, VA at an elevation of 524 m consisted of approximately 32 hectares (Figure 12). Saltville lies in the Ridge and Valley Province of the Appalachian Highlands. The Saltville Valley rises and narrows to the southwest and northwest leaving a triangular section of lowland bordered on the southern edge by the Saltville Thrust and on the north by a railway bed and a series of hills. Much of the low marshy area has been filled to create the roads that now bisect the brine fields.

Freshwater enters the basin from several springs and saltwater is produced from soil leaching during drainage. Old brine wells dot the fields. A series of ponds and pools exist within the brine fields and range in salinity from 0-33 parts per thousand. Salinity fluctuates with rainfall from season to season. The brine fields drain into the North Fork of the Holston River. The prevailing winds are from the southwest.

The geological Mississippi Maccrady formation is the major source of the fossiferous dark gray muck that surrounds the ponds (Ray et al. 1967) and provides an excellent larval habitat for immature _C. variipennis_. Soil tests showed the following ranges in ponds and pools sampled; pH 7.6-7.8 , Mg 82-199 ppm, K 28-64 ppm, Ca 1679+ ppm, P 1-13 ppm, NO_3 52-437 ppm, and organic matter 2.9-6.5%.

Salt appears to have been deposited from marine sedimentary origin ranging in age from the Cambrian to the Mississipian (Ray et al. 1967). The area is a syncline of Calcareous stratification (a cradle of limestone layers in which rock salt has accumulated from the evaporation of saline brine). In the 1700's one-third of the valley was a morass overlying brittle limestone (Lonn 1933). During subsequent years the area

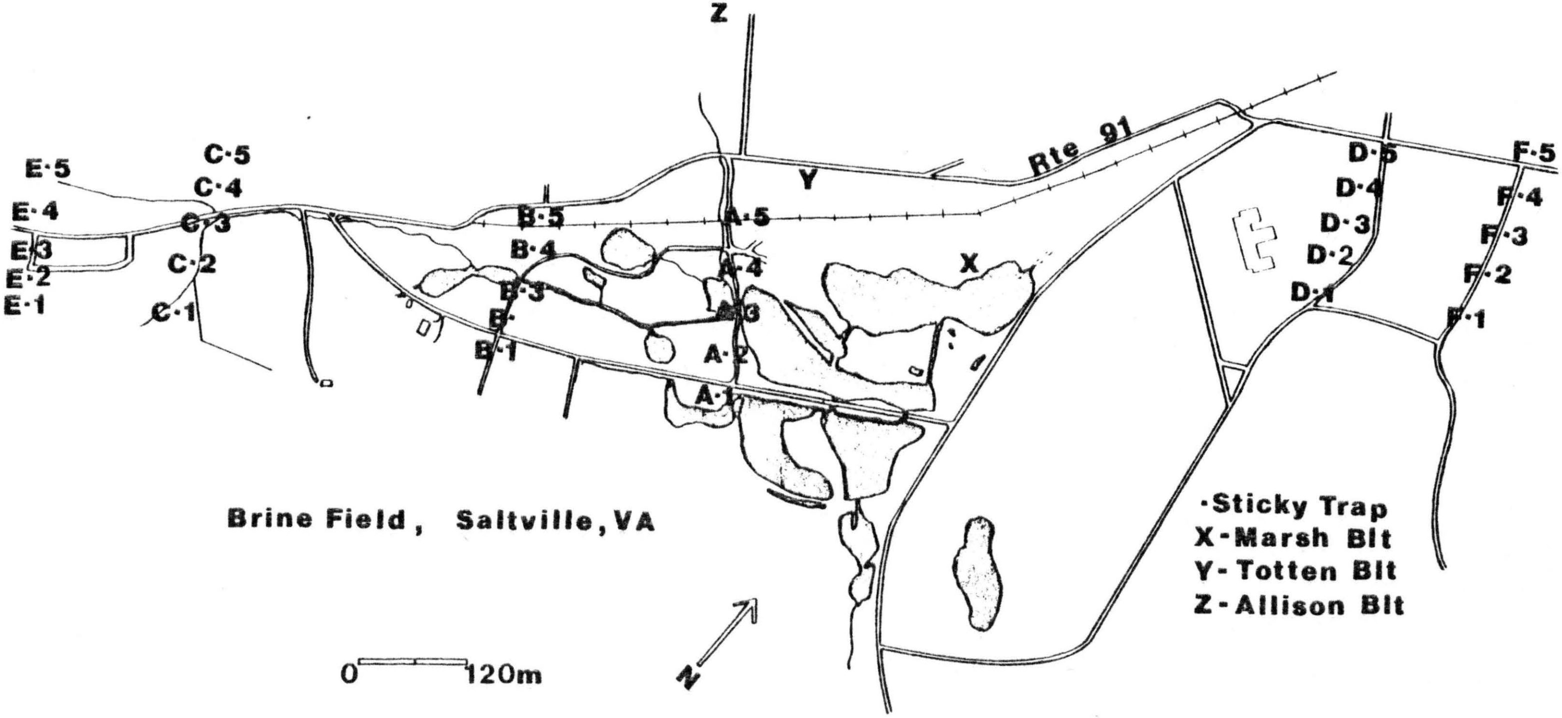

Figure 12. Collection sites of Culicoides variipennis in Saltville, VA. A-F, sticky traps, X,Y,Z blacklight traps (Blt).

was ditched, filled and cattle were grazed in the fields. Between 1893 and 1917 brine derricks became common (Ogle 1981). As the salt was removed the elevation dropped 5.2 m in 30 years (C. Totten pers. comm.) and the marshes were recreated.

The salt marsh habitat has several plant and animal species have a disjunct distribution. The flora has been described by Ogle (1981), and five halophytes were present with their next closest habitat along the eastern coast of Virginia. The halophytes are ; Atriplex patula L. (Orach), Eleocharis parvula (R&S)Link (Spikerush), Juncus gerardi Loiseleur (Blackgrass), Salicornia europaea L. (Glasswort) and Scirpus robustus Pursh. (Bulrush).

The unique fauna consists of several bird species which normally use the coastal flyway (Decker 1979) as well as three species of insects that are coastal in habitat. They are: a mosquito, Aedes sollicitans (Walk), a chironomid midge and a carabid beetle Bembidion constrictus Leconte. Two other insects frequent the inland saltmarsh: the brine fly Ephydra packardi Wirth and Culicoides variipennis.

MATERIALS AND METHODS

I primarily used Blacklight traps (BLT) and sticky traps to sample C. variipennis. In 1979, one light trap (Totten BLT) was placed 650 m from the nearest breeding site at a height of 1.83 m and operated each night from 1800-0800 hrs (Figure 12). A second BLT was placed near sticky trap D-5 and operated once a week. In 1980, the second BLT was discontinued because only 10 midges were collected in 10 trap nights. Two additional BLT's were placed 9 m (Marsh BLT) and 396 m (Allison BLT) from the nearest breeding site (Figure 12) and operated the same way as the Totten BLT. Adults were classified to sex, and females were further categorized as nulliparous, engorged, gravid or parous.

In 1979 and 1980, sticky traps were used similar to those described by Kettle (1951) for _Culicoides impunctatus_ Goet. Black cylinders 30.5 cm high and 8 cm in diameter were placed at 0.61 m, 1.83 m and 3.05 m and nailed to 3.05 m high wooden poles (5.8x5.8cm) (Figure 13). Black cylinders were used because this color appeared to be most attractive to _Culicoides_ (Hill 1947). All sticky traps had a 30 cm long pipe(10mm dia.) attached to the lower end with 15 cm extending beyond the base. This extension was placed into a 20 cm long pipe (15mm. dia.) which was flush with the ground. This construction provided easy removal and replacement of the poles. A 30 x 50 cm rectangular sheet of clear plastic film (6 mil) was attached to each black cylinder by a strip of 1.5 cm masking tape. A thin even layer of Tack Trap^R was applied to the plastic sheet with a 10 cm paint brush. The plastic sheets were removed once a week between 0730 and 1100 hrs using a standard rug cutter. Each sheet of plastic was covered with waxpaper and stored in a cool dry room. Midges were identified to sex on the plastic sheet using a binocular microscope (48x). During certain experiments females were removed from the plastic sheets on the date of collection by applying gasoline to disolve the the Tack Trap. They were sorted to gonotrophic age (see below).

Sticky traps were placed in six transects with 3-5 traps per transect (Figure 12). Transects were perpendicular to the prevailing winds which followed the curvature of the valley. Traps within transects were 60 m apart except at the southeastern end of the valley which narrowed considerably. There sticky traps E-1, E-2 and E-3 were only 24 m apart. Sticky traps E-1, E-2, F-1 and F-5 were not used until 1980. In 1980, at sites A-3, B-3, and C-2 additional black cylinders were put at heights of 4.27 m, 6.1 m and 9.15 m. This was done by coupling together three 3.1 m conduit pipes (60cm dia.). The top end was then flattened for right angle attachment, with a 1.5 m section of conduit pipe (60cm dia.) which was also flattened at one end. An eye bolt was attached to the distal end of the 1.5 m pipe so a 0.64 cm rope could be passed through it and act as a pulley. The three additional sticky cylinders were attached to the rope by passing the rope through two holes (at the upper and lower ends) in the sticky cylinders. As the rope passed through the lower hole a knot was made to prevent the cylinders from slipping out of position. This extension was placed in a 1.5 m (79mm dia.) long pipe which extended 0.75 m above ground. It was also offset 1.5 m from the 3.05 m sticky trap so that the eye bolt and rope lined up with the centers of the three lower sticky cylinders (Figure 13). The additional sticky cylinders were lowered by removal of the wooden pole and lowering of the rope. When the trap was in operation the rope was attached to a wooden stake positioned at the base of the wooden pole. Guide ropes were attached to eye bolts at the junction of the second and third conduit pipes.

Sticky traps were used for 17 weeks in 1979, starting on June 1 and ending on September 28. In 1980, they were used for 15 weeks starting on May 19 and ending on September 8. The extended sticky traps were used in 1980 for 13 weeks, starting on June 3 and ending September 8.

In July 1980, I discovered that the gonotrophic age of the females could be determined if they were removed from the sticky cylinders within 24 hrs of collection. The parous rates of the females collected on the sticky traps differed from the parous rates of the females collected in the Marsh BLT. Therefore, I set up a series of comparisons to examine these differences.

1. Only sticky trap A-3 vs the Marsh BLT for thirteen 24 hr trap periods.

2. Only sticky traps A-3 and D-5 at a height of 1.83 m and the 3 BLTs operated between 1800-0800 hrs, six times.

3. Only sticky trap A-3 at 1.83 m vs a sheep bait trap located 60 m northeast of the Marsh BLT (9 m from the nearest breeding site), vs the Marsh BLT. Traps were operated from one hour pre-sunset to one hour post-sunset as well as one hr pre-sunrise to one hr post-sunset for seven trap periods. This experiment was similar to that described on page 16.

Figure 13. The sticky trap showing the extension added in 1980, Saltville, VA.

Only sticky trap A-3 at 1.83 m, vs a standard model A-1 D-Vac, vs the Marsh BLT for three SS trap periods. The D-Vac was run with the opening positioned at a height of 1.83 m parallel to the ground, in front of the carrier. The D-Vac was carried over a course along the pond edge southwest of the Marsh BLT. Four 15 min periods were used: one starting at SS-30, SS, SS+30 and SS+60. Sticky trap A-3 and the Marsh BLT were run during this same time period.

5. A comparison between the Marsh BLT vs a similar New Jersy light trap equipped with a white circular fluorescent bulb (30.5cm dia.) rather than the circular blacklight was run for seven nights. The whitelight trap was positioned 60 m southwest of the blacklight trap and 10 m from the marsh edge at a height of 1.83 m. When operated neither trap could be seen from the other trap site because of a small woodlot. The traps were run from 1800 - 0800 hrs.

Vegetation was cleared in a 2 m circle around each sticky trap and was cut weekly. The height of the surrounding vegetation was measured weekly at the four compass points and means calculated.

Temperature and humidity were measured using a Bendix Model 594 Hygro-thermograph placed in a standard weather shelter 1.37 m above the ground. Rainfall was collected in a standard rain gauge.

Comparisons between midges collected from sticky traps at different heights were done using arcsine transformation of the proportion collected at each height. Differences in means were tested using Duncans Multiple Range Test.

RESULTS

Horizontal Dispersal

The number of Culicoides variipennis collected from sticky traps decreased as the distance from the nearest breeding site increased (Figure 14). Both sexes were collected on the farthest sticky trap (0.89 Km) from the nearest breeding site. In 1979 and in 1980, the largest number of females was collected on sticky trap A-3. Males were more abundant on sticky trap A-3 in 1979 and on sticky trap B-3 in 1980 (Tables 24 & 25). The lowest number of both sexes was collected on sticky trap F-2 in 1979 and E-2 in 1980.

Predictable numbers of adults were collected from most sticky traps if I assumed that numbers decreased with distance. However, sticky trap B-1 collected a smaller number of midges than some sticky traps located at an equal distance or further from the breeding sites (i.e B-4, B-5, C-2, C-4, D-1 (Tables 24 & 25)). In 1979, sticky trap C-1 collected fewer female midges than any other trap in C-transect as well as traps D-1, D-2, D-4, E-4, E-5 and F-4 (Table 24). But, in 1980, sticky trap C-1 collected more female midges than any other trap in C-transect as well as sticky traps B-1, B-5 and those mentioned above (Table 25).

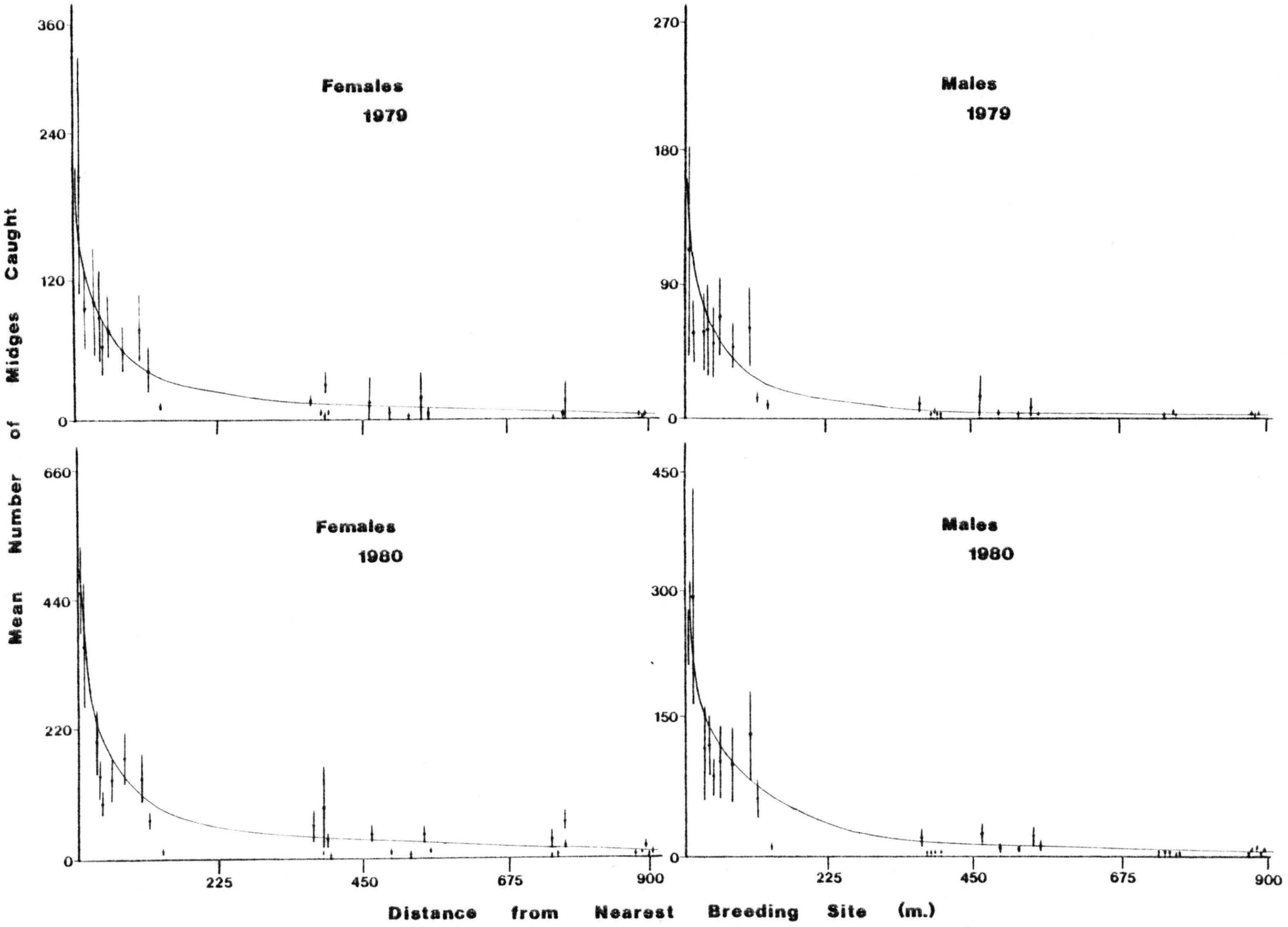

Figure 14. Mean number and confidence limits of Culicoides variipennis collected on sticky traps in Saltville, VA.

Sticky trap E-5 also collected more female midges than traps in the same transect, as well as some of the traps closer to the breeding grounds (Tables 24 & 25).

Vegetative growth around the sticky traps fluctuated in height from 5 cm to 15 m as the seasonal species composition changed. This did not appear to alter the number of midges collected (ANOVA $p < 0.05$).

The number of midges collected and distance from the nearest breeding site were converted to Log N+1 in order to examine the slopes of the regression models. Slopes differed between 1979 and 1980 for both sexes and between sexes in 1980 (Table 26).

Vertical Dispersal

Results of the vertical dispersal study for the sticky traps placed at heights of 0.61, 1.83 and 3.05 m are presented in Tables 24 & 25. Most midges were collected either at a height of 1.83 m or 3.05 m. Some traps collected more at 1.83 m than at 3.05 m the first year and more at 3.05 m than at 1.83 m the second year (e.g. B-5). Also, mean catches between 1.83 m and 3.05 m were sometimes significantly different one year and not the next (e.g B-3). For most traps the height of flight was shown to be dependent on site and date (ANOVA $p<0.05$). Also, change in the height of the vegetation did not influence height of midge collection (ANOVA $p <0.05$). At three sites the female catch was greater at 0.61 m. They were from sticky trap site E-5 in both years, site C-1 in

Table 24. Mean number of *Culicoides variipennis* collected from sticky traps at different heights in 1979, Saltville, VA.*

Site	Ht(m)	Female	Male	Site	Ht(m)	Female	Male	Site	Ht(m)	Female	Male
A-1	0.61	20^{b}	18^{a}	C-1	0.61	0.5^{b}	0.2^{a}	E-3	0.61	0.3^{a}	0.1^{b}
(28)**	1.83	39^{a}	21^{a}	(391)	1.83	1^{b}	0.5^{a}	(752)	1.83	0.6^{a}	0.1^{b}
	3.05	40^{a}	20^{a}		3.05	3^{a}	1^{a}		3.05	0.3^{a}	0.5^{a}
A-2	0.61	4^{b}	4^{a}	C-2	0.61	1^{b}	0.6^{a}	E-4	0.61	1^{b}	1^{b}
(32)	1.83	29^{a}	27^{b}	(368)	1.83	7^{a}	6^{b}	(767)	1.83	4^{a}	2^{a}
	3.05	28^{a}	20^{c}		3.05	7^{a}	3^{c}		3.05	4^{a}	0.8^{b}
A-3	0.61	58^{b}	37^{a}	C-3	0.61	0.3^{a}	0.0^{b}	E-5	0.61	11^{a}	0.2^{a}
(2)	1.83	84^{a}	43^{a}	(383)	1.83	2^{b}	1^{a}	(767)	1.83	5^{b}	0.2^{a}
	3.05	62^{ba}	34^{a}		3.05	4^{c}	0.7^{a}		3.05	2^{b}	0.1^{a}
A-4	0.61	9^{a}	9^{a}	C-4	0.61	3^{b}	0.8^{b}	F-2	0.61	0.2^{a}	0.0^{a}
(30)	1.83	47^{b}	35^{b}	(391)	1.83	11^{a}	2^{ba}	(895)	1.83	0.2^{a}	0.1^{a}
	3.05	30^{c}	16^{c}		3.05	15^{a}	2^{a}		3.05	0.2^{a}	0.0^{a}
A-5	0.61	26^{a}	26^{a}	C-5	0.61	1^{a}	0.1^{b}	F-3	0.61	0.4^{b}	0.5^{a}
(98)	1.83	31^{a}	23^{b}	(391)	1.83	2^{a}	0.5^{ba}	(895)	1.83	2^{b}	2^{a}
	3.05	18^{b}	13^{c}		3.05	2^{a}	1^{a}		3.05	2^{a}	1^{a}
B-1	0.61	1^{b}	0.8^{a}	D-1	0.61	5^{b}	3^{a}	F-4	0.61	1^{b}	0.8^{a}
(131)	1.83	4^{a}	3^{b}	(463)	1.83	12^{a}	7^{a}	(895)	1.83	3^{ba}	1^{a}
	3.05	5^{a}	5^{c}		3.05	9^{a}	4^{a}		3.05	3^{a}	0.8^{a}
B-2	0.61	4^{b}	4^{b}	D-2	0.61	0.5^{b}	0.3^{a}				
(71)	1.83	28^{a}	25^{a}	(492)	1.83	2^{ba}	1^{a}				
	3.05	28^{a}	20^{a}		3.05	4^{a}	2^{a}				
B-3	0.61	7^{a}	5^{b}	D-3	0.61	0.4^{a}	0.2^{a}				
(10)	1.83	35^{b}	25^{a}	(523)	1.83	0.7^{a}	0.9^{a}				
	3.05	52^{c}	29^{a}		3.05	2^{a}	1^{a}				
B-4	0.61	10^{a}	10^{a}	D-4	0.61	2^{b}	2^{a}				
(50)	1.83	39^{b}	41^{b}	(543)	1.83	9^{a}	3^{a}				
	3.05	26^{c}	18^{c}		3.05	8^{a}	2^{a}				
B-5	0.61	2^{a}	1^{a}	D-5	0.61	0.2^{b}	0.0^{b}				
(111)	1.83	10^{b}	5^{b}	(553)	1.83	2^{a}	0.8^{ba}				
	3.05	28^{c}	8^{c}		3.05	2^{a}	1^{a}				

*within a site, numbers with different letters are significantly different from each other (Duncans multiple range test $p<0.05$).

**distance from breeding site in meters.

Table 25. Mean number of *Culicoides variipennis* collected from sticky traps at different heights in 1980, Saltville, VA.*

Site	Ht(m)	Female	Male	Site	Ht(m)	Female	Male	Site	Ht(m)	Female	Male
A-1	0.61	47^{b}	49^{a}	C-1	0,61	48^{a}	0.7^{a}	E-1	0.61	7^{b}	2^{ba}
	1.83	81^{a}	48^{a}		1.83	26^{a}	1^{a}	(752)**	1.83	13^{a}	3^{a}
	3.05	70^{a}	27^{b}		3.05	13^{b}	0.8^{a}		3.05	13^{a}	1^{b}
A-2	0.61	6^{b}	7^{a}	C-2	0.61	6^{a}	3^{b}	E-2	0.61	0.2^{b}	0.1^{a}
	1.83	46^{a}	45^{b}		1.83	22^{b}	8^{a}	(752)	1.83	0.6^{b}	0.0^{a}
	3.05	44^{a}	39^{c}		3.05	28^{c}	9^{a}		3.05	1^{a}	0.2^{a}
A-3	0.61	128^{b}	81^{b}	C-3	0.61	0.6^{a}	0.1^{b}	E-3	0.61	0.9^{b}	0.1^{a}
	1.83	213^{a}	101^{a}		1.83	3^{b}	0.5^{b}		1.83	2^{b}	0.4^{a}
	3.05	118^{b}	70^{b}		3.05	6^{c}	2^{a}		3.05	4^{a}	0.3^{a}
A-4	0.61	11^{a}	14^{a}	C-4	0.61	4^{a}	1^{a}	E-4	0.61	3^{a}	1^{a}
	1.83	86^{b}	84^{b}		1.83	13^{b}	2^{a}		1.83	9^{b}	2^{a}
	3.05	46^{c}	31^{c}		3.05	16^{c}	1^{a}		3.05	11^{c}	0.9^{a}
A-5	0.61	37^{b}	44^{a}	C-5	0.61	1^{a}	0.3^{b}	E-5	0.61	37^{a}	0.6^{a}
	1.83	63^{a}	67^{b}		1.83	2^{a}	1^{a}		1.83	17^{b}	0.4^{a}
	3.05	37^{b}	27^{c}		3.05	1^{a}	1^{a}		3.05	8^{c}	0.7^{a}
B-1	0.61	0.9^{a}	0.9^{b}	D-1	0.61	9^{b}	6^{b}	F-1	0.61	4^{b}	2^{b}
	1.83	4^{b}	3^{a}		1.83	17^{a}	11^{a}	(895)	1.83	7^{b}	3^{ba}
	3.05	7^{c}	6^{a}		3.05	16^{a}	10^{a}		3.05	12^{a}	5^{a}
B-2	0.61	10^{a}	10^{a}	D-2	0.61	1^{b}	1^{b}	F-2	0.61	1^{a}	0.6^{b}
	1.83	74^{b}	60^{b}		1.83	3^{b}	3^{a}		1.83	1^{a}	0.8^{ba}
	3.05	88^{c}	36^{c}		3.05	7^{a}	4^{a}		3.05	2^{a}	1^{a}
B-3	0.61	37^{b}	40^{a}	D-3	0.61	0.9^{b}	1^{b}	F-3	0.61	1^{b}	0.9^{b}
	1.83	163^{a}	146^{b}		1.83	2^{b}	2^{a}		1.83	4^{a}	3^{a}
	3.05	164^{a}	108^{c}		3.05	4^{a}	3^{a}		3.05	4^{a}	2^{b}
B-4	0.61	20^{a}	24^{b}	D-4	0.61	6^{a}	6^{b}	F-4	0.61	2^{b}	1^{b}
	1.83	65^{b}	60^{a}		1.83	21^{b}	14^{a}		1.83	4^{a}	3^{a}
	3.05	49^{c}	26^{b}		3.05	12^{c}	6^{b}		3.05	6^{a}	2^{ba}
B-5	0.61	8^{a}	12^{b}	D-5	0.61	1^{b}	2^{a}	F-5	0.61	1^{a}	0.8^{a}
	1.83	23^{b}	24^{a}		1.83	5^{a}	6^{a}	(895)	1.83	2^{a}	1^{a}
	3.05	36^{c}	25^{a}		3.05	5^{a}	4^{a}		3.05	2^{a}	1^{a}

*within a site different letters are significantly different from each other (Duncans multiple range test $p<0.05$).

**distance from nearest breeding site same as Table 24.

TABLE 26. Log number x log distance regression models for *Culicoides variipennis* collected on sticky traps at Saltville, VA. *

Year	Sex	r^2	Model
1979	Female	.48	$Y = 2.308 - 0.579x^a$
	Male	.46	$Y = 2.149 - 0.584x^a$
1980	Female	.55	$Y = 2.734 - 0.627x^b$
	Male	.62	$Y = 2.804 - 0.735x^c$

[abc] models with different letters are significantly different (t-test $p < 0.01$).

* All F-tests, $p < 0.001$.

1980 and site A-3 during the last 3 weeks of 1980. In 1979 and during the first 12 weeks of 1980 site A-3 collected 25% of its total catch of females at 0.61 m. Then during the last three weeks of 1980, 47% of the total females collected were collected from the trap at 0.61 m. The number of males collected also increased to 45% of the total catch at 0.61 m at site A-3 during the last three weeks of 1980. Previously to this time 30% of all males were collected at 0.61 m.

A closer examination of the number of adults collected at different heights was done using the extended sticky traps at sites A-3, B-3 and C-2 (Figure 15 & Table 27). The number of midges collected at different heights depended on trap location. The extended sticky trap at site A-3 collected the most females at all heights and the extended trap at site C-2 the least. More males were found on the extended sticky trap at site B-3 from all heights, except 0.61 m. The extended sticky trap at site A-3 collected the most females at 0.61 m. The extended sticky trap at site C-2 also had the least number of males. The mean number of females collected from the extended sticky trap at site A-3, which was located next to the breeding site, was significantly greater on the sticky cylinder at a height of 1.83 m, followed by those at 0.61 and 3.0 m. The means for females collected at 0.61 and 3.05 m were not significantly different, then there was a decrease in numbers with height. Males showed similar results except significantly more were collected at 0.61 m than at 3.05 m.

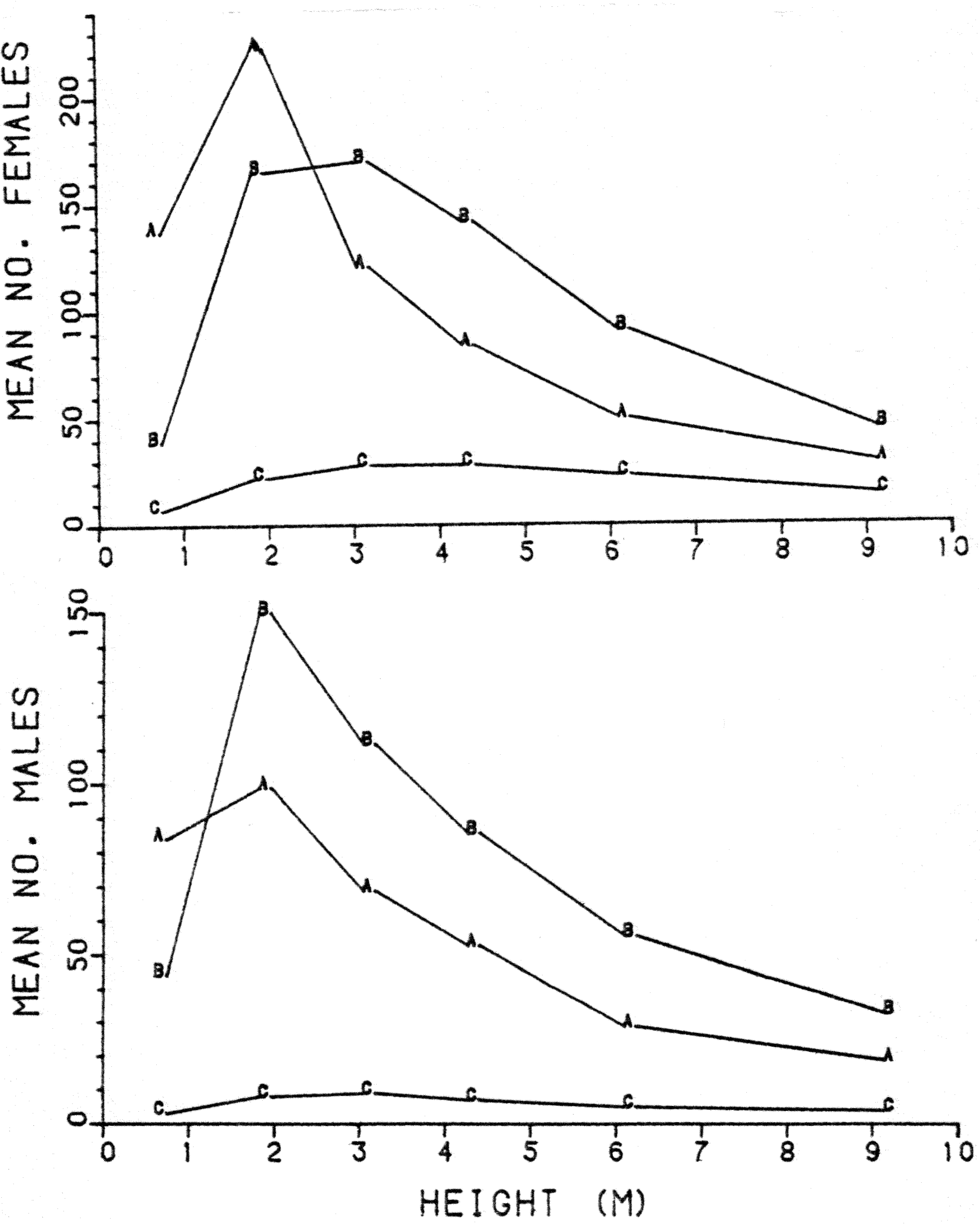

Figure 15. Mean number of Culicoides variipennis collected at different heights from the 9.15 m sticky traps in 1980, Saltville VA; sticky trap A-3 (A), sticky trap B-3 (B) and sticky trap C-2 (C).

Table 27. Mean number and(percent of total population)of *Culicoides variipennis* collected from the extended sticky traps at different heights (Ht) in 1980, Saltville, VA*

Trap Ht(m)	Site A-3		Site B-3		Site C-3	
	Female	Male	Female	Male	Female	Male
0.61	137^{b}(21)	84^{b}(24)	40^{d}(6)	44^{e}(9)	7^{d}(6)	3^{c}(8)
1.83	223^{a}(35)	99^{a}(28)	166^{a}(25)	150^{a}(31)	22^{b}(18)	8^{a}(22)
3.05	121^{b}(19)	69^{c}(20)	170^{a}(26)	112^{b}(23)	29^{a}(24)	9^{a}(25)
4.27	84^{c}(13)	53^{d}(15)	142^{b}(22)	86^{c}(18)	28^{a}(23)	7^{a}(19)
6.10	50^{d}(8)	29^{e}(8)	91^{c}(14)	56^{d}(12)	23^{b}(19)	5^{c}(14)
9.15	29^{d}(5)	19^{e}(5)	45^{d}(7)	33^{e}(7)	14^{c}(11)	4^{c}(11)

*within a site, numbers with different letters are significantly different from each other (Duncans multiple range test $p < 0.05$).

Results between heights were slightly different at site B-3 which was located 9 m from the nearest breeding site. More females were collected at 3.05 m than at 1.83 m, but the means were not significantly different. Midges collected decreased at higher heights, but the means were significantly different from each other. Means were significantly different between heights 0.61 and 9.15 m. Males were collected in significantly higher numbers at 1.83 m. They also decreased with height, and the means were all significantly different from each other except at 0.61 and 9.15 m.

The extended sticky trap at site C-2, which was located 366 m from the nearest breeding site, collected more females at 3.05 m than at 1.83 m and the means were significantly different. But, at this site there was no significant difference in means between 3.05 m and 4.27 m. The number of females collected then decreased with trap height. Males were collected in low numbers at this trap site, with no significant difference between means at 0.61, 1.83 and 4.27 m. The number of males also appeared to decrease with trap height.

Blacklight Traps (BLT)

Due to the mechanical failure of some of the light traps I could only compare 39 trap nights. If either the Marsh or Totten BLT began to malfunction, I moved the Allison BLT to either of the other sites until the malfunction was repaired. Nightly totals and gonotrophic age of catches are listed in Appendix C. Table 28 shows the seasonal totals and parous rates for the three blacklight traps. No engorged females were collected, and gravid and parous females decreased in number as the distance from the nearest breeding site increased. The Marsh BLT collected the largest number of adults of all gonotrophic ages and the Totten BLT collected slightly higher numbers of gravid and parous females compared to the Allison BLT. The Allison BLT collected more nulliparous females than the Totten BLT.

The differences in nulliparous and parous females were reflected in a comparison of parous rates from females collected from these traps. Parous rates were significantly different between each trap and decreased as the distance from the marsh increased (Table 28). Parous rates of females collected at the Totten site ranged from 50-100% in 1979 and from 32-82% in 1980. At the Marsh site, the parous rates ranged from 49-91% and the weekly mean parous rates for females collected at the Marsh site were always higher than those for the Totten site (parous rates were calculated from Appendix D). Though there were times on a daily basis, when the Totten BLT collections, as well as as the Allison BLT collections, resulted in higher parous rates when compared to the Marsh BLT collections (parous rates were calculated from Appendix C). Weekly totals and total females for each gonotrophic age for the Totten BLT (1979 & 1980) and the Marsh BLT (1980) are listed in Appendix D. No relationship was observed between peak numbers collected and decreases in parous rates.

TABLE 28. Number of female *Culicoides variipennis* of different gonotrophic ages collected in the three blacklight traps (BLT) in 1980, Saltville, VA.

	Gonotrophic age			
BLT	Nulliparous	Parous	Gravid	% Parous*
Marsh	2464	4663	19,108	64
Totten	466	473	227	51
Allison	1082	453	219	36

* Parous rates are significantly different at each trap (chi sq. $p < 0.05$).

COMPARISON BETWEEN STICKY TRAPS, BLACKLIGHT TRAPS, ANIMAL BAIT TRAP AND A D-VAC SAMPLER

I showed previously that the parous rates of females collected at sticky trap site A-3 and the Marsh BLT site were different (see Table 22). So, in order to properly interpret the results of the blacklight trap study and the sticky trap study, I examined the possible bias in proportion of nulliparous and parous females collected by the two trapping methods. Table 29 shows the results of 13 comparison dates between sticky trap A-3 and the Marsh BLT. On 11 of the 13 dates the Marsh BLT collected a significantly higher proportion of parous females. This agreed with the results shown in Table 24. Table 30 shows the percent parous and total numbers collected for six dates that could be compared between sticky trap sites A-3, and D-3, and the three blacklight traps during July and August. Results showed there was no significant difference in parous rates between sticky traps, the Totten BLT and the Allison BLT. However, the Marsh BLT collected a significantly higher proportion of parous females than any of the other traps. These comparisons suggested that the Marsh BLT may here be bias, either attracting more parous females or not attracting (or repelling) nulliparous females.

Since it was shown in the Culicoides survey that there was no significant difference between the parous rates of C. variipennis collected from the bait animals and those from the blacklight trap on the coastal plain (page 70), I decided that perhaps a comparison between the Marsh BLT and a bait animal (sheep) would help explain the difference in

TABLE 29. Comparison of the percent parous and (numbers collected) of *Culicoides variipennis* from sticky trap A-3 and the marsh blacklight trap in 1980, Saltville, VA.

Date	Marsh blacklight trap	Sticky trap A-3
July 7	83 (58)	39 (23)
14	50 (246)	8 (37)
16	38 (334)	23 (40)
21	53 (280)	20 (25)
22	59 (312)	17 (18)
23*	63 (344)	90 (10)
24	58 (60)	40 (10)
28	50 (210)	24 (25)
Aug. 5	42 (184)	21 (42)
10	59 (220)	8 (48)
11	8 (463)	25 (20)
18	52 (210)	15 (13)
19*	48 (141)	50 (4)
Total	46 (3134)	23 (315)

* dates when parous rates are not significantly higher in the marsh blacklight trap (chi sq. $p > 0.05$).

TABLE 30. Comparison of the percent parous and (number collected) of *Culicoides variipennis* from sticky trap A-3, sticky trap D-3 and the three blacklight traps in 1980, Saltville, VA.

Sticky trap		Blacklight trap		
A-3	D-3	Marsh	Totten	Allison
28 (128)[a]	22 (32)[a]	53 (1558)[b]	26 (137)[a]	22 (268)[a]

[ab] parous rates with different letters are significantly different (chi sq. $p < 0.01$).

parous rates between the above trapping methods. The results for seven trap times, during the first two weeks of September, showed that the parous rates for midges collected from the bait animal and from the sticky trap were similar, but parous rates were significantly different between these two traps and the Marsh BLT (Table 31). In addition no gravid females were collected from the bait animal. Ten males were collected from the host animal. This differed from the results of the midge survey (page 61), where only one male was collected with 2143 females.

The D-Vac is considered to be unbiased sampling method for collecting midges that are in flight (Tanner & Turner 1975). The D-Vac was therefore used as an additional method for comparison with results obtained from sticky trap A-3 and the Marsh BLT. The comparision was done three times during the first week of September. The parous rate of midges collected in the D-Vac and on the sticky trap were not significantly different, but the Marsh BLT collected a significantly higher proportion of parous females (Table 32). In addition there were differences in the proportionate number of gravid females collected. The sticky trap collected 2% gravids, the BLT 50% and the D-Vac 22%. The percent gravid females collected from the sticky trap and the Marsh BLT in the other experiments was similar to this comparison.

These comparisons further suggested that the Marsh BLT was biased toward parous females. To examine whether it was related to blacklight I made a comparison between the blacklight trap and a whitelight trap between September 10-16. The number of midges collected in the blacklight trap was higher, but there was no difference in parous rates (Table 33).

TABLE 31. Comparison of the percent parous and (number collected) of *Culicoides variipennis* from the bait animal, marsh blacklight trap and sticky trap A-3 in 1980, Saltville, VA.

Bait trap	Marsh blacklight trap	Sticky trap A-3
26 (31)[a]	85 (197)[b]	25 (100)[a]

[ab] parous rates with different letters are significantly different (chi sq. $p < 0.001$).

TABLE 32. Comparison of the percent parous and (number collected) of *Culicoides variipennis* from the D-Vac, marsh blacklight trap and sticky trap A-3 in 1980, Saltville, VA.

D-Vac	Marsh blacklight trap	Sticky trap A-3
22 (89)[a]	71 (293)[b]	24 (66)[a]

[ab] parous rates with different letters are significantly different (chi sq. $p < 0.001$).

TABLE 33. Comparison of the percent parous and (number collected) of *Culicoides variipennis* from the marsh blacklight trap and the whitelight trap in 1980, Saltville, VA.

Marsh blacklight trap	Whitelight trap
81 (1042)	80 (594)

SEASONAL POPULATION TRENDS

Based on adults collected in 1979, sticky trap A-3 showed three major population peaks, sticky trap A-5 showed three peaks and the Totten BLT showed two major peaks (Figure 16). No observable differences were seen in seasonal population trends between the two sticky traps. Population trends showed by the Totten BLT were similar to the sticky traps at the beginning of the trapping period, but the number of midges collected in the Totten BLT by the end of the season was too low for valid comparisons.

In 1980 , Sticky trap A-3 showed four to five major population peaks of midges, sticky trap A-5, four peaks, the Totten BLT, four peaks and the Marsh BLT, four peaks (Figure 17). Midges collected on the sticky traps did not increase or decrease concurrently in 1980 as in 1979 until the first week of July. Population trends for midges collected on sticky traps B-3, C-2 and E-5 were compared with A-3 and A-5 and fluctuated concurrently with sticky trap A-5. Also, population peaks varied between BLTs in 1980.

DISCUSSION

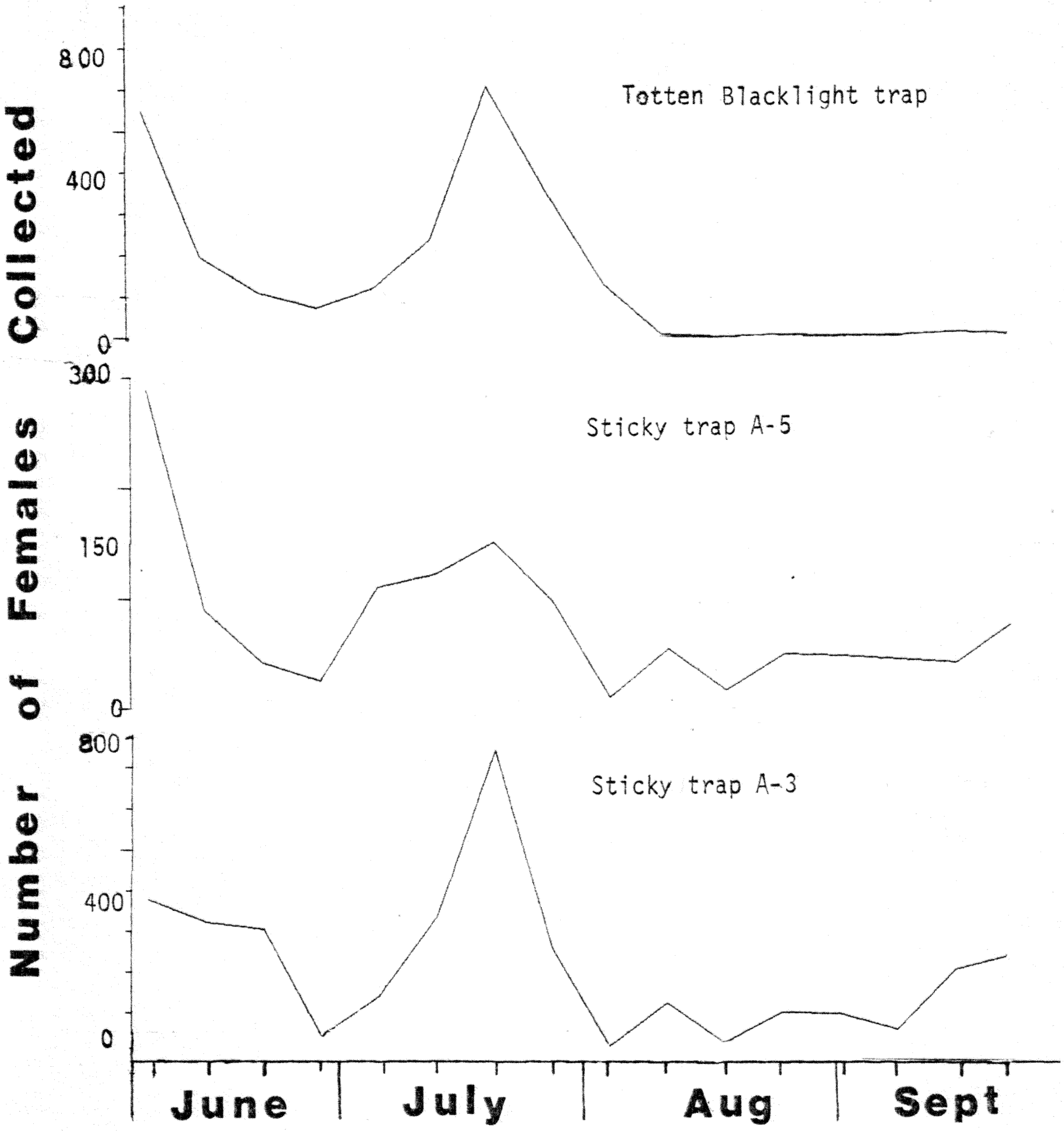

Figure 16. Weekly totals of *Culicoides variipennis* collected in the Totten blacklight trap, sticky trap A-5 and sticky trap A-3 in Saltville, 1979.

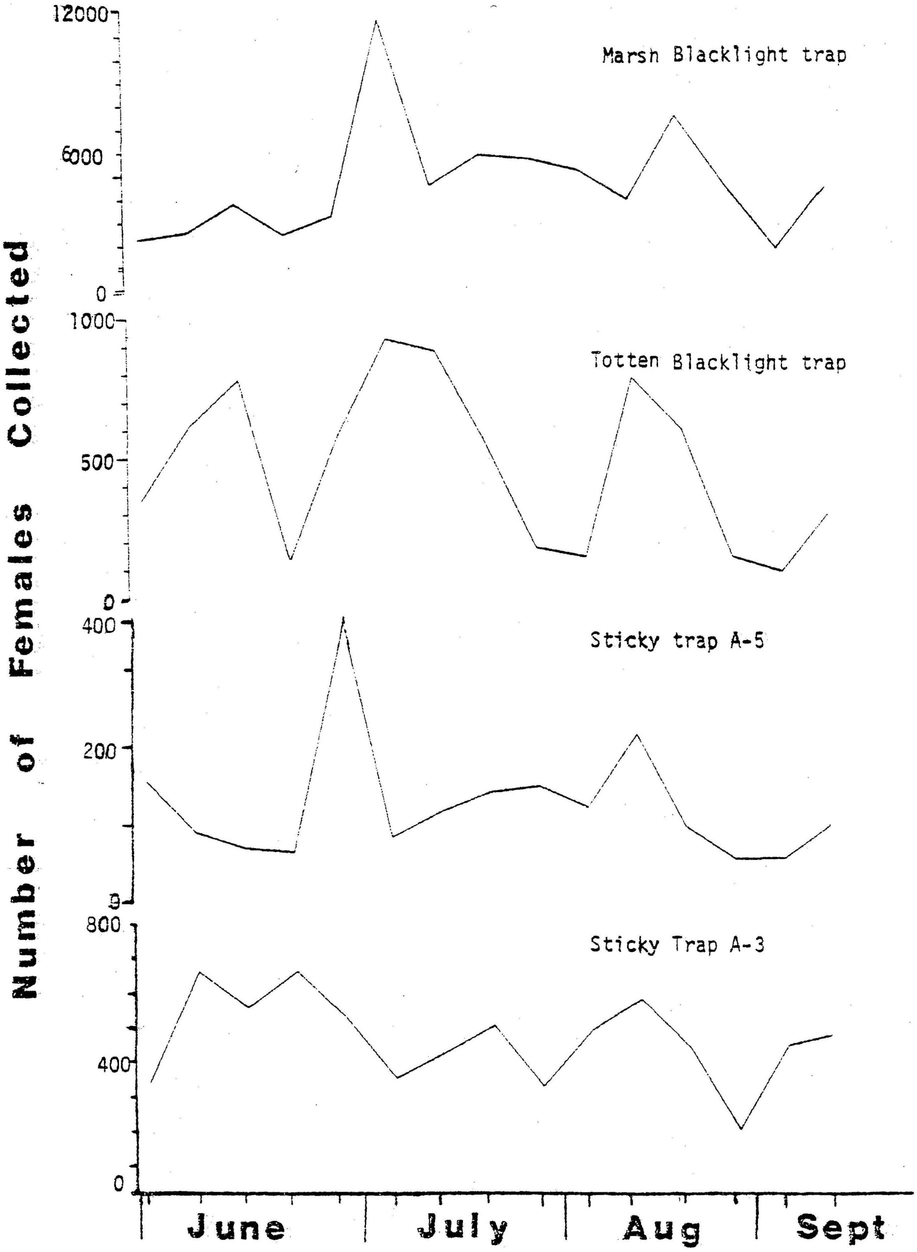

Figure 17. Weekly totals of Culicoides variipennis collected in the Marsh blacklight trap, Totten blacklight trap, sticky trap A-5 and sticky trap A-3 in Saltville, 1980

Horizontal Dispersal

The dispersal curves for C. variipennis in this study were similar to dispersal curves for other Culicoides reported by Wolfenbarger (1959). The maximum distance of dispersal recorded for this species was 4.0 Km for the females and 800 m for males (Lillie 1978). Other Culicoides species have been shown to travel greater than 8 Km (Johnson 1969) and it has been suggested that they can travel up to 50 Km when aided by the wind (Sellers et al. 1978). In this study, C. variipennis dispersed at least 0.89 Km. There appears to be a vertical plume of dispersal away from the breeding site which would contribute to the airborne spread of this species by the wind (discussed under Vertical Dispersal).

There is a positive correlation between insect density at a source and the number collected at greater distances (Taylor 1978, Taylor et al. 1978, Wolfenbarger et al. 1974). This also appeared to be the case for C. variipennis. In this study, weekly catches of midges on sticky traps increased concurrently. If more individuals dispersed farther when numbers were greater at the source then the regression models for 1980 should have x-intercepts (log-distance) greater than those for 1979. This occurred for both females and males. The x-intercepts for females and males in 1979 were 3.99 and 3.67, respectively. In 1980, the x-intercepts increased to 4.36 for females and 3.81 for the males. The increase in the x-intercept when numbers caught at the breeding site increase indicates that there was a positive correlation between insect density at the source and the number of individuals found farther away from the source.

The steepness of a slope may be affected by weather conditions that inhibit movement, thereby concentrating the midges near the breeding site. It was generally hotter and dryer in 1980 than in 1979. This could have restricted movement during 1980 and created steeper slopes, especially for males, which do not have as great a tendency to overcome harsh conditions as do host-seeking females. This also could be the reason why the slopes between sexes were significantly different in 1980. Since the steepness of slope is also affected by the number of midges at a source and the changes in aggregation that occur with changing densities, it would be necessary to examine aggregation in relationship to density in order to explain the differences in slope more fully.

There appears to be a relationship between catch and site or host presence for the three sticky traps that did not follow the expected decrease with distance relationship. For example, sticky trap B-1 was located 130 m from the nearest breeding site (Figure 12), along a narrow dirt roadway having 10 m hedgerows on each side. Fewer midges were collected at this site than at many traps located > 300 m from the breeding site (Tables 24 & 25). This may have been due to the hedgerows, though overall vegetation up to 1.5 m did not affect catch. Sticky trap C-1 collected lower numbers in 1979 when compared to traps at similar distances. But, in 1980, the area around sticky trap C-1 was frequented by cattle and the number of females collected was higher when compared to other traps at similar distances from the breeding site. Cattle were also present during the entire study at sticky trap site E-5, and the larger number of females collected there appeared due to host presence. The presence of a host in the vicinity of a sticky trap has been shown to affect the distributional pattern of male and female C. impunctatus (Kettle 1960). Male and female C. impunctatus increased at sites where hosts were present. At sticky traps C-1 and E-5 only females increased in numbers. The lack of an observed increase in male C. variipennis at sites C-1 and E-5 may have been due to my inability to detect differences in midges numbers. Low numbers were collected at these sites (Tables 24 & 25).

Vertical Dispersal

Culicoides variipennis when not influenced by host presence, was collected most on sticky traps at 1.83 m and 3.05 m. This is in agreement with the sticky trap study by Kettle (1951). Reuben (1963) used suction traps to study C. impunctatus. He found no difference in numbers collected at 0.91 m and 1.83 m but found a sharp decrease at 0.15 m. He combined his work with Kettle and suggested that between 0.91 m and 3.05 m C. impunctatus was more or less evenly distributed. Davies (1965) used wind traps to study the biting midge Leptoconops bequareti Kieffer. He found that more midges were present at 0.91 m. Results for C.variipennis were similar to C. impunctatus, but since sticky cylinders were not placed below 0.61 m or between 0.61 m and 1.83 m, I could not determine at which height, if any, a distinct decrease in catch would occur.

The population density at various heights is dependent on the density of the terrestrial population, the proportion taking flight and the behavior of the insect; including time of flight related to boundary layer effects and host-seeking (Johnson 1969, Taylor 1974). Results from this study add the factor of distance from its breeding source. The farther a trap was located from the breeding site, the greater the proportion of C. variipennis collected at increasing heights (Figure 15 & Table 27). Twenty-six percent of the total midges collected at site A-3 were collected above 3.05 m, 40% at site B-3 and 52% at site C-2. Vertical dispersion away from a source was not shown in either study on C. impunctatus (Kettle 1951, Reuben 1963). This may have been due to the lack of traps above 3.05 m. Also, Davies (1965) found 99% of the total catch of L. bequareti to be below 3.05 m, but no information is available on his placement of traps relative to breeding sites.

It has been shown that crepuscular insects are least subject to wind changes because dawn and dusk are the calmest parts of the day (Southwood 1962). During this period air currents are least likely to transport the insect away from favorable habitats. But the increase in proportion of midges collected at greater heights farther from the breeding site promotes dispersal. Vertical movement would increase the number of midges that would enter the upper layer of air and increase the possibility of passive transport by atmospheric turbulence. Transport by wind increases potential colonization of new and distant sites, increases interpopulation mixing and increase disease transmission.

Host presence also affected vertical distribution. Sticky traps at sites C-1 and E-5 collected larger numbers when hosts were present. But, the maximum catch was from 0.61 m instead of 1.83 or 3.15 m. (Tables 24 & 25). The increase in catch from the sticky cylinder at 0.61 m at trap site A-3 during the last three weeks of the study also appeared to be due to the presence of potential hosts. At that time, domestic geese and fishermen began to frequent the area around sticky trap site A-3 and the percent catch of females from the sticky cylinder at 0.61 m increased from 25 to 47%. The percent of males collected increased from 30 to 45%.

A change in the height of flight of a haematophagous insect may be caused by the insect's orientation towards the host. The attraction of mosquitoes to warm blooded mammal and CO2 has been classified into zones at which successive host stimuli were first encountered (Gillies & Wilkes 1969). The zones of stimulization were further examined for simulids by Bradbury and Bennett (1974). The general hypothesis is that haematophagous insects orientate upwind and follow host odor patterns until they enter close range orientation when host odors may be overridden by visual attraction. Perhaps this hypothesis can be applied to the change in flight path shown by C. variipennis. Knowing that this species is attracted to CO2 (Nelson 1965) and CO_2 is heavier than air, if midges detected the CO_2 before they encountered the host they may alter their height of flight in accordance with an increase in the concentration of CO_2. This factor would increase the potential catch of midges from traps placed at lower heights. A change in the height of flight due to host odors may be why C. variipennis has been found mainly on the belly regions of cattle (Jones 1961a, Schmidtmann et al. 1980a).

Males also were trapped in greater numbers at 0.61 m at sticky trap site A-3 when hosts were present. Does this mean that they are also attracted to host odors? The attraction of males to host animals is extremely rare in nature. In Russia, Culicoides nubeculosus males mated with females while females engorged on the host animals (Downes 1955). In this study, males were collected from host animals in Saltville, but no attempt was made to observe mating. If males did orientate to host odors like the females it may explain why more were collected at 0.61 m at sticky trap site A-3 when hosts were present.

Gonotrophic Age

In order properly to interpret the results of the blacklight trap study it is necessary to have a clear understanding of the gonotrophic ages of adults that were trapped. First, with the exception of the animal bait trap, no trapping method collected engorged females. This is not surprising since most engorged females stay near the host until the eggs are fully developed. Gravid females do not usually seek a host which is supported by their absence from the bait animal trap. Though some gravid C. variipennis were collected from the bait animal trap on the coastal plain (page 61). Trap bias can be determined by examining the proportionate number of gravid females collected by each trapping method. Twenty-two percent of the females collected in the D-Vac sampler were gravid. During the comparison tests, the sticky traps collected 2% gravid females and the Marsh BLT 50% gravid females. Assuming that the D-Vac is an unbiased sampling technique then gravid females appeared to avoid the sticky traps but were attracted to the blacklight trap.

The large number of gravid females collected in the Marsh BLT may have been influenced by the same factor(s) that caused an increase in the number of parous females collected in the Marsh BLT. Gravid females seek an oviposition site at night (Jones 1967, pers. obs.). They may also be aggregated near the mud-water interface where they oviposit. The oviposition site for this species was 9 m from the Marsh BLT. Aggregation by gravid females near oviposition sites has been suggested by Downes (1950) for C. nubeculosus and may occur in C. variipennis. This would increase their presence near the Marsh BLT. No information is known on when this species again seeks a host to begin subsequent gonotrophic cycles, but if recently parous females delay host-seeking they also would be concentrated around the breeding site. A concentration of gravid and recently parous females around the breeding site may have contributed to the high number of gravid and parous females collected in the Marsh BLT. Since the proportion of gravid and parous females collected in the Marsh BLT was not the same as the D-Vac, those collected in the Marsh BLT would have to be attracted to the artifical light when not in flight. Unless, gravid and parous females were attracted to light more than nulliparous.

The effect of artifical light on moths has been studied by several authors (Robinson & Robinson 1952 , Callahan 1965, Hsiao 1971) and the general trapping effect of artificial light was reviewed by Verheijen (1960). Various hypothesis have been proposed to explain attraction to light including a dazzle effect, attraction to a dark area near the light, illumination etc.. In house flies it has been shown that the wavelength of light used affected the physiological age of the catch (Pickens et al. 1969). The effect of artifical light on the catch of Culicoides has been limited to tests with different types of lights. The wavelength of light affected the number collected and the species composition (Belton & Pucat 1967, Rowley & Jorgensen 1967), but no information is available on the mechanism of attraction or the influnce of artifical light.

Habitat can not be excluded from consideration. It was shown that the parous rates from females collected in the the blacklight trap and in the bait animal trap located on the coastal plain did not differ (page 70). The BLT was located 60 m from the nearest known breeding site and was hidden from view of the breeding site by a barn. The bait animals, sheep and cattle, were positioned 50 and 80 m from the nearest breeding site, respectively. The bait animals were also hidden from the breeding site by the barn. There, the position of the trap may have prevented recently parous females from being attracted to the BLT. It would be interesting to compare parous rates of a BLT and a bait animal at various distances from the breeding site in Saltville to examine further the effect of site.

From these observations it appears that in certain locations and with the use of different trapping methods, _C. variipennis_ will differ in relation to the proportion of each gonotrophic age collected. It is difficult to do more than propose the idea that light may effect the age of catch in _C. variipennis_ under certain situations; though the possibility is likely and is supported by the results of studies on mosquitoes (Morris & DeFoliart 1969, Service 1976).

Seasonal Population Trends

The movement of insects away from and towards breeding grounds has important evolutionary, life history and disease transmission implications. The blacklight study showed that gonotrophic age composition differed depending on the distance from the nearest breeding site. Midge parous rates decreased with distance (Table 18). This implied that nulliparous females moved away from the breeding site while parous females were found closer to the breeding site. Therefore, the potential for disease transmission was higher near the breeding site. But, it was discovered that the Marsh BLT may have been biased for gravid and parous females. Therefore, one must use caution when interpreting the results from the blacklight traps. Only if all the light traps had equal bias for different gonotrophic ages would the results represent true midge movement.

However, several other results support the above conclusions. First, though low numbers were obtained, the sticky trap placed closest to the breeding site (A-3) collected midges with a higher parous rate than did the the one located 590 m from the breeding site (D-3) (Table 30). Second, the large number of nulliparous females collected in the Allison BLT compared to the number collected in the Totten BLT indicated movement of nulliparous females (Table 28). Third, a recent mark-recapture study on C. variipennis showed that the females could fly up to 2 Km within the first eight hrs after release (Lillie 1978). These three results support the conclusion that nulliparous females move soon after emergence.

Population peaks occurred at different times not only between sticky trap A-3 and the Marsh BLT, but also between different sticky traps, and different light traps. At some times they were in concordance with each other and at other times they were not. Variation in population trends often occurred when different trapping methods were used to study the biology of Culicoides (Bidlingmayer 1961, Linley et al. 1970, Kline & Axtell 1976). In most studies weather is considered the most important factor to explain catch differences between trap types. In this study, it was observed that the type of habitat, the location of trap and the proportion of each gonotrophic age the trapping method collected should be considered.

SUMMARY

Culicoides variipennis dispersed a minimum of 0.89 Km from the nearest breeding site. Dispersal was also vertical. A higher proportion of midges were collected at a height of 1.83 m near the breeding site while farther away a higher proportion of midges were collected at a height of 3.05 m. The percent of total catch on sticky traps located higher than 3.05 m increased as the distance from the nearest breeding site increased. Female midges converged around host animals and females and males were captured at lower levels when hosts were present.

Comparisons indicated that the Marsh blacklight trap was biased for gravid and parous females while gravid females tended to avoid sticky traps. Though the Marsh blacklight trap was biased, several observations indicated that nulliparous females moved away from the breeding site and parous females were found in higher numbers near the breeding site. Since epidemiological risk (vector potential) is directly related to the number of parous females in a given location, the vector potential of *C. variipennis* was greatest near the breeding site.

Chapter VII

GENERAL CONCLUSIONS

Trap type, time and site influenced the species composition, number collected and female age. Thirty species were collected in the BLTs and 17 in the bait traps. _Culicoides biguttatus_, _C. stellifer_, _C. variipennis_, and _C. venustus_ were the most abundant species collected in both traps. They were uni(bi)voltine, multivoltine, multivoltine and multivoltine respectively. _C. obsoletus_ was also collected in high numbers in the BLTs but not in the bait traps. This species was multivoltine.

Eight new host records were reported in this study. The drop trap collected more midges than did the vacuum trap, and the cattle trap collected more midges than did the sheep trap. Surface area formulas are inaccurate in explaining differences in catch from hosts.

Culicoides stellifer began host-seeking activity at SS-30 in bait traps and continued to be collected in the blacklight trap the remainder of the night. Earlier in the evening, nulliparous females were collected in higher percentages than parous females. Time of flight for _C. stellifer_ was delayed when the temperature was above 25°C.

C. biguttatus and _C. variipennis_ peaked at SS+30 in the bait traps but continued to be collected in the BLTs after dark. _C. venustus_ peaked in the bait traps at SS+60 and continued to be collected in the BLTs after dark. The parous rate of _C. variipennis_ was higher in the cow trap than the sheep trap at the CP site. This was also true for _C. stellifer_ at the PP site.

A morphometric study of C. variipennis in Virginia showed that taxonomic characters used to separate species or subspecies in Virginia were to variable. Temperature, salinity and rainfall affected this variability. Therefore, I concluded that in Virginia using the present taxonomic characters that only one species is present.

Culicoides variipennis males swarmed 15-20 min before sunset and 99% of the females that entered the swarm were nulliparous. One mating pair dropped to the ground every 10 sec and remain in copula from 30-120 sec. The time spend on the ground by mating pairs significantly increased successful insemination ($p<0.01$).

Females collected from swarms could not always be separated to C. occidentalis or C variipennis using the width of the third maxillary palp as stated in Downes (1978). The presence of intermediate widths supports the conclusion that C. variipennis is one ecotypic species in Virginia.

Dispersal of C. variipennis was at least 0.89 Km. More midges were collected at 1.83 m and 3.05 m than at 0.61 m. When hosts were near the sticky traps more midges were collected at 0.61 m. Host presence also increased the number collected.

Extended sticky traps showed that C. variipennis can be found at 9.15 m and that there was vertical movement away from the breeding site. There was a positive correlation between numbers collected at the breeding site and those collected at traps farther away.

Comparison tests showed that sticky traps, D-Vac, and bait traps collected similar percentages of parous individuals. The Marsh BLT collected a higher percentage of parous females than did the above sampling methods. The parous rate of midges collected in the Marsh BLT were also higher than the parous rates from the Totten or Allison BLTs. I concluded that the Marsh BLT was biased for parous individuals. This bias may be due to site and/or attractive properties of light traps.

Gravid females were collected in higher percentages from the Marsh BLT (50%) when compared to sticky traps (2%) and the D-Vac (22%). This suggested that the sticky traps were avoided by the gravid female and attracted to the Marsh BLT (assuming that the D-vac was an unbiased sampling technique).

Though the Marsh BLT was biased for parous and gravid females, other data indicated that nulliparous females moved away from the breeding site. Since more parous females were closer to the breeding site, this would be the area of concern for maximum disease control.

Chapter VIII

LITERATURE CITED

Anderson, J. R. 1974. Symposium on reproduction of arthropods of medical and veterinary importance. II. Meeting of the sexes. J. Med. Entomol. 11: 7-19.

Atchley, W. R. 1967. The Culicoides of New Mexico (Diptera: Ceratopogonidae). Univ. Kans. Sci. Bull. 46: 937-1020.

Atchley, W. R. 1971. A comparative study of the cause and significance of morphological variation in adults and pupae of Culicoides: a factor analysis and multiple regression study. Evol. 25: 563-583.

Barker, R.R. 1978. The Evolutionary Ecology of Animal Migration. Hodder and Stoughton Press, London, 1012p.

Barnard, D. R. and R. H. Jones. 1980a. Culicoides variipennis: Seasonal abundance, overwintering, and voltinism in northeastern Colorado. Environ. Entomol. 9: 709-712.

Barnard, D. R., and R. H. Jones. 1980b. Diel and seasonal patterns of flight activity of Ceratopogonidae in Northeastern Colorado: Culicoides. Environ. Entomol. 9: 446-451.

Battle, F. V. and E. C. Turner, Jr. 1971. A systematic review of the genus Culicoides (Diptera: Ceratopogonidae) in Virginia with a geographic catalog of the species occurring in the Eastern United States north of Florida. Va. Polytech. Inst. & State Univ. Res. Div. Bull. 44. 129p.

Belton, P. and A. Pucat. 1967. A comparison of different lights in traps for Culicoides (Diptera: Ceratopogonidae). Can. Ent. 99: 267-272.

Bennett, G. F. 1960. On some ornithophilic blood-sucking Diptera in Algonquin Park, Ontario, Canada. Can. J. Zool. 38: 377-389.

Bidlingmayer, W. L. 1961. Field activity studies of adult Culicoides furens. Ann. Entomol. Soc. Am. 54: 149-156.

Bidlingmayer, W. L. 1971. Mosquito flight paths in relation to the environment. 1. Illumination levels, orientation, and resting areas. Ann. Entomol. Soc. Am. 64: 1121-1131.

Bidlingmayer, W.L. and D.G Hem. 1979. Mosquito (Diptera: Culicidae) flight behavior near conspicuous objects. Bull. Entomol. Res. 69: 691-700.

Bidlingmayer, W. L. and D. G. Hem. 1981. Mosquito flight paths in relation to the environment, effect of the forest edge upon trap catches in the field. Mosq. News 41: 55-59.

Birley, M. H. and P. K. Rajagopalan. 1981. Estimation of the survival and biting rates of Culex quinquefasciatus (Dipera: Culicidae). J. Med. Entomol. 18: 181-186.

Blanton, F. S. and W. W. Wirth. 1979. The sand flies (Culicoides) of Florida (Diptera: Ceratopogonidae) IN Arthropods of Florida and neighboring land areas. 10: 204p.

Bowne, J. G. 1973. Is bluetongue an important disease in cattle? J. Am. Vet. Med. Assoc. 163: 911-914.

Bradbury, W. C. and G. F. Bennett. 1974. Behavior of adult Simuliidae (Diptera). II. Vision and olfaction in near-orientation and landing. Can. J. Zool. 52: 1355-1364.

Brody, S. 1945. Bioenergetics and growth with special reference to the efficiency complex in domestic animals. Reinhold Publishing Corp. New York, New York, 1023p.

Callahan, P. S. 1965. Far infrared emission and detection by night-flying moths. Nature, Lond. 207: 1172-1173.

Campbell, M. M. and D. S. Kettle. 1979. Swarming of Culicoides brevitarsis Kieffer (Diptera: Ceratopogonidae) with reference to markers, swarm size, proximity of cattle, and weather. Aust. J. Zool. 27: 17-30.

Childers, C. C. and C. W. Wingo. 1968. Genus Culicoides (Diptera-Ceratopogonidae) in Central Missouri. Univ. Missouri Res. Bull. 934: 32p.

Crans, W. J., J. D. Downing and M. E. Slaff. 1976. Behavioral changes in the salt marsh mosquito, Aedes sollicitans, as a result of increased physiological age. Mosq. News 36: 437-445.

Davies, J. B. 1965. Studies on the dispersal of Leptoconops bequaerti Kieffer (Diptera: Ceratopogonidae) by means of wind traps. XII Int. Congr. Ent. London, 12: 753-755.

Davies, J. E. and M. E. C. Giglioli. 1977. Water levels and the emergence of Culicoides furens (Diptera: Ceratopogonidae) from mangrove swamps in Grand Cayman. Mosq. News 37: 426-435.

Davies, L. 1963. Seasonal and diurnal changes in the age-composition of adult Simulium venustum Say (Diptera) populations near Ottawa. Can. Ent. 95: 654-667.

Decker, A.M. 1979. An annotated list of some of the birds of the Saltville ponds. 607 Lincoln Ave. Marion, VA Unpublished Ms.

Detinova, T. S. 1962. Age-grouping methods in Diptera of medical importance. W.H.O. Monograph 47: 1-216.

Downes, A. E. R. 1962. Some aspects of host selection by Mansonia perturbans (Walk) (Diptera: Culicidae). Canadian Jour. of Zoology 40: 725-732.

Downes, J. A. 1950. Habits and life-cycle of Culicoides nubeculosus Mg. Nature 166: 510-511.

Downes, J. A. 1955. Observations on the swarming flight and mating of Culicoides (Diptera: Ceratopogonidae). Trans. R. Ent. Soc. Lond. 106: 213-236.

Downes, J. A. 1958. Assembly and mating in the biting Nematocera. Int. Congr. Entomol. Proc. 10th Montreal, 1956. 2: 425-434.

Downes, J. A. 1969. The swarming and mating flight of Diptera. Ann. Rev. Entomol. 14: 271-298.

Downes, J. A. 1978. The Culicoides variipennis complex: A necessary re-alignment of nomenclature (Diptera: Ceratopogonidae). Can. Ent. 110: 63-69.

Duke, B. O. L. 1960. Studies on the biting habits of Chrysops VII.- The biting-cycles of nulliparous and parous C. silacea and C. dimidiata (Bombe form). Ann. Trop. Med. Parasit. 54: 147-155.

Duval, J. 1971. Etude ecologique du ceratopogonide halophile Stylоconops spinosifrons (Carter 1921) des plages de Nossi-Be en vue d'une lutte rationnelle au moyen d'insecticide. Cah. ORSTOM Ser. Entomol. Med. Parasitol. 9: 203-220.

Dyce, A. L. 1969. The recognition of nulliparous and parous Culicoides (Diptera: Ceratopogonidae) without dissection. J. Australian Entomol. Soc. 8: 11-15.

Dyce, A. L. and H. A. Standfast. 1972. A comparison of the flight patterns of Culicoides and other blood-sucking Nematocera. Abstr. Int. Congr. Entomol. 14th, Canberra, 279-280.

Fontaine, R. E., D. H. Green, and L. M. Smith. 1957. Ecological observations of the valley black gnat, Leptoconops torrens Townsend. J. Econ. Entomol. 50: 764-767.

Foster, N. M. and R. H. Jones. 1973. Bluetongue virus transmission with Culicoides variipennis via embryonating chicken eggs. J. Med. Entomol. 10: 529-532.

Foulk, J. D. 1969. Attack activity of two species of gnats in southern California. Ann. Entomol. Soc. Am. 62: 112-116.

Gillies, M. T. and T. J. Wilkes. 1969. A comparison of the range of attraction of animal baits and of carbon dioxide for some West African mosquitoes. Bull. Ent. Res. 59: 441-456.

Glick, P. A. 1939. The distribution of insects, spiders and mites in the air. Tech. Bull. U.S. Dep. Agric. No. 673, 150p.

Haddow, A. J. 1956. Observations on the biting-habits of African mosquitos in the genus Eretmapodites Theobald. Bull. Ent. Res. 46: 761-762.

Hair, J. A. and E. C. Turner, Jr. 1968. Preliminary host preference studies on Virginia Culicoides (Diptera: Ceratopogonidae). Mosq. News 28: 103-107.

Hair, J. A., E. C. Turner, Jr. and D. H. Messersmith. 1966. Larval habitats of some Virginia Culicoides (Diptera: Ceratopogonidae). Mosq. News 26: 195-204.

Haufe, W. O. 1966. Synoptic correlation of weather with mosquito activity. Proc. 3rd Inter. Biometeorol. Cong., France, 1963: 523-540.

Helwig, J.T. and K.A. Council eds. 1979. SAS Users Guide. SAS Institute Inc. Raliegh, North Carolina. 494p.

Hensleigh, D. A. and W. R. Atchley. 1977. Morphometric variability in natural and laboratory populations of Culicoides variipennis. J. Med. Entomol. 14: 379-386.

Hill, M. A. 1947. The life-cycle and habits of Culicoides impunctatus Goetghebuer and Culicoides obsoletus Meigen, together with some observations on the life-cycle of Culicoides odibilis Austen, Culicoides pallidicornis Kieffer, Culicoides cubitalis Edwards and Culicoides chiopterus Meigen. Ann. Trop. Med. and Parasitol. 41: 55-115.

Hoffman, R. L. 1969. The biotic regions of Virginia IN The Insects of Virginia: No 1, Part II. Res. Div. Bull. 48: 23-62.

Hsiao, H. S. 1973. Flight paths of night-flying moths to light. J. Insect Physiol. 19: 1971-1976.

Humphreys, J. G. 1969. Factors affecting feeding activity in adult *Culicoides* spp. (Diptera: Ceratopogonidae). Ph.D. dissertation, Virginia Polytechnic Institute, Blacksburg, 52p.

Humphreys, J. G. and E. C. Turner, Jr. 1973. Blood-feeding activity of female *Culicoides* (Diptera: Ceratopogonidae). J. Med. Entomol. 10: 79-83.

James, M.T. and R.F. Harwood. 1969. Herms's Medical Entomology. MacMillan Publishing Co. New York. 484p.

Jamnback, H. 1961. Observations on *Culicoides obsoletus* (Meigen) in the laboratory (Diptera:Ceratopogonidae). Mosq. News 21: 48-53.

Jamnback, H. 1965. The *Culicoides* of New York State. N. Y. St. Mus. Sci. Bull. No. 399, 154p.

Jamnback, H., and T. Watthews. 1963. Studies of populations of adult and immature *Culicoides sanguisuga* (Diptera: Ceratopogonidae). Ann. Entomol. Soc. Am. 56: 728-732.

Jamnback, H. and W. W. Wirth. 1963. The species of *Culicoides* related to *obsoletus* in eastern North America (Diptera: Ceratopogonidae). Ann. Entomol. Soc. Am. 56: 185-198.

Johnson, C.G. 1969. Migration and Dispersal of Insects by Flight. Methuen & Co. Ltd, London, 763p.

Jones, R. H. 1957. The laboratory colonization of *Culicoides variipennis* (Coq.). J. Econ. Entomol. 50: 107.

Jones, R. H. 1961a. Some observations on biting flies attacking sheep. Mosq. News 21: 113-115.

Jones, R. H. 1961b. Observations on the larval habitats of some North American species of *Culicoides* (Diptera: Ceratopogonidae). Ann. Entomol. Soc. Am. 54: 702-710.

Jones, R. H. 1964. Mass production methods in rearing *Culicoides variipennis* (Coquillett). Bull. Wld. Hlth. Org. 31: 571-572.

Jones, R. H. 1967. Some irradiation studies and related biological data for *Culicoides variipennis* (Diptera: Ceratopogonidae). Ann. Entomol. Soc. Am. 60: 836-846.

Jones, R. H. and D. H. Akey. 1977. Biting flies attacking Holstein cattle in a bluetongue enzootic area in Colorado, 1976. Mosq. News 37: 372-375.

Jones, R. H. and N. M. Foster. 1971. Transovarian transmission of bluetongue virus unlikely for Culicoides variipennis. Mosq. News 31: 434-437.

Jones, R. H. and E. T. Schmidtmann. 1980. Colonization of Culicoides variipennis variipennis from New York. Mosq. News 40: 191-193.

Jones, R. H., H. W. Potter, Jr. and H. A. Rhodes. 1972. Ceratopogonidae and other biting flies attacking horses in south Texas during the 1971 vee epidemic. Mosq. News 32: 507-509.

Jones, R. H., R. O. Hayes, H. W. Potter, Jr. and D. B. Francy. 1977. A survey of biting flies attacking equines in three states of the southwestern United States, 1972. J. Med. Entomol. 14: 441-447.

Jorgensen, N. M. 1969. The systematics, occurrence and host preference of Culicoides (Diptera: Ceratopogonidae) in southeastern Washington. Melanderia 3: 1-46.

Kettle, D. S. 1951. The spatial distribution of Culicoides impunctatus Goet. under woodland and moorland conditions and its flight range through woodland. Bull. Ent. Res. 42: 239-291.

Kettle, D. S. 1956. Rainfall and larval density of Culicoides impunctatus Goetghebuer (Diptera: Ceratopogonidae). Proc. R. Ent. Soc. Lond. (A)31: 173-177.

Kettle, D. S. 1960. The flight of Culicoides impunctatus Goetghebuer (Diptera, Ceratopogonidae) over moorland and its bearing on midge control. Bull. Ent. Res. 51: 461-489.

Kettle, D. S. 1962. The bionomics and control of Culicoides and Leptoconops (Diptera, Ceratopogonidae=Heleidae). Ann. Rev. Entomol. 7: 401-418.

Kettle, D. S. 1965. Biting Ceratopogonids as vectors of human and animal diseases. Acta. Trop. 22: 356-362.

Kettle, D. S. 1969. The biting habits of Culicoides furens (Poey) and C. barbosai Wirth and Blanton. II. Effect of meteorological conditions. Bull. Ent. Res. 59: 241-258.

Kettle, D. S. 1977. Biology and bionomics of bloodsucking Ceratopogonids. Ann. Rev. Entomol. 22: 33-51.

Kettle, D. S. and J. R. Linley. 1967. The biting habits of Leptoconops bequaerti II. Effect of meteorological conditions on biting activity; 24 hour and seasonal cycles. J. Applied Ecol. 4: 397-420.

Kline, D. L. and R. C. Axtell. 1976. Salt marsh Culicoides (Diptera: Ceratopogonidae): species, seasonal abundance and comparisons of trapping methods. Mosq. News 36: 1-10.

Klowden, M. J. and A. O. Lea. 1980. "Physiologically old" mosquitoes are not necessarily old physiologically. Am. J. Trop. Med. Hyg. 29: 1460-1464.

Koch, H. G. and R. C. Axtell. 1979a. Attraction of Culicoides furens and C. hollensis (Diptera: Ceratopogonidae) to animal hosts in a salt marsh habitat. J. Med. Entomol. 15: 494-499.

Koch, H. G. and R. C. Axtell. 1979b. Correlation of hourly suction trap collections of Culicoides furens and C. hollensis (Diptera: Ceratopogonidae) with wind, temperature and habitat. J. Med. Entomol. 15: 500-505.

Lillie, T. H. 1978. The flight range of Culicoides variipennis (Diptera: Ceratopogonidae). M.S. thesis, Colorado State University, Ft. Collins, Colorado, 75p.

Linley, J. R. and G. M. Adams. 1972. A study of the mating behavior of Culicoides melleus (Coquillett) (Diptera: Ceratopogonidae). Trans. R. Entomol. Soc. Lond. 124: 81-121.

Linley, J. R. and M. J. Hinds. 1976. Seasonal changes in size, female fecundity and male potency in Culicoides melleus (Diptera: Ceratopogonidae). J. Med. Entomol. 13: 151-156.

Linley, J. R., F. D. S. Evans, and H. T. Evans. 1970. Seasonal emergence of Culicoides furens (Diptera: Ceratopogonidae) at Vero Beach, Florida. Ann. Entomol. Soc. Am. 63: 1332-1339.

Lonn, E. 1933. Salt as a factor in the Confederacy. Walter Neale, Publisher. New York.

Luedke, A. J., R. H. Jones and M. M. Jochim. 1967. Transmission of bluetongue between sheep and cattle by Culicoides variipennis. Am. J. Vet. Res. 28: 457-460.

Luedke, A. J., R. H. Jones, and M. M. Jochim. 1976. Serial cyclic transmission of bluetongue virus in sheep and Culicoides variipennis. Cornell Vet. 66: 535-549.

Luedke, A. J., R. H. Jones and T. E. Walton. 1977. Overwintering mechanism for bluetongue virus: Biological recovery of latent virus from a bovine by bites of Culicoides variipennis. Am. J. Trop. Med. Hyg. 26: 313-325.

Mayr, E. 1969. Principles of Systematic Zoology. McGraw-Hill Co. New York, 428p.

Messersmith, D. H. 1966. Report of a collection of Culicoides (Diptera: Ceratopogonidae) from western Virginia. Va. J. Sci. 17: 83-104.

Metcalf, H.E. 1978. Current status of bluetongue of sheep and goats in the United States. Proc. Sheep and Goat Practice Symposium 1976:157-187.

Morris, C. D. and G. R. DeFoliart. 1969. A comparison of mosquito catches with miniature light traps and CO2-baited traps. Mosq. News 29: 424-426.

Morris, C. D., R. H. Zimmerman and J. D. Edman. 1980. Epizootiology of eastern equine encephalomyelitis virus in upstate New York, U.S.A. J. Med. Entomol. 17: 453-465.

Murray, W. S. 1957. Investigations on the bionomics of Culicoides obsoletus (Meigen) and other biting midges at Mount Solon, Virginia. Mosq. News 17: 77-82.

Nathan, M. B. 1981. A study of the diurnal biting and flight activity of Culicoides phlebotomus (Williston) (Diptera: Ceratopogonidae) using three trapping methods. Bull. Ent. Res. 71: 121-128.

Nelson, R. L. 1965. Carbon dioxide as an attractant for Culicoides. J. Med. Entomol. 2: 56-57.

Nelson, R. L. and R. E. Bellamy. 1971. Patterns of flight activity of Culicoides variipennis (Coquillett) (Diptera: Ceratopogonidae). J. Med. Entomol. 8: 283-291.

Nevill, E. M. 1978. The use of cattle to protect sheep from bluetongue infection. J. South African Vet. Assoc. 49: 129-130.

Nicholas, W. L. 1953. The dispersal of Culicoides grahamii and C. austeni from their breeding-sites prior to their taking a blood-meal. Ann. Trop. Med. Parasit. 47: 309-323.

Nielsen, E. T. and H. Greve. 1950. Studies on the swarming habits of mosquitoes and other Nematocera. Bull. Ent. Res. 41: 227-258.

Ogle, D. W. 1981. Long-distance dispersal of vascular halophytes: the marshes of Saltville, Virginia. Castanea 46: 8-15.

Pantin, C. F. A. 1959. Notes on microscopical technique for zoologists. Cambridge Univ. Press, 79p.

Parker, A. H. 1949. Observations on the seasonal and daily incidence of certain biting midges (Culicoides Latreille-Diptera: Ceratopogonidae) in Scotland. Trans. Roy. Ent. Soc. Lond. 100: 179-190.

Pickard, E., and W. E. Snow. 1955. Light trap collections of punkies (family Heleidae, genus Culicoides) McMinn County, Tennessee, April-September, 1952. J. Tennessee Acad. Sci. 30: 15-18.

Pickens, L. G., N. O. Morgan, and R. W. Thimijan. 1968. House fly response to fluorescent lamps: Influenced by fly age and nutrition, air temperature, and position of lamps. J. Econ. Entomol. 62: 536-539.

Potter, H. W. and D. H. Akey. 1978. Pigmentation associated with oogenesis in the biting midge Culicoides variipennis: changes in abdominal tergite patterns. Mosq. News 38: 499-504.

Precht, H., J. Christophersen, H. Hensel, and W. Larcher. 1973. Temperature and life. Springer-Verloy, New York 779p.

Provost, M. W. 1952. The dispersal of Aedes Taeniorhynchus I. preliminary studies. Mosq. News 12: 174-190.

Quraishi, M. S. 1965. Swarming, mating, and density in nature of Anopheles stephensi mysorensis. J. Econ. Entomol. 58: 821-824.

Ray, C. E., B. N. Cooper, and W. S. Benninghoff. 1967. Fossil mammals and pollen in a late Pleistocene deposit at Saltville, Virginia. Jour. of Paleontology 41: 608-622.

Reeves, W. C. 1971. Mosquito vector and vertebrate host interaction: The key to maintenance of certain arboviruses, from Ecology and physiology of parasites. A. M. Fallis, ed., Univ. of Toronto Press. 258p.

Reisen, W. K., Y. Aslam and T. F. Siddiqui. 1977. Observations on the swarming and mating of some Pakistan mosquitoes in nature. Ann. Entomol. Soc. Am. 70: 988-995.

Reuben, R. 1963. A comparison of trap catches of Culicoides impunctatus Goetghebeur (Diptera: Ceratopogonidae) with meteorological data. Proc. Roy. Ent. Soc. Lond. 38: 181-193.

Robinson, H. S. and P. J. M. Robinson. 1950. Some notes on the observed behavior of Lepidoptera in flight in the vicinity of light sources. Ent. Gaz. 1: 3-20.

Ross, H. H. 1962. A synthesis of evolutionary theory. Prentice- Hall Inc., Englewood Cliffs, N.J. 387p.

Roth, L. M. 1948. A study of mosquito behavior. Am. Midl. Nat. 40: 265-352.

Rowley, W. A. 1965. The occurrence and bionomics of blood-sucking midges in the Central Columbia basin. unpublished Ph.D. thesis, Wash. State Univ. 92p.

Rowley, W. A., and N. M. Jorgensen. 1967. Relative effectiveness of three types of light traps in collecting adult Culicoides. J. Econ. Entomol. 60: 1478-1479.

Schmidtmann, E. T., C. J. Jones and B. Gollands. 1980a. Comparative host-seeking activity of Culicoides (Diptera: Ceratopogonidae) attracted to pastured livestock in central New York State, USA. J. Med. Entomol. 17: 221-231.

Schmidtmann, E. T., J. F. Abend and M. E. Valla. 1980b. Nocturnal blood-feeding from pastured calves by the ceratopogonid midge, Culicoides venustus, in New York State. Mosq. News 40: 571-577.

Sellers, R. F., D. E. Pedgley and M. R. Tucker. 1978. Possible windborne spread of bluetongue to Portugal, June-July 1956. J. Hyg., Camb. 81: 189-196.

Sellers, R. F., E. P. J. Gibbs, K. A. J. Herniman, D. E. Pedgley and M. R. Tucker. 1979. Possible origin of the bluetongue epidemic in Cyprus, August 1977. J. Hyg., Camb. 83: 547-555.

Service, M. W. 1969a. Light-trap catches of Culicoides spp. (Dipt., Ceratopogonidae) from southern England. Bull. Ent. Res. 59: 317-322.

Service, M. W. 1969b. Studies on the biting habits of Culicoides impunctatus Goetghebuer, C. obsoletus (Meigen), and C. punctatus (Meigen) (Diptera: Ceratopogonidae) in southern England. Proc. Roy. Soc. Lond. 44: 1-10.

Service, M. W. 1976. Mosquito Ecology: Field Sampling Methods. John Wiley & Sons, New York-Toronto, 583p.

Smith, W. W., and J. H. Varnell, Jr. 1967. Hydrogen-ion concentration (pH) as related to the occurrence and abundance of treehole dwelling Culicoides spp. (Diptera: Ceratopogonidae) in northern Florida. Mosq. News 27: 519-521.

Snow, W. E. 1955. Feeding activities of some blood-sucking Diptera with reference to vertical distribution in bottomland forest. Ann. Ent. Soc. Am. 48: 512-521.

Snow, W. E., E. Pickard, and J. B. Moore. 1957. The Heleidae of the Tennessee River Basin. J. Tenn. Acad. Sci. 32: 18-36.

Snow, W. E., E. Pickard, and C. M. Jones. 1958. Observations on the activity of Culicoides and other Diptera in Jasper County, South Carolina. Mosq. News 18: 18-21.

Southwood, T. R. E. 1962. Migration of terrestrial arthropods in relation to habitat. Biol. Rev. 37: 171-214.

Taylor, L. R. 1974. Insect migration, flight periodicity and the boundary layer. J. Animal Ecol. 43: 225-238.

Taylor, L. R., I. P. Woiwod, and J. N. Perry. 1978. The density-dependence of spatial behaviour and the rarity of randomness. J. Animal Ecol. 47: 383-406.

Taylor, R. A. J. 1978. The relationship between density and distance of dispersing insects. Ecol. Entomol. 3: 63-70.

Tanner, G. D. 1971. Vertical activity, host preference, and population studies of adult Culicoides (Diptera: Ceratopogonidae). Ph.D. dissertation, Virginia Polytechnic Institute, Blacksburg, 84p.

Tanner, G. D. and E. C. Turner, Jr. 1974. Vertical activities and host preferences of several Culicoides species in a southwestern Virginia forest. Mosq. News 34: 66-70.

Tanner, G. D. and E. C. Turner, Jr. 1975. Seasonal abundance of Culicoides spp. as determined by three trapping methods. J. Med. Entomol. 12: 87-91.

Verheijen, F. J. 1960. The mechanisms of the trapping effect of artificial light sources upon animals. Archs. Neerland. Zool. 13: 1-107.

Walker, A. R. 1977. Adult lifespan and reproductive status of Culicoides species (Diptera: Ceratopogonidae) in Kenya, with reference to virus transmission. Bull. Ent. Res. 67: 205-215.

Williams, C. B. 1937. The use of logarithms in the interpretation of certain entomological problems. Ann. Appl. Biol. 24: 404-414.

Williams, R.W. 1955. Observations on the bionomics of some Culicoides of Cheboygan County, Michigan (Diptera: Heleidae). Bull. Brooklyn Entomol. Soc. 50: 113-120.

Wirth, W. W., and A. A. Hubert. 1962. The species of Culicoides related to piliferus Root and Hoffman in eastern North America (Diptera, Ceratopogonidae). Ann. Entomol. Soc. Am. 55: 182-195.

Wirth, W. W. and R. H. Jones. 1957. The North American subspecies of Culicoides variipennis (Diptera, Heleidae). Tech. Bull. U. S. Dept. Agric. 1170, 35p.

Wolfenbarger, D. O. 1959. Dispersion of small organisms: Incidence of viruses and pollen; dispersion of fungus spores and insects. Lloydia 22(1): 1-106.

Wolfenbarger, D. O., J. A. Cornell, and D. A. Wolfenbarger. 1974. Dispersal distances attained by insect populations of different densities. Res. Popul. Ecol. 16: 43-51.

Appendix A: *Culicoides* collected in blacklight traps during 1978 and 1979 in Virginia; listed by year, site, trap time and reproductive status.

TABLE A-1. *Culicoides* collected in Blacklight traps (12 trap nights) at the Coastal Plain site, from 30 minutes pre sunset to 90 minutes post sunset during 1978, in Virginia.

Species	Nulliparous	Gravid	Parous	Engorged	Total
C. alexanderi	1	0	0	0	1
C. arboricola	1	1	0	0	2
C. bickleyi	4	0	0	0	4
C. biguttatus	37	0	80	7	124
C. crepuscularis	3	4	0	0	7
C. debilipalpis	2	1	1	0	4
C. furens	5	0	2	4	11
C. guttipennis	1	0	0	0	1
C. haematopotus	2	1	0	0	3
C. obsoletus	6	13	2	1	22
C. sanguisuga	3	0	0	0	3
C. spinosus	6	0	0	0	6
C. stellifer	949	4	429	17	1399
C. travisi	2	1	0	0	3
C. variipennis	80	214	218	7	519
*C. venustus	0	1	0	2	160
Total	1259	240	732	38	2269

*Parity was not determined for this species.

TABLE A-2. _Culicoides_ collected in Blacklight traps (15 trap nights) at the Coastal Plain site from 30 minutes pre sunset to 60 minutes post sunrise during 1978, in Virginia.

Species	Nulliparous	Gravid	Parous	Engorged	Total
C. alexanderi	1	0	0	0	1
C. arboricola	3	1	0	0	4
C. bickleyi	13	0	0	0	13
C. biguttatus	1253	18	571	107	1949
C. crepuscularis	26	21	30	0	77
C. debilipalpis	2	1	1	0	4
C. furens	24	0	63	4	91
C. guttipennis	2	0	0	0	2
C. haematopotus	24	10	0	0	34
C. mulrennani	9	0	0	0	9
C. obsoletus	33	55	5	1	94
C. piliferus	2	0	0	0	2
C. sanguisuga	34	0	4	8	46
C. spinosus	674	0	14	1	689
C. stellifer	5091	29	2650	54	7824
C. testudinalis	0	0	1	0	1
C. travisi	10	3	2	1	16
C. variipennis	1527	1508	3581	170	6786
*C. venustus	0	7	0	7	1259
Totals	9973	1653	6922	353	18901

*Parity was not determined for this species.

TABLE A-3. *Culicoides* collected in Blacklight traps (15 trap nights) at the Piedmont Plateau (Bennett Farm), from 30 minutes pre sunset to 90 minutes post sunset during 1978, in Virginia.

Species	Nulliparous	Gravid	Parous	Engorged	Total
C. alexanderi	1	0	0	0	1
C. arboricola	2	0	0	0	2
C. bickleyi	6	0	0	0	6
C. biguttatus	20	1	8	3	32
C. crepuscularis	6	3	0	0	9
C. guttipennis	3	5	0	0	8
C. haematopotus	1	6	1	0	8
C. obsoletus	48	48	19	0	115
C. sanguisuga	4	0	6	0	10
C. spinosus	9	0	2	0	11
C. stellifer	605	66	196	10	877
C. variipennis	7	8	6	0	21
*C. venustus	0	0	0	0	50
Total	762	137	238	13	1150

*Parity was not determined for this species.

TABLE A-4. _Culicoides_ collected in Blacklight traps (15 trap nights) at the Piedmont Plateau (Farris Farm), from 30 minutes pre sunset to 90 minutes post sunset during 1978, in Virginia.

Species	Nulliparous	Gravid	Parous	Engorged	Total
C. alexanderi	1	1	7	0	21
C. arboricola	2	1	1	0	4
C. baueri	0	1	0	0	1
C. bickleyi	18	1	3	0	22
C. biguttatus	9	15	20	0	44
C. crepuscularis	8	66	1	1	76
C. debilipalpis	2	0	0	0	2
C. guttipennis	0	1	0	0	1
C. haematopotus	33	7	2	0	42
C. husseyi	1	0	0	0	1
C. jamnbacki	17	9	7	0	33
C. nanus	1	1	0	0	2
C. niger	1	0	0	0	1
C. obsoletus	26	26	21	1	74
C. parapiliferus	11	1	5	0	17
C. piliferus	4	12	3	0	19
C. sanguisuga	6	2	7	0	15
C. spinosus	1	0	0	0	1
C. stellifer	28	29	14	0	71
C. testudinalis	1	1	2	0	4
C. travisi	7	8	4	1	20
C. variipennis	5	5	4	0	14
*C. venustus	0	1	0	0	2
C. villosipennis	16	0	0	0	16
Totals	211	188	101	3	503

*Parity was not determined for this species.

TABLE A-5. *Culicoides* collected in Blacklight traps (11 trap nights) at the Ridge and Valley Province from 30 minutes pre sunset to 90 minutes post sunset during 1978, in Virginia.

Species	Nulliparous	Gravid	Parous	Engorged	Total
C. alexanderi	9	1	1	0	11
C. bergi	1	1	0	0	2
C. bickleyi	10	1	0	0	11
C. bigattatus	0	1	1	0	2
C. crepuscularis	3	10	2	0	15
C. haematopotus	0	2	0	0	2
C. jamnbacki	3	0	0	0	3
C. obsoletus	27	149	2	1	179
C. parapiliferus	4	0	0	0	4
C. piliferus	1	0	0	0	1
C. sanguisuga	3	0	0	0	3
C. spinosus	0	0	1	0	1
C. stellifer	59	11	19	1	90
C. travisi	0	6	1	0	7
C. variipennis	0	0	2	0	2
*C. venustus	0	0	0	0	14
Total	132	182	31	2	347

*Parity was not determined for this species.

TABLE A-6. *Culicoides* collected in Blacklight traps (12 trap nights) at the Ridge and Valley Province from 30 minutes pre sunset to 60 minutes post sunrise during 1978, in Virginia.

Species	Nulliparous	Gravid	Parous	Engorged	Total
C. alexanderi	9	0	0	0	9
C. baueri	0	15	0	0	15
C. bergi	3	7	0	0	10
C. bickleyi	4	2	0	0	6
C. bigattatus	93	18	11	4	126
C. crepuscularis	16	43	1	3	63
C. guttipennis	5	0	0	0	5
C. haematopotus	1	35	0	1	37
C. obsoletus	110	378	68	2	558
C. piliferus	0	2	0	0	2
C. sanguisuga	14	0	2	1	17
C. spinosus	43	14	1	2	60
C. stellifer	154	28	45	4	231
C. travisi	5	50	1	0	56
C. variipennis	14	7	22	0	43
*C. venustus	0	4	0	2	91
Total	534	603	173	19	1329

*Parity was not determined for this species.

TABLE A-7. *Culicoides* collected in Blacklight traps (18 trap nights) at the Coastal Plain site, 30 minutes pre sunset to 90 minutes post sunset, during 1979, in Virginia.

Species	Nulliparous	Gravid	Parous	Engorged	Total
C. arboricola	0	2	1	0	3
C. bergi	1	0	0	0	1
C. bigattatus	57	0	92	19	168
C. crepuscularis	0	3	1	1	5
C. debilipalpis	1	0	1	0	2
C. furens	22	0	2	0	24
C. guttipennis	0	0	5	0	5
C. haematopotus	2	1	0	0	3
C. husseyi	0	0	0	0	0
C. jamnbacki	0	0	0	0	0
C. niger	0	0	1	0	1
C. obsoletus	0	1	0	0	1
C. piliferus	0	5	0	0	5
C. sanguisuga	2	0	0	0	2
C. spinosus	9	0	1	0	10
C. stellifer	1365	1	569	13	1948
C. testudinalis	0	1	2	0	3
C. travisi	1	0	2	0	3
C. variipennis	571	650	646	27	1894
*C. venustus	0	0	0	0	101
Total	2133	664	1324	60	4179

*Parity was not determined for this species.

TABLE A-8. *Culicoides* collected in Blacklight traps (18 trap nights) at the Coastal Plain site, from 30 minutes pre sunset to 60 minutes post sunrise, during 1979, in Virginia.

Species	Nulliparous	Gravid	Parous	Engorged	Total
C. arboricola	12	2	1	0	15
C. bergi	1	0	0	0	1
C. biguttatus	492	24	655	28	1199
C. crepuscularis	29	23	3	1	56
C. debilipalpis	1	0	1	0	2
C. furens	170	0	115	2	287
C. guttipennis	0	0	5	0	5
C. haemotopotus	9	6	0	0	15
C. husseyi	0	0	12	0	12
C. jamnbacki	1	0	1	0	2
C. niger	0	0	9	0	9
C. obsoletus	0	1	0	0	1
C. piliferus	2	5	0	0	7
C. sanguisaga	14	0	1	0	15
C. spinosus	17	0	1	0	18
C. stellifer	9435	22	6215	61	15733
C. testudinalis	1	11	2	0	14
C. travisi	11	1	12	0	24
C. variipennis	2369	2568	2887	78	7902
*C. venustus	0	9	0	12	989
Total	13532	2672	9920	182	26306

*Parity was not determined for this species.

TABLE A-9. Culicoides collected in Blacklight traps (12 trap nights) at the Piedmont Plateau from 30 minutes pre sunset to 90 minutes post sunset during 1979, in Virginia.

Species	Nulliparous	Gravid	Parous	Engorged	Total
C. arboricola	0	7	6	0	13
C. bigattatus	99	14	95	0	208
C. crepuscularis	2	6	0	0	8
C. debilipalpis	0	1	0	0	1
C. guttipennis	4	0	0	0	4
C. haematopotus	3	12	1	1	17
C. husseyi	0	6	0	0	6
C. jammbacki	3	0	5	0	8
C. obsoletus	55	137	26	1	219
C. piliferus	0	3	6	3	12
C. sanguisuga	6	0	3	0	9
C. scanloni	0	3	0	0	3
C. spinosus	20	4	6	0	30
C. stellifer	826	267	357	10	1460
C. testudinalis	3	5	2	0	10
C. travisi	3	24	0	0	27
C. variipennis	7	36	33	0	76
*C. venustus	0	0	0	0	51
Total	1082	525	540	15	2162

*Parity was not determined for this species.

TABLE A-10. *Culicoides* collected in Blacklight traps (9 trap nights) at the Ridge and Valley Province from 30 minutes pre sunset to 90 minutes post sunset during 1979, in Virginia.

Species	Nulliparous	Gravid	Parous	Engorged	Total
C. alexanderi	0	0	1	0	1
C. arboricola	0	7	4	0	11
C. baueri	3	13	0	0	16
C. bergi	0	1	0	0	1
C. bickleyi	6	1	20	0	27
C. bigattatus	4	19	1	1	25
C. crepuscularis	1	65	3	0	69
C. haematopotus	1	6	0	0	7
C. hinmani	1	0	0	0	1
C. obsoletus	11	38	1	0	50
C. piliferus	0	6	0	0	6
C. spinosus	10	42	0	0	52
C. stellifer	16	53	3	3	75
C. testudinalis	0	0	2	0	2
C. travisi	7	153	3	3	166
C. variipennis	3	58	11	1	73
*C. venustus	0	2	0	0	6
Total	67	464	49	8	588

*Parity was not determined for this species.

Appendix B: *Culicoides* collected from bait animals during 1978 and 1979 in Virginia; listed by year, site, bait and trap period.

TABLE B-1 *Culicoides* collected in bait traps at sunset minus 30 minutes (SS-30), sunset (SS), sunset plus 30 minutes (SS+30), and sunset plus 60 minutes (SS+60), at the Coastal Plain site, in Virginia, 1978.

Species	Trap	SS-30	SS	SS+30	SS+60	Total
C. arboricola	Cow	0	0	1	0	1
	Sheep	0	0	0	0	0
C. biguttatus	Cow	3	7	0	5	15
	Sheep	0	4	0	0	4(+1?)
C. crepuscularis	Cow	0	0	2	0	2
	Sheep	0	1	0	0	1
C. furens	Cow	1	0	0	0	1
	Sheep	0	0	0	0	0
C. hollensis	Cow	0	0	0	1	1
	Sheep	0	0	0	0	0
C. niger	Cow	0	0	0	0	0
	Sheep	0	1	0	0	1
C. obsoletus	Cow	0	0	0	0	0
	Sheep	0	0	1	0	1
C. sanguisuga	Cow	0	0	0	2	2
	Sheep	0	0	0	0	0
C. stellifer	Cow	10	6	5	2	23
	Sheep	1	26	2	0	29
C. variipennis	Cow	24	38	72	77	211
	Sheep	4	8	13	3	28

TABLE B-2 *Culicoides* collected in bait traps at sunset minus 30 minutes (SS-30), sunset (SS), sunset plus 30 minutes (SS+30), and sunset plus 60 minutes (SS+60), at the Piedmont Plateau, in Virginia, 1978.

Species	Trap	SS-30	SS	SS+30	SS+60	Total
C. biguttatus	Cow	0	0	0	0	0
	Sheep	0	1	0	0	1
C. debilipalpis	Cow	0	0	0	0	0
	Sheep	0	2	0	0	2
C. guttipennis	Cow	0	0	1	0	1
	Sheep	0	0	0	0	0
C. obsoletus	Cow	0	1	2	0	3
	Sheep	0	0	0	2	2
C. sanguisuga	Cow	0	1	0	0	1
	Sheep	0	0	0	0	0
C. stellifer	Cow	1	0	0	1	2
	Sheep	12	18	17	1	48
C. variipennis	Cow	0	1	1	0	2
	Sheep	0	0	0	0	0

TABLE B-3 *Culicoides* collected in bait traps at sunset minus 30 minutes (SS-30), sunset (SS), sunset plus 30 minutes (SS+30), and sunset plus 60 minutes (SS+60), at the Ridge and Valley Province, in Virginia, 1978.

Species	Trap	SS-30	SS	SS+30	SS+60	Total
C. bickleyi	Cow	0	0	0	0	0
	Sheep	1	0	0	0	1
C. biguttatus	Cow	0	0	0	0	0
	Sheep	0	2	7	0	9
C. crepuscularis	Cow	0	0	0	1	1
	Sheep	0	0	0	0	0
C. obsoletus	Cow	0	0	0	0	0
	Sheep	0	0	1	0	1
C. sanguisuga	Cow	0	0	0	0	0
	Sheep	0	0	1	0	1
C. spinosus	Cow	0	0	0	0	1?
	Sheep	0	0	0	0	0
C. stellifer	Cow	0	0	2	0	2
	Sheep	12	12	3	1	28
C. variipennis	Cow	0	0	0	0	0
	Sheep	0	0	1	0	1

TABLE B-4 *Culicoides* collected in bait traps at sunset minus 30 minutes (SS-30), sunset (SS), sunset plus 30 minutes (SS+30), and sunset plus 60 minutes (SS+60), at the Coastal Plain site, in Virginia, 1979.

Species	Trap	SS-30	SS	SS+30	SS+60	Total
C. biguttatus	Cow	13	55	72	25	165
	Sheep	1	3	27	2	33
C. crepuscularis	Cow	1	0	0	0	1
	Sheep	0	0	0	0	0
C. furens	Cow	3	4	6	2	15
	Sheep	1	0	1	0	2
C. niger	Cow	2	0	0	0	2
	Sheep	0	0	1	0	1
C. piliferus	Cow	0	1	0	0	1
	Sheep	0	0	0	0	0
C. sanguisuga	Cow	0	0	1	0	1
	Sheep	0	0	0	0	0
C. spinosus	Cow	1	0	3	0	4
	Sheep	0	2	2	0	4
C. stellifer	Cow	264	225	187	47	723
	Sheep	33	41	62	5	141
C. travisi	Cow	6	0	3	2	11
	Sheep	3	0	0	0	3
C. variipennis	Cow	34	300	1154	233	1721
	Sheep	1	7	156	18	182
C. venustus	Cow	1	1	21	21	44
	Sheep	0	0	4	2	6

TABLE B-5 *Culicoides* collected in bait traps at sunset minus 30 minutes (SS-30), sunset (SS), sunset plus 30 minutes (SS+30), and sunset plus 60 minutes (SS+60), at the Piedmont Plateau, in Virginia, 1979.

Species	Trap	SS-30	SS	SS+30	SS+60	Total
C. biguttatus	Cow	7	0	4	0	11
	Sheep	2	0	3	0	5
C. obsoletus	Cow	2	1	0	0	3
	Sheep	1	0	0	0	1
C. sanguisuga	Cow	0	0	1	0	1
	Sheep	0	0	0	0	0
C. spinosus	Cow	0	0	2	0	2
	Sheep	0	0	0	0	0
C. stellifer	Cow	21	25	26	9	81
	Sheep	10	13	34	1	58
C. variipennis	Cow	0	0	0	1	1
	Sheep	1	0	7	0	8
C. venustus	Cow	0	0	0	2	2
	Sheep	0	0	0	0	0

TABLE B-6 *Culicoides* collected in bait traps at sunset minus 30 minutes (SS-30), sunset (SS), sunset plus 30 minutes (SS+30), and sunset plus 60 minutes (SS+60), at the Ridge and Valley Province, in Virginia, 1979.

Species	Trap	SS-30	SS	SS+30	SS+60	Total
C. biguttatus	Cow	0	0	1	0	1
	Sheep	0	0	0	0	0
C. piliferus	Cow	0	0	1	0	1
	Sheep	0	0	0	0	0
C. spinosus	Cow	0	0	2	0	2
	Sheep	1	0	0	0	1
C. stellifer	Cow	4	0	3	0	7
	Sheep	0	0	0	0	0
C. travisi	Cow	0	1	0	0	1
	Sheep	0	0	0	0	0
C. variipennis	Cow	0	0	1	0	1
	Sheep	0	0	0	0	0
C. venustus	Cow	0	0	1	0	1
	Sheep	0	0	0	0	0

Appendix C: <u>Culicoides</u> <u>variipennis</u> collected from the 3 blacklight traps used in the comparison of parous rates in 1980 Saltville, VA.

TABLE C-1. *Culicoides variipennis* collected in the Marsh blacklight trap used in the comparison of parous rates between blacklight traps in 1980, Saltville VA.

Date	Nulliparous	Gravid	Parous	Total
June 16	5	93	93	191
17	84	604	112	800
30	66	1479	110	1655
July 1	66	1479	110	1655
16	208	386	126	720
17	60	653	105	818
18	24	650	82	756
19	24	560	68	652
20	77	843	109	1029
21	130	1125	150	1405
22	128	632	184	944
23	126	138	218	482
24	25	685	35	745
25	33	254	28	315
27	90	785	73	948
30	14	240	46	300
31	61	254	193	508
Aug. 1	70	695	290	1055
2	72	548	140	760
3	70	775	240	1085
4	55	333	28	416

TABLE C-1 cont.

Date	Nulliparous	Gravid	Parous	Total
Aug. 5	101	631	73	805
19	73	990	68	1131
21	86	935	103	1124
23	3	59	5	67
24	4	204	7	215
25	5	198	13	216
26	10	57	16	83
27	13	86	83	182
28	34	106	110	250
30	30	76	74	180
31	67	162	100	329
Sept. 1	119	357	477	953
2	12	397	397	806
3	143	248	143	534
4	167	770	14	951
5	65	365	153	583
6	14	64	88	166
9	30	192	199	421
Total	2464	19108	4663	26235

TABLE C-2. *Culicoides variipennis* collected in the Totten blacklight trap used in the comparision of parous rates between blacklight traps in 1980, Saltville VA.

Date	Nulliparous	Gravid	Parous	Total
June 16	1	3	8	12
17	6	24	20	50
30	0	11	6	17
July 1	13	35	35	83
16	33	16	10	59
17	21	13	23	57
18	3	5	4	12
19	10	7	15	32
20	15	7	10	32
21	7	8	7	22
22	57	7	17	81
23	4	2	2	8
24	4	1	1	6
25	4	1	9	14
27	15	8	4	27
30	9	2	4	15
31	4	8	10	22
Aug. 1	2	5	3	10
2	14	1	4	19
3	1	0	3	4
4	0	1	0	1

TABLE C-2 cont.

Date	Nulliparous	Gravid	Parous	Total
Aug. 5	104	6	70	180
19	10	3	11	24
21	6	1	18	25
23	0	0	0	0
24	1	1	2	4
25	4	1	1	6
26	7	1	4	12
27	7	1	1	9
28	6	1	12	19
30	2	0	2	4
31	3	3	4	10
Sept. 1	11	2	17	30
2	26	16	44	86
3	10	7	13	30
4	5	5	3	13
5	14	2	19	35
6	9	11	30	50
9	18	1	27	46
Total	466	227	473	1166

TABLE C-3. *Culicoides variipennis* collected in the Allison blacklight trap used in the comparision of parous rates between blacklight traps in 1980, Saltville VA.

Date	Nulliparous	Gravid	Parous	Total
June 16	0	0	2	2
17	2	2	7	11
30	4	4	0	8
July 1	13	8	4	25
16	81	14	13	108
17	48	14	28	90
18	19	6	14	39
19	79	13	26	118
20	85	4	37	126
21	57	3	17	77
22	32	2	4	38
23	11	7	10	28
24	4	0	11	15
25	15	0	10	25
27	10	17	6	33
30	18	4	14	36
31	94	0	20	114
Aug. 1	23	3	23	49
2	36	2	14	52
3	22	4	9	35
4	8	2	3	13

TABLE C-3. cont.

Date	Nulliparous	Gravid	Parous	Total
Aug. 5	2	0	1	3
19	28	2	6	36
21	9	11	15	35
23	5	3	3	11
24	7	8	6	21
25	58	2	9	69
26	69	3	7	79
27	11	3	11	25
28	11	2	5	18
30	5	5	5	15
31	35	0	13	48
Sept. 1	47	7	17	71
2	43	38	25	106
3	13	5	19	37
4	23	7	11	41
5	35	7	19	61
6	11	4	5	20
9	9	3	4	16
Total	1082	219	453	1754

Appendix D: Weekly mean numbers of _Culicoides_ _variipennis_ collected in the Totten BLT (1979 & 1978), and the Marsh BLT (1980) in Saltville VA.

TABLE D-1. Weekly mean numbers of *Culicoides variipennis* collected in the Totten BLT in 1979, Saltville VA.

Date	Nulliparous	Gravid	Parous	Total
6/1 - 6/7	170	58	321	549
6/8 - 6/14	20	94	83	197
6/15 - 6/21	8	53	49	110
6/22 - 6/28	15	31	27	73
6/29 - 7/5	30	29	61	120
7/6 - 7/12	41	70	125	236
7/13 - 7/19	147	144	315	606
7/20 - 7/26	60	99	195	354
7/27 - 8/2	39	50	39	128
8/3 - 8/9	0	5	1	6
8/10 - 8/16	0	1	0	1
8/17 - 8/23	0	0	5	5
8/24 - 8/30	1	2	0	3
8/31 - 9/6	0	1	1	2
9/7 - 9/13	1	4	4	9
9/14 - 9/21	1	0	4	5
Total	533	641	1230	2404

TABLE D-2. Weekly mean numbers of *Culicoides variipennis* collected in the Totten BLT in 1980, Saltville VA.

Date	Nulliparous	Gravid	Parous	Total
5/26 - 6/1	9	12	28	49
6/2 - 6/8	18	20	50	88
6/9 - 6/15	16	14	81	111
6/16 - 6/22	2	9	7	18
6/23 - 6/29	22	32	29	83
6/30 - 7/6	43	33	56	132
7/7 - 7/13	58	22	45	125
7/14 - 7/20	37	12	29	78
7/21 - 7/27	13	5	6	24
7/28 - 8/3	9	4	6	19
8/4 - 8/10	36	16	58	110
8/11 - 8/17	44	12	27	83
8/18 - 8/24	6	2	9	17
8/25 - 8/31	5	1	4	10
9/1 - 9/7	13	7	20	40
Total	331	201	455	987

TABLE D-3. Weekly mean numbers of *Culicoides variipennis* collected in the Marsh BLT in 1980, Saltville VA.

Date	Nulliparous	Gravid	Parous	Total
5/26 - 6/1	9	238	80	327
6/2 - 6/8	30	230	103	363
6/9 - 6/15	14	382	139	535
6/16 - 6/22	14	291	36	341
6/23 - 6/29	18	402	37	457
6/30 - 7/6	66	1479	110	1655
7/7 - 7/13	96	446	92	634
7/14 - 7/20	116	565	140	821
7/21 - 7/27	87	598	104	789
7/28 - 8/3	56	510	146	712
8/4 - 8/10	75	373	80	528
8/11 - 8/17	174	609	254	1037
8/18 - 8/24	48	497	52	597
8/25 - 8/31	27	117	70	214
9/1 - 9/7	75	322	189	586
Total	905	7059	1632	9596

Seasonal abundance and host preference of *Culicoides* in Virginia; with emphasis on the ecology of *Culicoides variipennis* (Diptera: Ceratopogonidae)

by Robert Henry Zimmerman

Abstract

Trap type, time and site influenced the species composition, number collected and female age. Thirty species were collected in the BLTs and 17 in the bait traps. *Culicoides biguttatus*, *C. obsoletus*, *C. stellifer*, *C. variipennis*, and *C. venustus* were the most abundant species collected in both traps.

Eight new host records were reported in this study. The drop trap collected more midges than did the vacuum trap, and the cattle trap collected more midges than did the sheep trap.

Culicoides stellifer began host-seeking activity at SS-30 in bait traps and continued to be collected in the blacklight trap the remainder of the night. Earlier in the evening, nulliparous females were collected in higher percentages than parous females. Time of flight for *C. stellifer* was delayed when the temperature was above 25°C.

Culicoides biguttatus and *C. variipennis* peaked at SS+30 in the bait traps but continued to be collected in the BLTs after dark. *C. venustus* peaked in the bait traps at SS+60 and continued to be collected

in the BLTs after dark. The parous rate of *C. variipennis* was higher in the cow trap than the sheep trap at the CP site. This was also true for *C. stellifer* at the PP site.

A morphometric study of *C. variipennis* indicated that in Virginia using the present taxonomic characters that only one species is present.

Culicoides variipennis males swarmed 15-20 min before sunset and 99% of the females that entered the swarm were nulliparous. One mating pair dropped to the ground every 10 sec and remain *in copula* from 30-120 sec. The time spend on the ground by mating pairs significantly increased successful insemination ($p<0.01$).

Dispersal of *C. variipennis* was at least 0.89 Km. More midges were collected at 1.83 m and 3.05 m than at 0.61 m. When hosts were near the sticky traps more midges were collected at 0.61 m and midges collected increased.

C. variipennis was collected at least as high as 9.15 m and vertical movement occurred away from the breeding site. There was a positive correlation between numbers collected at different sites. Comparison tests showed that sticky traps, D-Vac, and bait traps collected similar percentages of parous individuals. The Marsh BLT collected a higher percentage of parous females than did the above sampling methods. Midge parous rates from the Marsh BLT were also higher than the parous rates from the Totten or Allison BLTs.

Gravid females were collected in higher percentages in the Marsh BLT (50%) than on the sticky traps (2%) or in the D-Vac (22%). Though the Marsh BLT was biased for parous and gravid females, other data indicated that nulliparous females moved away from the breeding site.