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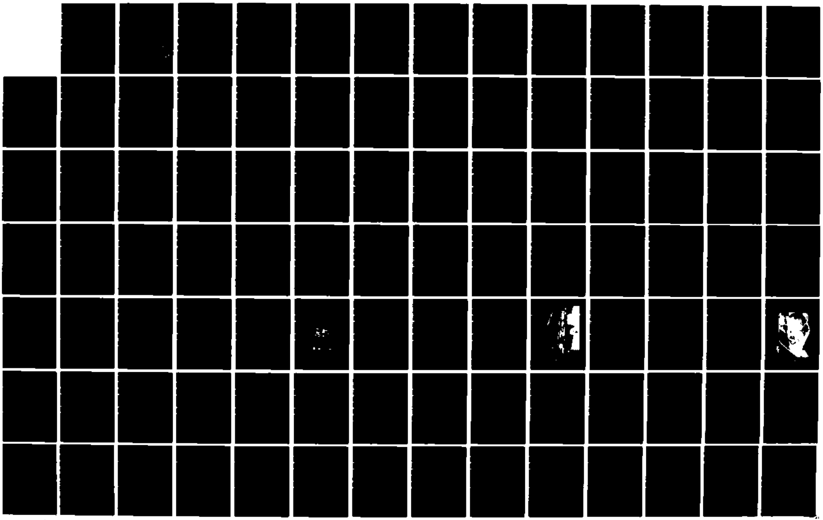

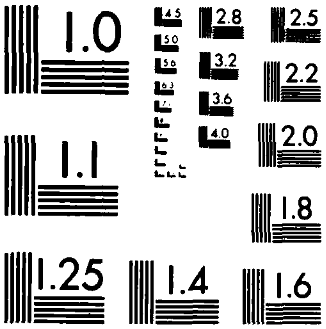

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DIEL AND SEASONAL ACTIVITIES
OF CULICOIDES SPP.
NEAR YANKEETOWN, FLORIDA

BY

THOMAS HENRY LILLIE

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1985

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER AFIT/CI/NR 85-47D	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Diel And Seasonal Activities of Culicoides SPP. Near Yankeetown, Florida		5. TYPE OF REPORT & PERIOD COVERED T~~HESIS~~/DISSERTATION
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Thomas Henry Lillie		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS AFIT STUDENT AT: University of Florida		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS AFIT/NR WPAFB OH 45433		12. REPORT DATE 1985
		13. NUMBER OF PAGES 145
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) UNCLASS
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

APPROVED FOR PUBLIC RELEASE; DISTRIBUTION UNLIMITED

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

APPROVED FOR PUBLIC RELEASE: IAW AFR 190-17

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19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

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DIEL AND SEASONAL ACTIVITIES OF *CULICOIDES* SPP. NEAR YANKEETOWN, FLORIDA

By

Thomas Henry Lillie, Capt., USAF, BSC

Seasonal occurrence and diel and lunar periodicities of adult *Culicoides mississippiensis* Hoffman, *C. furens* (Poey), *C. barbosai* Wirth and Blanton, and *C. floridensis* Beck were studied near Yankeetown, Levy County, Florida, from May 1983 to July 1984. A vehicle-mounted trap was used to collect 3,360 samples of individuals in flight and an aspirator was used to collect 1,120 samples of individuals attracted to a human host. Over 300,000 specimens were sorted and identified from these collections. *Culicoides mississippiensis* was the only species active throughout the year. It was also the most abundant, representing over 65% of the total catch. *Culicoides furens* and *C. barbosai* were collected in spring, summer, and fall while *C. floridensis* was primarily a summer species.

Diel and lunar periodicities varied seasonally for most species. Peaks of activity occurred most frequently during morning and evening twilight periods. The morning peak was greater of the two for *C. barbosai* while *C. furens* and *C. mississippiensis* were more abundant during evening twilight. Female activity persisted throughout the night on full moon but declined after sunset when the moon was in some other phase. Male activity gradually declined after evening twilight during all phases of the moon.

The dispersal of female *C. mississippiensis* was also examined by marking approximately 40,000 specimens with fluorescent dust and releasing them in a saltmarsh habitat. About 1.5% (567) of the marked individuals were recaptured. They traveled a mean distance of 2.0 km from the release point. The maximum distance traveled by an individual was 3.2 km.

University of Florida, 1985
Doctor of Philosophy in Medical Entomology

145 pages

(Abstract of Dissertation)

Dedicated to
Michelle, Carrie, and Carla

ACKNOWLEDGEMENTS

I am most grateful to the USAF for providing me with such an incomparable education opportunity through their civilian institutions program. I am also grateful to Dr. D.L. Shankland and the staff of the Department of Entomology and Nematology, University of Florida, and the personnel of the Insects Affecting Man and Animals Research Laboratory, USDA, Gainesville, Florida, for providing moral and material support for my research. The resources, time, and suggestions provided by these organizations are greatly appreciated.

I wish to express sincere gratitude to the members of my committee: Dr. E.C. Greiner, Associate Professor of Parasitology, for his critique of the research proposal and review of the dissertation; Dr. D.L. Kline, USDA, ARS, for his encouragement throughout the research project and for reviewing the dissertation; and a special note of thanks to my advisor, Dr. D.W. Hall, Department of Entomology and Nematology, for his guidance and encouragement, particularly during the qualifying exam. Dr. C.S. Barfield is acknowledged for his participation in the qualifying exam.

I thank A. Wilkening, K.F. Baldwin, and N. Pierce, USDA, ARS, for their technical assistance. The residents of Granny's Footprint Island and Allen's Hickory Island are also acknowledged for their friendship during the field project.

I owe my utmost gratitude to my wife, Michelle, and daughters, Carrie and Carla, who served as cheering section throughout my doctoral

program. Their understanding, encouragement, and love made the entire curriculum more enjoyable.

Finally, I would like to thank the persons who assisted me in my endeavors to attend graduate school: C.E. Thalken, Lieutenant Colonel, USAF; Dr. M.E. Dakin, University of Southwestern Louisiana; Dr. D.W. Fronk, Colorado State University; and my advisor for my M.S. degree, Dr. W.C. Marquardt, Colorado State University.

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Jones, 1957; Boorman, 1974; Jones and Schmidtmann, 1980). Three general mating strategies have been described for ceratopogonids: species that mate in swarms near the host; species that mate in swarms away from the host; and species that do not mate in swarms (Glukhova and Dubrovskaya, 1974; Kettle, 1977). Culicoides brevitarsis will form swarms either in the presence or absence of the host but swarms are larger when the host is present (Campbell and Kettle, 1979). Culicoides variipennis will mate without swarming but swarms have been observed away from the host and described in detail (Downes, 1978a; Zimmerman et al., 1982). The swarming behavior of C. variipennis is similar to that of C. nubeculosus Meigen (Downes, 1955). Culicoides melleus is a species that does not form mating swarms (Linley and Adams, 1972).

In species that swarm, the swarm is formed by males. Females fly into a swarm and a mated pair exit in copula. The ratio of females to males may be as low as 1:167 (Zimmerman et al., 1982). A typical swarm is usually formed over some type of marker (Nielsen and Greve, 1950) which may be an area of high contrast such as grass clumps surrounded by barren soil (Zimmerman et al., 1982). The males tend to face into the wind and oscillate upwind and downwind across the marker (Downes, 1969). The antennal plumes on the male are held in the erect position (Downes, 1955) to sense the wing beat frequency of the female (Roth, 1948). However, auditory recognition may not be the only cue for mate selection. Kremer et al. (1979) have detected a highly volatile material that is released by female C. nubeculosus to attract males. The pheromone also stimulates copulation. A mating pheromone has been detected in C. melleus as well but it is a non-volatile contact pheromone (Linley and of rainfall in coastal North Carolina (Kline and Axtell, 1976). In Florida, the emergence was greatly reduced during cold periods but increased during brief warm spells (Linley et al., 1970a). When the diel emergence of C. furens was examined, the majority of adults (97%) emerged during daylight hours and no emergence occurred between 1900 and 0300 h (Linley, 1966a).

In most instances the pattern of emergence is seasonal but strongly rhythmic in response to the moon or sunlight. Culicoides variipennis has a bimodal rhythmic emergence pattern. One group of both sexes emerge in equal numbers about 4 h after sunrise and the other group, which contains more males, emerge in the hour following sunset (Barnard, 1980a). For L. becquaerti, 98% of the individuals emerge between 0300 and 1100 h while no adults emerge from 1900 to 0300 h (Linley, 1968b). Kelson et al. (1980) showed that the emergence of C. occidentalis Wirth and Jones is seasonal but during a given season it peaks when the moon is full. Lunar periodicity has also been noted for C. peliliouensis Tokunaga in mangrove swamps of the Palau Islands (Tokunaga and Esaki, 1936).

Barnard and Jones (1980a) used data from larval population surveys and adult emergence studies to determine that C. variipennis is multivoltine in northeastern Colorado. This species had 7 generations, of which 1 was the overwintering population, during 12 months of observation. Additional reports of voltinism are available from laboratory studies. Under optimum conditions, the ceratopogonids studied have been multivoltine (Jones, 1966).

Mating Behavior

Mating usually occurs in flight but some species have been shown to mate in confined spaces where flight is not possible (Downes, 1955;

and Charnov (1978) have used computer simulation to show that natural selection could favor seasonal shifts in the sex ratio.

The immatures are naturally subjected to biological factors that affect their survival. Wirth (1977) covered most of them in a review of the pathogens and parasites of biting midges. Mermithid nematodes have been removed from late instar larvae (Glukhova, 1967; Mullens and Rutz, 1982) and rarely from pupae (Rubstov, 1974). Microsporidia have also been identified from the larvae of biting midges (Chapman et al., 1968; Kline et al., 1985). Predation is another source of mortality in the immature stages. A variety of predatory insects, including other ceratopogonids, attack the larvae and pupae but few cases have been published (Chan and LeRoux, 1967; Rieb et al., 1980).

Adult Emergence

The emergence of ceratopogonid adults has been assessed by using several trap designs (Dove et al., 1932; Williams, 1955; Breeland, 1960; Corbet, 1965; Davies, 1966; Braverman, 1970). The major characteristics of all traps are a lower section that covers the pupal habitat and an upper section for collecting emerged adults. The traps have been used either to examine an area for the occurrence of ceratopogonids or to monitor the time and frequency of emergence.

Corbet (1964) defines 4 basic temporal patterns of emergence in insects: continuous, rhythmic, sporadic, and seasonal. Tropical species appear to have a continuous pattern but periodic seasonal peaks occur (Davies and Giglioli, 1977). The seasonal peaks of C. furens were related to fluctuations in the water level in the breeding sites. This same species has a more distinct seasonal pattern in a temperate climate. The frequency of adult emergence was strongly correlated with the amount

Culicoides larvae by several other workers (Koch and Axtell, 1977; 1978; Mullens and Schmidtmann, 1981; Linley, 1981a). Linley (1966a, 1968a, 1969) used nematodes and oatmeal, alone and mixed together, to rear *C. furens*. He concluded that *C. furens* larvae are omnivorous (Linley, 1966a).

Larvae develop to the obtect pupal stage in 4 instars (Barnard and Jones, 1980a). In some species, pupation does not occur until after the larvae have overwintered (Jones, 1967a; Rowley, 1967). Larvae tend to move into less saturated areas, usually at the perimeter of the breeding site, when ready to pupate. Thus, the greatest concentration of pupae can be found on the margin of streams or ponds for species such as *C. hieroglyphicus* Malloch (Jones, 1961a). Even the larvae of *C. denningi*, a river-breeding species, move to the shore to pupate (Fredeen, 1969). Linley (1966a) recorded detailed observations of pupal formation in *C. furens*. Pupation could not occur when the larvae were completely below water; however, once formed, the pupae were capable of floating (Linley, 1966a). In contrast, *C. melleus* larvae are able to pupate underwater and the pupae do not float. The inability to float is not detrimental because the pupae of *C. melleus* can survive complete submersion for 4 days (Linley and Adams, 1972).

An unusual feature of *C. melleus* is that seasonal changes occur in the sex ratio of the pupae while that of the larvae is 1:1. Linley and Mook (1978) concluded that the seasonal shift, which is biased towards predominance of males, was most likely due to differential mortality of unknown origin in the larval stage. Reports on other species indicate that the sex ratio deviates little from 1:1 (Kettle, 1955), but Werren the larvae are aspirated 10-15 at a time into a disposable pipet (Barnard and Jones, 1980a). The inverted funnel would concentrate the larvae and simplify the process.

Reports of food requirements during larval development vary greatly. Most aquatic species with prognathous heads are carnivorous while those with hypognathous heads are herbivorous (Peterson, 1979). Species in the genus *Palpomyia* feed on larvae of aquatic insects including those of their own species (Grogan and Wirth, 1979). Cannibalism has been reported for species in the genus *Culicoides* as well (Becker, 1958); however, such behavior may be the result of conditions under which the larvae are maintained (Kettle, 1977). Direct examination of the gut contents and organisms in the habitat of *L. spinosifrons* indicates that individuals feed on microorganisms and decaying organic material (Laurence and Mathias, 1972). Such a diet was also reported for 3 tree hole *Culicoides* (Foote and Pratt, 1954) and larvae of other ceratopogonid genera (Weerekoon, 1953).

Other reports of larval diet were obtained when developing laboratory colonization techniques (Jones, 1966). Some workers have used a mixture of materials such as loam, barley, and water (Jobling, 1953); yeast, soil, and charcoal (Megahed, 1956); soil, fresh cow manure, and yeast (Jones, 1957); and leaf mold soaked in distilled water (Hair and Turner, 1966). Jones et al. (1969) use a mixture of commercial products as a larval medium for *C. variipennis*. Kettle et al. (1975) reported that they observed 12 species of *Culicoides* feeding on small nematodes in the laboratory. Linley (1979a) developed a technique for culturing nematodes and other organisms for use as food for predaceous *Culicoides* larvae. Nematodes, usually *Panagrellus redivivus* (L.), were also fed to

Jamnback and Wall, 1958; Wall and Doane, 1965), but Linley and Adams (1972) showed that the larvae are virtually confined to the region between the limits of high and low tides. Linley and Adams (1972) also noted diel changes in the vertical distribution of _C. melleus_ larvae between depths of 0 and 5 cm, probably in response to heat and light.

The method used to find and collect larvae depends on the type of habitat. Usually a portion of the substrate is moved into the laboratory and the immatures are reared to the adult stage. A shovel or posthole digger is often used to sample for mud-inhabiting species (Kline et al., 1981b) but a variable depth sampler was designed specifically for collecting _C. melleus_ larvae (Linley and Adams, 1972). Once the samples are obtained, different methods are available for extracting the larvae. Soil-inhabiting _Culicoides_ spp. have been removed from saltmarsh samples by using sieve/flotation (Kettle and Lawson, 1952; Wirth, 1952b; Kettle et al., 1956), sand flotation (Bidlingmayer, 1957; Williams, 1960), direct flotation (Linley and Adams, 1972), and Berlese funnels (Jamnback and Wirth, 1963). Kline et al. (1975) compared these methods and found that sieve/flotation was the best method for obtaining _C. hollensis_ (Melander and Brues) but sand flotation was the best method when _C. furens_ (Poey) was required.

An agar technique (Kline et al., 1981b) and an inverted funnel procedure (Boreham, 1981) were later found to be cleaner, less variable, and more rapid when compared with sand flotation. The two new methods have yet to be directly compared. The inverted funnel procedure may be more advantageous when larvae develop in a substrate other than saltmarsh soil such as _C. variipennis_ which develop in mud on pond margins. Mud samples containing _C. variipennis_ larvae are simply diluted in water and in typical aquatic habitats such as shallow streams (Jones, 1965; Grogan and Wirth, 1979), tree-holes (Wirth and Jones, 1956; Smith and Varnell, 1967), leaf axils (Means, 1973), and saltmarshes (Atchley and Hull, 1936).

The larvae usually remain within the upper 30 mm of the substrate in which they develop and the water is usually shallow (Kettle, 1977). Culicoides denningi Foote and Pratt, however, has been collected from river beds in water as deep as 10 m although the majority of the larvae were found on shore along the margin of the water (Fredeen, 1969). Barnard and Jones (1980a) conducted a thorough study of the ecology of immature C. variipennis Coquillett and found that most larvae remain in the upper 10 mm of mud. Prior to their work, several workers had noted that the larvae of various species have a horizontal stratification relative to the shoreline (Jones, 1961a; Hair et al., 1966; Rowley, 1967; Kardatzke and Rowley, 1971). Barnard and Jones (1980a) demonstrated quantitatively that such a stratification exists and concluded that the larvae were most abundant in mud 7 cm below the shoreline of ponds in Colorado. Isaev (1974) also found a horizontal stratification for larvae of C. odibilis Austen in the Soviet Union. He demonstrated that the stratification varied seasonally. The larvae were concentrated slightly above the shoreline in the spring, moved to the water's edge in the summer, and migrated underwater in the fall. The rate of migration was influenced by the temperature of the water.

The shoreline is difficult to use as a reference point when studying saltmarsh or beach inhabiting species because of the changing tides. Several workers collected C. melleus (Coquillett) larvae in intertidal sand in situations where wave action is minimal (Jamnback et al., 1958;

(Williams, 1951), but most reports are based on laboratory investigations where the temperature and other conditions varied little from a constant setting. Davis et al. (1983b) showed that the development time of *C. mississippiensis* Hoffman eggs was inversely related to temperature. At 10°C the eggs hatched in an average time of 20 days while at 30°C only 3 days were required. Linley (1965a) studied *Leptoconops becquaerti* (Kieffer) and also showed an inverse relationship between temperature and duration of the egg stage.

Most attempts at colonization have shown that the eggs hatch in 2-4 days (Megahed, 1956; Jones, 1960; Linley, 1965b; Linley, 1968a; Mullens and Schmidtmann, 1981). *Leptoconops spinosifrons* (Carter) is an exception to this generalization; the egg stage lasts 12 days at 30°C and may extend for six months at 95% relative humidity (Kettle, 1977). Another exception is *C. vexans* (Staeger); the eggs enter diapause and hatching is delayed for several months (Jobling, 1953).

The larvae lack spiracles (apneustic) and thus rely on cutaneous respiration. They are generally small (<10 mm) and occur in habitats which vary from aquatic to terrestrial (Peterson, 1979). The only requirements for survival are air, food, and moisture. *Forcipomyia* spp. have been collected under bark and on wet or damp wood (Wirth et al., 1977). Species of the genus *Palpomyia* inhabit pond algae and feed on chironomid larvae or larvae of phytophagous ceratopogonids (Chan and LeRoux, 1967). Other species, primarily in the genus *Culicoides*, have been found in rotting cacti (Jones, 1962), human sewage (Wirth and Bottimer, 1956; Jones, 1959), cocoa pods (Williams, 1964; 1966), dung (Kettle and Lawson, 1952; Neville, 1968), and on the surface of damp rocks (Dipeolu and Ogunrinade, 1976). The majority of species are found

Ceratopogoninae (formerly Heleinae) (Wirth et al., 1974). The largest subfamily is the Ceratopogoninae.

The differentiation between species is based on antennae, wing structure, reproductive organs, and coloration on the wings, body, and appendages (Blanton and Wirth, 1979). In 1974, there were 3,870 catalogued species of ceratopogonids in the world (Wirth et al., 1974). The current number probably exceeds 4,000 because contributions are being made periodically. From 1962 to 1974 the number of species in the genus Culicoides alone increased from 800 (Arnaud and Wirth, 1964) to 924 (Wirth et al., 1974) and by 1979 over 1,000 had been described (Blanton and Wirth, 1979). There are 60 genera recognized in the family; the genus Culicoides contains the most species. When compared with other genera, the adult Culicoides have small tarsal claws without hairy empodia, large thoracic humeral pits, macrotrichia on the wings, 2 radial wing cells of about equal length, and the costa usually extends slightly past midlength of the wing (Johannsen, 1952).

In North America there are approximately 400 species of ceratopogonids of which 137 are in the genus Culicoides (Downes, 1978c). Forty-seven species of Culicoides are represented in the state of Florida (Blanton and Wirth, 1979). An excellent account of the distribution, biology, and habits of Culicoides species in Florida was prepared by Blanton and Wirth (1979).

Biology and Bionomics of the Immature Stages

The ceratopogonids are holometabolous insects that develop in a variety of habitats. The eggs may be oviposited individually or in a gelatinous mass, but only on rare occasions have the eggs been recovered in nature (Kettle, 1977). The duration of the egg stage is usually short

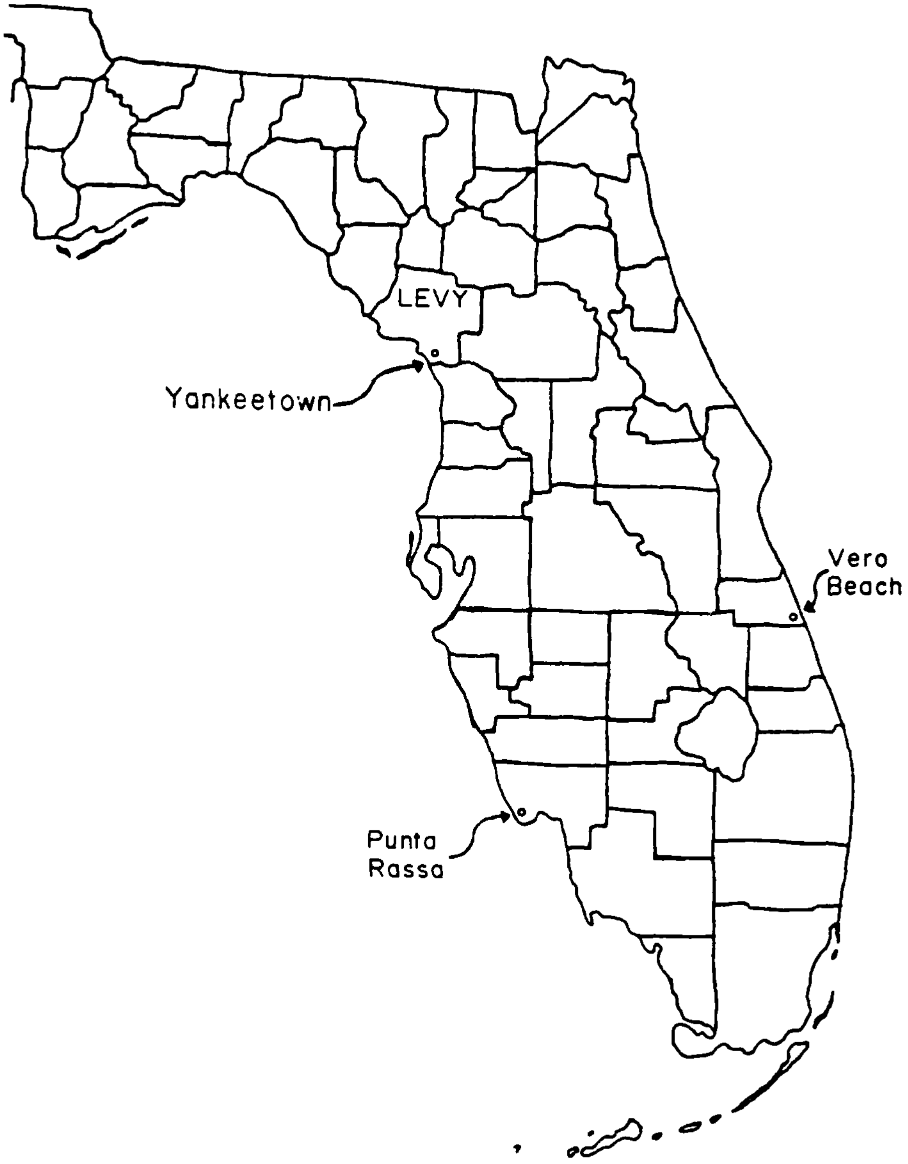

Figure 1. Location of research site near Yankeetown, Levy County, Florida, and research sites used by other workers.

studies along the Gulf Coast in Levy County near Yankeetown (Figure 1). Topics which require further study in the Yankeetown area include the diel host-seeking activity, seasonal periodicity, diel flight activity, lunar periodicity, and flight range of biting midges.

Literature Review

Classification

The biting midges are small (1-4 mm) nematocerous flies. The group was initially classified by Meigen in 1803 as the genus Ceratopogon in the family Chironomidae but in 1917 Malloch gave them family status (Kwan and Morrison, 1974). The family was occasionally referred to as Heleidae but the International Commission for Zoological Nomenclature classifies them in the family Ceratopogonidae (Freeman, 1973). They differ from the Chironomidae in having complete mouthparts and wings with forked media (Battle and Turner, 1971; Wirth et al., 1977). When differentiated from other Diptera, the ceratopogonids have long (usually 15 segmented) antennae, no ocelli, lack of a median furrow or keel on the scutellum, well developed mouthparts fitted for piercing and blood sucking, and the costal wing vein ending before the wing tip (Blanton and Wirth, 1979; Borror et al., 1976).

Further classification of the Ceratopogonidae has been controversial because some workers used larval characteristics to separate groups while others used adult characteristics (Wirth et al., 1974). Wirth (1952a) attempted to resolve the issue by using previously proposed nomenclaturally formalized subfamily and tribal names based on priority. The subfamily classification is currently being used and contains the following: Leptoconopinae, Forcipomyiinae, Dasyheleinae, and

CHAPTER ONE
BACKGROUND INFORMATION

Introduction

Biting midges (Diptera: Ceratopogonidae) have hampered land development in many coastal areas of the Gulf and South Atlantic states (Dove et al., 1932). Much of the coast of Florida remains unsuitable for tourist activities because of the annoyance caused by these small biting midges. Their biting habits have even had an impact on tourism in the Bahamas and Caribbean area (Linley and Davies, 1971). The midges are so annoying that U.S. Marine Corps personnel commonly refer to them as "flying teeth" (Roberts and Kline, 1980). Other common names include no-see-ums, punkies, gnats, sand flies, and sandfleas (Blanton and Wirth, 1979). The most widely accepted common name is biting midges which separates them from the phlebotomine sand flies (Diptera: Psychodidae) and the non-biting midges (Diptera: Chironomidae) (Freeman, 1973).

The biting midges have been studied most extensively in Florida because of the severity of the problem. The long coastline, extensive beaches, and tidal waterways serve as ideal areas for their development. State laboratories at Vero Beach and Panama City, and the U.S. Department of Agriculture (USDA), Insects Affecting Man and Animals Laboratory at Gainesville have been involved in the studies. Information on species composition, seasonal occurrence, and geographic distribution has been gathered throughout the state, but most of the ecological studies have been restricted to the Atlantic Coast. In 1977 the USDA initiated field twilight periods. The morning peak was greater of the two for *C. barbosai* while *C. furens* and *C. mississippiensis* were more abundant during evening twilight. Female activity persisted throughout the night on full moon but declined after sunset when the moon was in some other phase. Male activity gradually declined after evening twilight during all phases of the moon.

The dispersal of female *C. mississippiensis* was also examined by marking approximately 40,000 specimens with fluorescent dust and releasing them in a saltmarsh habitat. About 1.5% (567) of the marked individuals were recaptured. They traveled a mean distance of 2.0 km from the release point. The maximum distance traveled by an individual was 3.2 km.

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

DIEL AND SEASONAL ACTIVITIES OF *CULICOIDES* SPP. NEAR YANKEETOWN, FLORIDA

By

Thomas Henry Lillie

May, 1985

Chairman: D.W. Hall
Major Department: Entomology and Nematology

Seasonal occurrence and diel and lunar periodicities of adult *Culicoides mississippiensis* Hoffman, *C. furens* (Poey), *C. barbosai* Wirth and Blanton, and *C. floridensis* Beck were studied near Yankeetown, Levy County, Florida. From May 1983 to July 1984, a vehicle-mounted trap was used to collect 3,360 samples of individuals in flight and an aspirator was used to collect 1,120 samples of individuals attracted to a human host. Over 300,000 specimens were sorted and identified from these collections. *Culicoides mississippiensis* was the only species active throughout the year. It was also the most abundant, representing over 65% of the total catch. *Culicoides furens* and *C. barbosai* were collected in spring, summer, and fall while *C. floridensis* was primarily a summer species.

Diel and lunar periodicities varied seasonally for most species. Peaks of activity occurred most frequently during morning and evening

Carlson, 1978). The difference in chemical properties with that in C. nubeculosus is not surprising because C. melleus does not mate in swarms.

The sexual behavior of C. melleus has been investigated in great detail. Males outnumber females at emergence (Linley and Hinds, 1974). Mating occurs on the ground, probably near the breeding site soon after emergence (Hinds and Linley, 1974). In laboratory studies, wingless females elicit less male response than dead females even though mating does not occur in flight (Linley and Carlson, 1983). Apparently, tarsal/wing contact is a necessary part of the mating process.

Virgin female C. melleus are receptive of the male soon after emergence but become less so with age or upon a second attempt at mating (Linley and Adams, 1974). A sexually experienced female will resist mating by kicking the male. The effect of such kicking is dependent upon the major grooming spines and combs of the tibia (Linley and Cheng, 1974). Resistance by the female reduces the number of sperm transferred (Linley and Hinds, 1975a, b). Male sensitivity to the kicking declines with passage of time since his last successful copulation (Linley and Mook, 1975). The termination of copulation is described in detail by Linley (1975a). The female will remove the male by kicking and the spermatophore will remain attached to either sex, usually the male.

Additional studies of reproduction in C. melleus have investigated ejaculation, spermatophore formation, and sperm motility. Spermatophore formation and the passage of sperm through the ejaculatory ducts occurs in the first 2 min of coitus (Linley and Adams, 1971; Linley, 1981b). The transfer of spermatozoa and male accessory fluids to the spermathecae accounts for the remaining 8 min of copulation (Linley, 1981c). The spermathecae create an incoming current by fluid absorption to accomplish sperm transfer. This current is essential since sperm motility (Linley, 1979b) contributes little to spermathecal filling in C. melleus or other lower Diptera (Linley and Simmons, 1981). In some instances, multiple copulations by a single female take place even though the female resists such encounters. In such cases, complete mixing of sperm occurs in the female storage organs before oviposition (Linley, 1975b).

Mating is not required for the production of viable eggs in all species. Culicoides bermudensis Williams (Williams, 1961) and C. bambusicola Lutz (Lee, 1968) are able to reproduce parthenogenically. The former may even be capable of multiplying during the immature stage (paedogenesis) (Kline and Axtell, 1977).

Adult Feeding Habits

The Ceratopogonidae have a broad range of feeding habits which probably exceeds the range of any other family of Diptera (Downes, 1971). The general types of feeding behavior are predaceous, vegetarian, and ectoparasitic but the diversity is great within each category.

Palpomyia spp. prey on insects as adults including males of their own species (Downes, 1978b). The female seeks out an insect swarm, flies into the swarm, and exits with a meal. If the swarm happens to contain males of her own species then simultaneous mating by the male and feeding upon him by the female will result. An empty male cuticle remains when the process is complete.

The vegetarian forms feed on pollen or other flower parts. Atrichopogon glaber Macfie vists flowers of the rubber tree in Brazil and may function in its pollination (Wirth, 1956a). Forcipomyia spp. feed on certain nodules on the petals of the cocoa plant but also play an important role in pollinating the plant (Leston, 1970). In both cases the female is involved in the process and the feeding behavior of the male is not reported.

The most common means of feeding is ectoparasitic. Species in this category will feed on invertebrates as well as warm- or cold-blooded vertebrates. *Forcipomyia* spp. have been observed feeding on caterpillars, stick insects, dragonflies, and lacewings (Wirth, 1956b). On insects with large wing veins such as dragonflies, the female biting midge will pierce a wing vein and suck the body fluid. A group of *Atrichopogon* spp. are known to attack blister beetles (Wirth, 1956c). There is even a group of *Culicoides* spp. that obtain nourishment by piercing the abdomens of recently engorged mosquitoes (Wirth and Hubert, 1959). Such behavior could contribute toward the transmission of human, animal, or insect pathogens from one vector to another.

The genera *Culicoides*, *Leptoconops*, *Forcipomyia*, and *Austroconops* contain the only vertebrate feeders in the family Ceratopogonidae. Some species feed only on cold-blooded vertebrates such as *C. testudinalis* which feeds on turtles (Wirth and Hubert, 1962) while others attack only birds (Bennett, 1960) or mammals (Tempelis and Nelson, 1971). *Culicoides piliferus* Root and Hoffman has been observed to feed on both birds and mammals (Humphreys and Turner, 1973).

The range of hosts that a given species feeds upon is important for epidemiological reasons. When a specific vector-borne disease is being studied, susceptible animals may be used as bait to find potential vectors. Such was the case when *C. variipennis* was collected from horses during an epidemic of Venezuelan equine encephalitis in 1971 (Jones et al., 1972; 1977). *Culicoides variipennis* was also collected from cattle and sheep during studies of the epidemiology of bluetongue virus in Colorado (Jones, 1961b; Jones and Akey, 1977).

In other instances a tethered animal or an animal-baited trap may be used simply to learn more about the biology of ceratopogonids in an area. Koch and Axtell (1979a) determined that *C. furens* and *C. hollensis* will feed on a variety of hosts in North Carolina. The frequency of response of the 2 species to trapped animals was related more to the size of the animal than to the kind of animal. Tanner and Turner (1974) came to the same conclusion in Virginia but added that the height of the host above ground level was also an important factor regulating host preference. A broader range of hosts was used by Hair and Turner (1968). Fourteen animal species were used including man. Most ceratopogonids collected showed no specific host preference but *C. hollensis* was attracted to man only. Once again, the attraction of *C. furens* was more related to the size of the animal than to the kind of animal.

A more recent study in Virginia concentrated on the attraction of ceratopogonids to livestock (Zimmerman and Turner, 1983). Cattle and sheep were used and the most abundant species were *C. variipennis*, *C. biguttatus* (Coquillett), *C. stellifer* (Coquillett), and *C. venustus* Hoffman. Livestock were also used to investigate the host-seeking activity of species in New York state (Schmidtmann et al., 1980b; 1981). A uniform group of species was collected from pastured calves at several different trapping locations. The species were *C. obsoletus* (Meigen), *C. stellifer*, *C. venustus*, *C. variipennis*, and *C. spinosus*. Such results warrant further study of the interaction of these species as they compete for the same resource.

More specific studies of the ecology of biting midges have addressed the location where adults prefer to feed on a host as well as the daily and seasonal biting habits. *Culicoides barbosai* prefer to feed on the arm of a human host during daylight hours but at night they have a preference for the leg (Kettle and Linley, 1969a). *Culicoides furens* were more abundant on the leg regardless of the time of day (Kettle and Linley, 1969b). The preference for legs occurred even when the host was sitting on the ground and it was more pronounced at night (Kettle, 1969a, b). Both species attacked in greatest numbers at dusk and dawn. Less activity occurred during the night and practically no activity was observed during the day. *Leptoconops becquaerti*, which bites only during the day, also showed a preference for legs when compared with arms (Kettle and Linley, 1967a, b). All three species had a preference for a particular human host when 4 individuals were exposed simultaneously. This phenomenon is probably related to some type of chemical cue that varies between individuals of the host species.

The diel biting activity has been looked at for other species. In California, *C. variipennis* attacks hosts at dusk and dawn while *L. knowltoni* Clastrier and Wirth has 2 diurnal peaks (Foulk, 1969). Nathan (1981) found that *C. phlebotomus* (Williston) seeks hosts during the day but the majority of biting occurs during crepuscular periods. In Wisconsin, *C. guttipennis* (Coquillett) is most active at dusk and dawn (Scholl et al., 1979). The general pattern of crepuscular biting activity does not hold true for all *Culicoides* spp. *Culicoides paraensis* Goeldi is only active during the day in Wisconsin (Scholl et al., 1979) and *C. venustus* Hoffman is most abundant during the night in New York (Schmidtmann et al., 1980a).

The seasonal biting activity of a species has been determined by using animal-baited traps or human hosts for a duration of one year or more. Studies of such length are rare for blood-sucking ceratopogonids. Kettle and Linley (1967b) collected biting *L. becquaerti* females on a weekly basis in Jamaica and determined that the species is active throughout the year. Midge abundance was related to the periods of greatest rainfall. *Culicoides furens* and *C. barbosai* were also active during the entire year in Jamaica (Kettle, 1972). The biting rate of *C. furens* was maximal in September and minimal in March. *Culicoides barbosai* was most abundant in March-June. Seasonal changes in the host-seeking activity of *C. barbosai* were negatively correlated with mean sea level. The seasonal distribution for *Culicoides* spp. in Virginia was reported after collecting specimens in an animal-baited trap, but the trap was operated for only 4 months during 1970 (Tanner and Turner, 1975).

The biting habits of a species can also be influenced by meteorological conditions. Temperature thresholds definitely exist but wind speed and light intensity have the most noticeable effects. The biting activity of *C. furens* and *C. barbosai* practically ceases at 20°C (Kettle, 1969b). The attack rate of *L. knowltoni* was reduced by winds in excess of 8 km/h (Foulk, 1969). For *C. furens* and *C. barbosai*, a wind speed of ca. 10 km/h caused complete cessation of biting activity (Kettle, 1969b). *Leptoconops becquaerti* was much more tolerant of the wind while seeking a bloodmeal. It remained active in wind speeds of 10-15 km/h and biting did not cease until the wind velocity was in excess of 24 km/h (Kettle and Linley, 1967b). Studies of the interaction between feeding and light intensity were performed on *C. guttipennis* which is active during crepuscular and nocturnal periods. The greatest feeding activity occurred between 0.1 and 9.0 fc (Humphreys and Turner, 1971). These intensities correspond to full moon and dawn/dusk conditions, respectively.

Other studies regarding adult feeding habits have involved examining the blood of engorged specimens to determine its source. The small size of ceratopogonids sometimes hampers the process but current immunological techniques are quite sensitive. Antigen/antibody interactions are the basis for the tests. The precipitin test has been the most widely used method (Braverman et al., 1971; Tempelis and Nelson, 1971; Neville and Anderson, 1972; Walker and Boreham, 1976). Antiserum to the blood of a given species is produced in a different species. Highly specific antiserum can be obtained by using closely related species. The blood obtained from an engorged fly is then exposed to the antiserum. The formation of a precipitate (i.e., antibody/antigen reaction) is a positive reaction which identifies the source of the bloodmeal (Clark, 1980). A problem with the precipitin test is that only 4-5 tests can be conducted with a single specimen. The problem can be overcome by using hemagglutination inhibition or gel diffusion tests (Murray, 1970), but they require sophisticated laboratory equipment to perform. Boorman et al. (1977) have found that the latex agglutination test can be used to compare a single specimen with a variety of antisera and it shows promise for application in the field. This method involves the production of antiserum in the manner described above but then the antiserum is bound to latex beads. Agglutination will occur when the coated latex is exposed to the proper antibody (Clark, 1980).

The bloodmeal or other protein meal ingested by ectoparasitic and predaceous species is used to complete egg maturation. Only the females ingest a protein meal, but both sexes are known to ingest carbohydrate materials such as nectar or sugar. Adult longevity is greatly increased if a carbohydrate source is available (Jamnback, 1961; Linley, 1966b). The material is also essential for sustained flight by males in mating swarms (Downes, 1969). Soaked raisins or cotton soaked with a sucrose solution are used to feed individuals in the laboratory (Jones, 1966). Nectar of flowers, honey dew, extrafloral nectaries, and sap flowing from plant wounds are possible sources in nature (Downes, 1958).

The Ovarian Cycle

Like other insects, the ovaries of ceratopogonids consist of several ovarioles in which the oocytes differentiate and mature (Downes, 1971). More specifically, the ovaries are of the meroistic type. That is, each developing oocyte is accompanied by a set of nurse cells which nourish it during the early stages of development (Linley, 1965a).

Cocyte formation begins while the midges are larvae and progresses to a resting stage. Further development does not occur until the female of bloodsucking species ingests a bloodmeal. Linley (1965a, 1966b) determined that sufficient blood is ingested during a single feeding by _L. becquaerti_ and _C. furens_ for practically all resting oocytes to mature. In contrast with these species, the small bloodmeal of _C. barbosai_ will ultimately yield very few eggs or no eggs at all. This implies that _C. barbosai_ females probably require multiple feedings to complete a gonotrophic cycle with all ovarioles in complete harmony. The sequence of events following the bloodmeal is described in detail by Linley (1965a, 1966b).

The entire process of oocyte development beyond the resting stage through oviposition is called a gonotrophic cycle. Jones (1967b) observed during laboratory tests that *C. variipennis* females ingest a bloodmeal for every egg batch and may complete as many as 7 gonotrophic cycles. The number of cycles can also be determined by dissecting and examining the ovaries (Linley, 1965c). This technique, called physiological aging, was first developed by using mosquitoes (Beklemishev et al., 1959; Detinova, 1962). It is based on the fact that after oviposition a dilatation remains at the end of the ovariole once occupied by an egg. Each egg produced by an ovariole and oviposited will result in a dilatation. Thus 2 dilatations will be present if 2 gonotrophic cycles have been completed. It is not necessary to examine all ovarioles for the condition because harmonious development occurs in most species (Linley, 1965a; 1966b).

Linley (1965c) used the dissection technique to show that *C. furens* and *C. barbosai* complete at least 1 gonotrophic cycle while *L. becquaerti* completes at least 2. *Culicoides melleus* and *C. hollensis* were also shown to complete only a single gonotrophic cycle (Magnarelli, 1981). It is highly probable, however, that a second cycle occurs in these species because the first cycle is completed without a bloodmeal (autogenous) (Linley, 1983). The fact that they are known to ingest blood would indicate the start of a second gonotrophic cycle. Mullens and Schmidtmann (1982) found relics from as many as 3 cycles in *C. variipennis*.

Dissection is a very tedious process but it can be avoided if one is simply interested in separating individuals that have not oviposited (nulliparous) from individuals that have completed one or more

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Dissection is a very tedious process but it can be avoided if one is simply interested in separating individuals that have not oviposited (nulliparous) from individuals that have completed one or more gonotrophic cycles (parous). Dyce (1969) noted changes in the ventral abdominal pigmentation that persisted after oviposition. The condition occurred in anautogenous as well as autogenous species. Akey and Potter (1979) applied the technique to *C. variipennis* populations but also reported changes in the pigmentation of abdominal tergites (Potter and Akey, 1978). Akey (1981) found that the method can even be used on pinned specimens. Mullens and Schmidtmann (1982) compared the method of Dyce (1969) with that of Potter and Akey (1978) and found the former method to be more reliable for eastern strains of *C. variipennis*. When applied to other species, a change in tergal pigmentation was found to occur only in abdominal segment 2 of *C. furens* (Linley and Braverman, 1984).

As mentioned previously, some species are able to complete a gonotrophic cycle without ingesting blood. The nutritional requirements for egg development are provided by material ingested during the larval stage and stored primarily in the fat body (Downes, 1971). The oocytes sometimes develop beyond the typical resting stage before adult eclosion (Linley, 1982). In biting flies in general, autogeny ranges from a facultative condition such as in many *Culicoides* spp. (Linley, 1983) to an obligate condition such as in the northern strain of the mosquito *Wyeomyia smithii* (Coquillett) (Bradshaw, 1980). Nutrient reserves in ceratopogonids are usually sufficient for only 1 egg batch but *L. carteri* Hoffman is known to complete more than 1 gonotrophic cycle autogenously (Schmidtmann and Washino, 1982).

All species in the genus *Dasyhelea* appear to be autogenous for the first cycle (Downes, 1971). In other ceratopogonids, the expression of autogeny varies among individuals of the same species. The rate of autogeny in *C. furens* ranged from 0 to 91% for collections obtained from 6 breeding sites within a few miles of one another (Linley, 1966b). Likewise, *C. hollensis* obtained from North Carolina were autogenous (Koch and Axtell, 1978) but populations in South Carolina were all anautogenous (Henry and Adkins, 1973). It is unknown whether differences between populations are due to genetic or environmental factors.

Linley (1968c) reported that autogeny is associated with polymorphism for winglength in *L. becquaerti*. Smaller individuals are autogenous and larger individuals anautogenous. This relationship is opposite of that reported for the mosquito *Aedes togoi* Theobald (Laurence, 1964). Linley (1968c) concluded that autogeny and winglength polymorphism are under genetic rather than environmental control. Environmental factors are more likely to influence certain aspects of autogeny such as fecundity rather than the complete expression of autogeny. Seasonal changes occur in the fecundity of autogenous *C. furens* near Vero Beach, Florida (Linley et al., 1970b). Individuals emerging during cooler times of the year are larger and oviposit about twice as many eggs as individuals emerging during warmer periods. Fecundity in autogenous species can also be affected by nutritional factors in the larval habitat. Lang (1978) and Lillie and Nakasone (1982) showed that the fecundity of *Wy. smithii* is influenced by the protein content of the larval diet.

Oviposition occurs about 7-10 days after emergence in autogenous species but may occur in as little as 1-2 days in species with precocious autogeny (Linley, 1982). In anautogenous species a period of 3-12 days, which is inversely related to temperature, is required between completion of a bloodmeal and oviposition (Linley, 1965a; 1966b). The number of eggs produced during that time varies between species and within species. It can be affected by temperature, season, and larval density. *Culicoides melleus* females produce more eggs during cooler times of the year (Linley and Hinds, 1976). A maximum of 150 eggs/female reaches maturity in February while only 50 eggs/female mature in August. The number of eggs matured is inversely related to temperature in *C. barbosai*, *C. furens* (Linley, 1966b), and *L. becquaerti* (Linley, 1965a). At 25°C, *C. barbosai* produced the fewest eggs (10/female) and *L. becquaerti* produced the most eggs (85/female). The number of eggs matured was also affected by the larval density during the rearing of *C. variipennis* (Akey et al., 1978). Larvae reared under crowded conditions ultimately yielded adults that produced about half as many eggs as individuals derived from less crowded conditions. The study by Akey et al. (1978) also demonstrates that fecundity in an anautogenous species can be affected by larval rearing conditions.

Diel and Seasonal Flight Activity

The major reasons for flight in ceratopogonids and other biting flies are to find food, a mate and a suitable oviposition site. Diel and seasonal patterns of ceratopogonid attraction to a host have been discussed previously in this report. Patterns of flight activity away from a host have been studied for several species by using light traps or vehicle-mounted traps. The effectiveness of the 2 methods varies with respect to species and location. These and other collection methods will be discussed in a later section.

Light traps are widely used for sampling insect populations. Various models have been introduced over the years. The New Jersey trap (Mulhern, 1942) was one of the first designs and it was followed by a portable unit, the CDC trap (Sudia and Chamberlain, 1962). The traps have undergone several modifications to make them more attractive to insects (Service, 1970), better suited for use in remote locations (Driggers et al., 1980; Kardatzke et al., 1980), and more efficient for segregating diel collections (Mitchell, 1982). Other traps have been designed specifically for collecting ceratopogonids (McDonald, 1970; Lillie et al., 1979). The traps all take advantage of the attraction of insects to a light source.

Extensive studies of biting midges have been conducted by using light traps in Louisiana (Khalaf, 1966; 1967), Florida (Beck, 1952; 1958), and other areas along the Gulf of Mexico (Khalaf, 1969). The distribution and seasonal abundance of approximately 33 species are reported from these areas. The majority of species are active primarily from March to July.

Seasonal and diel periodicities have been determined in other areas by using suction traps that automatically cycle at preset intervals (Koch et al., 1977) or by manually changing the collection bag on light traps (Brenner and Wargo, 1984). The traps may be baited with CO_2 (dry ice) to attract host-seeking individuals (Nelson, 1965) and to make the traps useful during daylight hours. Studies of this nature typically show that bloodsucking ceratopogonids have a bimodal activity pattern with peaks at dawn and dusk. Culicoides furens and C. hollensis have this type of activity but C. furens is active at night as well, and C. hollensis is most active at sunrise (Koch and Axtell, 1979b). The dawn peak for C. barbosai is also greater than that observed at dusk (Kline and Roberts, 1982). The activity of L. torrens Townsend, L. foulki Clastrier and Wirth, and L. knowltoni is diurnally bimodal rather than crepuscular.

temperature of the substrate to increase. This could shorten the development time and bias the results. He provides a systematic method of randomly moving traps during a study to avoid such a problem.

The ground suction device or D-vac® is used less often than the other methods for collecting ceratopogonids. It is essentially a portable suction trap which is ideal for sampling various surfaces in the environment for the presence of adults. Bidlingmayer (1961) used the device to study C. furens in Florida. He found that males preferred to rest in the trees whereas females occurred in about equal numbers in the trees and on the ground. Males remained closer to the saltmarsh than females. Tanner and Turner (1975) used it for a study of Culicoides spp. in Virginia. Their results indicate that the D-vac® collected fewer specimens than light traps or animal-baited traps but the species diversity was greatest when the D-vac® was used. The increased diversity most likely occurred because the portability of the D-vac® allows it to be used over a broader area and range of habitats than the other 2 methods.

It is obvious from this review that the selection of a sampling device depends upon the objectives of the study for which it will be used. Each trap is best suited for a particular situation. Bidlingmayer (1974) compared most of these methods and concluded that the vehicle-mounted trap and the suction trap are less subject to environmental and meteorological influences. These two methods can, therefore, provide a less biased sample of airborne insect population levels.

changes in the flight activity of species that frequent open areas. The catch is clean, easy to sort, and the specimens can be used for virus isolation tests.

The sticky cylinder trap is another means of collecting specimens without the use of an attractant. A typical trap is composed of plastic sewer pipe coated with Tanglefoot® (Kline and Axtell, 1976). The color of the trap will affect the results. Black cylinders and red cylinders collect significantly more specimens than white (Castle, 1965). The trap is positioned a given height above ground level and any biting midges that intercept it are retained in the Tanglefoot®. Sticky traps are a reliable method of monitoring seasonal changes in adult ceratopogonid populations but the cleanup and sorting of specimens is tedious. Kline and Axtell (1976) found that this technique was more sensitive than light traps for detecting the beginning and end of the seasonal occurrence of C. furens and C. hollensis. The sticky trap is also less expensive to construct and operate than light and suction traps.

Emergence traps are easy to construct and are also more sensitive than light traps for detecting the commencement and termination of seasonal occurrence of a species (Kline and Axtell, 1976). Males are usually collected more frequently with this method than any other method previously discussed. The sex ratio of the sample is typically 1:1 but diel (Barnard, 1980a) and seasonal (Linley and Mook, 1978) changes occur in some species. Emergence traps are best suited for locating breeding sites and for comparing the productivity of different sites. They do not require a power source but the size of the area sampled is usually small and the specimens are often in poor condition (Kline and Axtell, 1976). Barnard (1980a) noted that the presence of the trap causes the areas had a reduced catch during full moon while those in wooded areas did not.

The effects of moonlight can be eliminated by removing the light source and operating the trap simply as a suction device but with a larger fan to increase the volume of air sampled. Results with this technique contradict the data from light trap collections. Mosquito activity increased during full moon (Bidlingmayer, 1967). The suction trap is best suited for studies of the diel and seasonal flight activities of a species. The collection is not biased towards host-seeking individuals since no attractant is used.

The vehicle-mounted trap also showed a positive correlation between moonlight and the number of biting midges collected (Nelson and Bellamy, 1971). This trap, like the suction trap, does not employ an attractant. It tends to collect more males than light traps and is not influenced by moonlight (Barnard, 1980b). The results could be misinterpreted, however, because the trap is operated over open roads where males of some species form swarms. Nathan (1981) observed swarms of C. phlebotomus being intercepted by a vehicle-mounted trap. Data from the diel trapping of this species are actually an account of male swarming times over the collection route rather than general flight activity of males over other areas. Thus, detailed observations should be recorded when operating this and other traps to accurately interpret the results.

Initial costs to construct the vehicle-mounted trap are not high compared to light traps but the operating cost can be prohibitive since the vehicle must be driven to use the trap. Vehicle maintenance, fuel, and pay for the operator must be considered when planning a study. When used, however, it is an excellent method for monitoring diel and seasonal standardized to reduce the number of extraneous factors. Results will vary with the species of host employed (Koch and Axtell, 1979a), the individuals involved (Kettle and Linley, 1967a), the part of the host sampled (Kettle, 1969a), and the location of the host (Tanner and Turner, 1974). Animal-baited traps also have many of these biases plus the collection can be influenced by the design of the trap. Aspirator or animal-baited trap techniques are best suited for instances where the host range or diel biting activity are being studied.

Light traps are commonly used for the collection and surveillance of a variety of insects. Collections from the same location over a long period of time can provide useful data about the seasonal trends of a given insect population. The number of specimens collected can be influenced by the color of the trap (Kohler and Fox, 1951), the color of the light (Gui et al., 1942), the brightness of the light (Barr et al., 1963), and the location of the trap (Bidlingmayer, 1967). The addition of attractants such as CO_2 (Nelson, 1965; Reeves, 1951) and animal extracts (Fallis and Smith, 1964) increases the number of individuals collected and allows the trap to function as a sampling device during daylight hours.

Moonlight has been shown to have an effect on light trap collections. The background light provided by the moon reduces the degree of contrast created by the light trap. Provost (1959) determined that this reduces the number of mosquitoes collected when the moon is full. Other workers have noted the same phenomenon for several types of insects (Bowden, 1973; 1981; Bowden and Church, 1973) including ceratopogonids (Kline and Axtell, 1976). The effect of moonlight can vary with trap location (Bidlingmayer, 1967). Light traps placed in open

In some instances, specimens from laboratory colonies have been used in an attempt to document the potential of biting midges as vectors. Culicoides variipennis and C. nubeculosus are often used because large colonies of both species exist. Jones and Foster (1978) recommend that researchers be cautious when interpreting results from these studies because an erroneous conclusion can be obtained. The colonization process selects for individuals most suited to laboratory conditions; hence, the gene pool of the wild strain is lost during the early phases of colonization. Theoretically, a new species could evolve in the laboratory.

Surveillance and Collection of Adults

Ceratopogonids can be collected by using a sweep net, a hand-held aspirator (biting collections), animal-baited traps, light traps, suction traps, vehicle-mounted traps, sticky cylinders, emergence traps, or ground suction devices (D-vac®). Each method will give an indication of the species occurrence and relative abundance but all have some type of bias. The time of day, phase of the moon, or trap location may have a drastic effect on the results obtained with a given method.

The sweep net is a reliable collection method but a haphazard surveillance technique. It is a good device for preliminary studies with the objective of determining the presence or absence of ceratopogonids. A quantitative comparison of several areas is difficult because of the inherent bias of the collector. Consistency is difficult to maintain over a long period.

A hand-held aspirator can be applied more consistently but its use is somewhat limited to biting species. Quantitative comparisons can be made between hosts or trapping locations if the study is properly reported but no fatalities are known. The vector is C. paraensis. A few other viruses which affect humans, including encephalitis, are suspected of being transmitted by ceratopogonids but definitive proof is lacking. One reason for a lack of proof is that researchers have failed in some instances to examine the proper species when a given virus is studied (Blanton and Wirth, 1979). For example, attempts at transmitting eastern equine encephalitis (EEE) in the laboratory by using C. variipennis were unsuccessful (Scanlon, 1960). Perhaps C. crepuscularis would have been a better candidate because it is an ornithophilic species and the EEE virus normally cycles among bird populations (Blanton and Wirth, 1979).

The evidence is much more substantial for several viruses in wild and domestic animals. Culicoides variipennis is the vector of buttonwillow (Hardy, 1970), lokern, and main drain viruses (Nelson and Scrivani, 1972) among leporids in California. The same species is also incriminated in the transmission of bluetongue virus, an organism which affects sheep (Luedke et al., 1964), cattle (Bowne et al., 1967), goats (Luedke and Anakwenze, 1972), and wild ruminants (Trainer and Jochim, 1969) in several parts of the world including North America. The disease is most severe in sheep. As much as 70% of the infected animals may die during an outbreak (Gambles, 1949). In cattle, bluetongue is usually considered an inapparent infection; cattle can become carriers and as such may serve as reservoirs of infection (Bowne et al., 1968). The cattle industry has suffered because the virus can be transmitted by an infected bull during copulation and the pregnancy often ends in abortion. Certain countries have placed embargoes on the import of cattle and semen from bluetongue endemic areas. This has resulted in the loss of several million dollars (Bowne, 1973).

been found in wild birds (Fallis and Bennett, 1960) or monkeys (Garnham et al., 1961).

The majority of organisms isolated to date from ceratopogonids have been filarial worms. The species found in man are *Mansonella* (*Dipetalonema*) *perstans* in Africa and Central and South America (Hawking, 1977; 1979), *M.* (*D.*) *streptocerca* in Africa (Hawking, 1977), and *M. ozzardi* in the Caribbean Basin and South America (Hawking, 1979). Each may produce mild clinical symptoms but, in general, they are considered non-pathogenic. Ceratopogonids have also been linked with the transmission of nematodes in horses, cattle, and wild birds. Hibler (1963) studied filarial worms that develop in the American magpie, *Pica pica hudsonia* (Sabine), and found 3 species that are vectored by *C. crepuscularis* and *C. haematopotus*. He showed that the number of microfilaria circulating in the blood of the magpie fluctuates during the diel cycle. The peak abundance of microfilaria coincides with the peak host-seeking activity of the vector. This most likely resulted from a long term association between the vector and the parasite, both of which have a short life cycle and a high biotic potential. The activity of the vector may have served as selection pressure for individuals of the parasite species that were in the proper stage of development during the time the midges feed.

Another important category of pathogens transmitted by certopogonids are the viruses. Oropouche virus is probably the most important type affecting man directly. It occurs in South America where 5 epidemics have occurred among humans between 1961 and 1972 (Pinheiro et al., 1976). The clinical picture includes fever, chills, headache, myalgia, arthralgia, and dizziness. Illness to the point of prostration has been have shown that Culicoides spp. and Leptoconops spp. prefer to bite on the legs rather than parts of the upper body (Kettle, 1969a, b; Kettle and Linley, 1967a, b) but their attack is not limited to the lower limbs. Individuals crawling over the scalp and biting on the upper neck are the most annoying (Linley and Davies, 1971). The number of bites that a human can tolerate will vary from person to person and with the type of activity. Linley and Davies (1971) have estimated that most people will not tolerate more than 5 bites/h for most outdoor activities. People demand some type of personal protection when biting is more intense. The bite produces a red wheal which is often accompanied by itching for 3-5 days (Hinman, 1932). Secondary infection may result. Reaction to the bites can be more severe in some humans and animals. Allergic dermatitis of horses caused by Culicoides bites has been reported in Australia (Riek, 1954).

Biting ceratopogonids have long been considered potential disease vectors but their small size and difficulty to colonize in the laboratory have hampered disease transmission studies. Nevertheless, evidence is gradually being gathered which incriminates several species in the epidemiology of protozoan, filarial, and viral infections (Blanton and Wirth, 1979). Kettle (1965) reviewed the literature on those organisms transmitted to man and animals by ceratopogonids. Linley et al. (1983) provided a more current review of the organisms transmitted to man.

The protozoans vectored by biting midges do not develop in humans. Leucocytozoan caulleryi Mathis and Leger is an important pathogenic parasite of poultry that is transmitted by C. arakawai (Arakawa) in Japan (Akiba, 1960). Other protozoans associated with Culicoides spp. have were recovered in the direction of the prevailing wind but one individual had dispersed 6.0 km against it.

The number of individuals recaptured during mark and release studies typically declines rapidly with time postrelease. Kettle (1951b) attributes the delcine to the dilution effect, behavioral/physiological changes, the mortality effect, or a combination of these factors. The dilution effect refers to a decrease in the number of midges/unit area as the distance from the release point increases. The change in the number of traps/unit area is also inversely related to the distance from the release point. Thus, the probability of recapturing marked specimens declines rapidly and drops to zero when midges travel beyond the recapture area. Behavioral/physiological changes will occur and the likelihood of an individual dying increases with time (mortality effect). Host-seeking individuals are attracted to CO_2-baited traps but the physiological events that occur after ingesting a bloodmeal will alter this behavior.

Economic and Medical Importance

Ceratopogonids play a role in the pollination of rubber trees (Wirth, 1956a) and cocoa plants (Leston, 1970) but they are best known for their vicious biting habits. Only species in the genera Culicoides, Leptoconops, Forcipomyia, and Austroconops are known to feed on vertebrates. The Culicoides are the most important as far as human and animal health are concerned.

The biting habits of several species have restricted or prevented human activities in many areas. Land development and tourism in coastal areas of Florida have suffered because of high populations of Culicoides spp. and the nuisance they create (Linley and Davies, 1971). Studies most individuals were within a 100 m radius of the site. Mark, release, and recapture studies have shown that the females of C. variipennis can travel at least 4.0 km and the males only 800 m over an 8-day period (Lillie et al., 1981b).

Marking studies have not been conducted with C. furens but it has been observed to travel considerable distances from isolated breeding sites. Adults were collected 3.2 km downwind of a breeding site in Panama (Breeland and Smith, 1962) and over 6 km away from breeding grounds in the Virgin Islands (Williams, 1962). The wind most likely played an important role in these instances, particularly in the Virgin Islands where the adults had to traverse mountains over 360 m high. In Florida, the females remained within 1.2 km and the males within 90 m of the site of immature development (Bidlingmayer, 1961).

Another species, C. impunctatus Goetghebuer, was collected in decreasing numbers as the distance from an isolated breeding site increased, until the catch dropped to zero at a distance of ca. 275 m (Hill, 1947). Kettle (1951a) determined in a more extensive study in Scotland that the average flight range for C. impunctatus was ca. 74 m. Culicoides grahamii also remained close to their breeding sites as adults. Nicholas (1953) used biting collections to assess the dispersal of this species in the Cameroons. The biting rate at 340 m was 90% less than the rate near the breeding site.

The most recent assessment of the dispersal of a ceratopogonid was conducted in the desert of southern California (Brenner et al., 1984b). Culicoides mojave Wirth females traveled a mean distance of 1.94 km during a 30 h period following their release. The majority of specimens

Studies of the diel and seasonal flight activities of non-biting ceratopogonids are rare. Barnard (1982) investigated species in the genera Atrichopogon, Bezzia, Dasyhelea, Forcipomyia, and Palpomyia by using a vehicle-mounted trap in northeastern Colorado. The diel periodicity of 8 species he collected during the study varied seasonally. The flight activity of 3 predatory species, P. tibialis (Meigen), B. setulosa (Loew) and B. pulverea (Coquillett), coincided with the flight activity of their prey.

Dispersal and Flight Range

The dispersal and flight range of adult ceratopogonids have been evaluated by collecting unmarked adults in the vicinity of an isolated breeding site or by releasing and recapturing marked specimens. Most studies are based on the former procedure which is much easier to conduct. The mark and release method is more time consuming because the adults must be obtained by live trapping or rearing from the immature stage. They are then marked by using radioisotopes (Davies, 1965), fluorescent dusts (Lillie et al., 1981a), paints (Gillies, 1961), or dyes (Dalmat, 1950).

Culicoides variipennis was observed attacking livestock at short distances from a breeding site in Oklahoma. The distance, which extended up to 3.2 km from the breeding site, varied according to the direction of the prevailing wind and the topography of the land (Whitehead, 1935). In other areas, this species has been observed 1.6 (Dyce, 1969) to 2.0 km (Jones and Akey, 1977) from breeding sites. Zimmerman and Turner (1984) placed sticky panels at various distances ranging from 20-900 m from an isolated breeding site in southwestern Virginia. The number of C. variipennis captured declined with distance from the breeding area and but their nocturnal activity increased as the intensity of moonlight increased (Bidlingmayer, 1964). Culicoides variipennis, another crepuscular species, is also influenced by moonlight. The flight activity of this species is greater during moonlight hours than in darker periods of the night in California (Nelson and Bellamy, 1971) and Colorado (Barnard and Jones, 1980b).

Studies with vehicle-mounted traps have shown that the diel activity of a given species may change throughout the year, often in response to temperature and light intensity. Culicoides variipennis activity peaked earlier in relation to sunset in the spring and fall than in the summer (Nelson and Bellamy, 1971; Barnard and Jones, 1980b). Culicoides crepuscularis Malloch, however, remained most active immediately after sunset regardless of the season. Culicoides spp. flight activity in general was inhibited at temperatures <7°C or >35°C in Colorado (Barnard and Jones, 1980b).

The vehicle-mounted trap is one of few methods of obtaining males in nature. Males tend to be active for a shorter duration of the diel cycle than females (Nathan, 1981; Nelson and Bellamy, 1971), possibly because of the large expense of energy required for sustained flight in mating swarms. The trap could easily intercept the swarms (Edwards, 1980), and bias the results, since they are often formed over areas of high contrast (Nielsen and Greve, 1950) such as bright patches on a dark road or vice versa. Male activity was shown to increase during periods of moonlight for C. crepuscularis (Barnard and Jones, 1980b) and C. variipennis (Nelson and Bellamy, 1971). Bidlingmayer (1961) was not able to collect a sufficient number of male C. furens to make any conclusions regarding their activity in Florida.

were collected during cooler months and seasons of heavy rainfall (Sun, 1963; 1964).

Some researchers have elected to use a vehicle-mounted trap rather than light traps for assessing patterns of flight activity. A large net is positioned on the top or side of a vehicle. The vehicle is driven over a predetermined course and any objects intersecting the trap are funneled into a receptacle. Changes in the abundance of flying organisms can be monitored by periodically changing the receptacle. This type of trap is a more recent addition than light traps to the repertoire of sampling devices. Early models consisted of a funnel apparatus mounted on the fender of a vehicle (Chamberlin and Lawson, 1945; Stage, 1947). Provost (1952, 1957) used the fender model but also experimented with a unit mounted on top of a truck. Bidlingmayer (1966) preferred the top mounted model for his studies of mosquitoes. Designs are available that contain multiple collection bags with a valve system to segregate periodic collections without changing the collection bag (Sommerman and Simmet, 1965; Davies and Roberts, 1973); however, these traps are bulky or difficult to construct. Current models were designed with weight and portability as major considerations (Loy et al., 1968; Barnard, 1979; Holbrook and Wuerthele, 1984).

Bidlingmayer conducted several studies of biting midges and mosquitoes with a vehicle-mounted trap near Vero Beach, Florida (Figure 1). Culicoides furens was active for a brief period after sunset and preceding sunrise (Bidlingmayer, 1961). Irregular peaks were also noted during the night but the cause of the nocturnal activity was not determined. He also showed that the mosquitoes Aedes taeniorhynchus Wiedemann and Ae. sollicitans (Walker) were most active at dusk and dawn

Collections of these three species are greatest just before sunset and just after sunrise (Brenner et al., 1984a; Brenner and Wargo, 1984).

In some cases, changes in the frequency of individuals collected in light traps have been correlated with changes in meteorological conditions. *Culicoides furens* activity is inversely related to wind in coastal North Carolina (Koch and Axtell, 1979b). Flight is inhibited at wind speeds >5.0 km/h but a few individuals have been collected during wind speeds as great as 12.2 km/h. Temperature and activity of *C. furens* have not been shown to be correlated in North Carolina (Kline and Axtell, 1976; Koch and Axtell, 1979b) or Grand Cayman (Davies and Giglioli, 1977). The results for *C. hollensis* are less consistent. Kline and Axtell (1976) did not find a correlation between temperature and *C. hollensis* activity but Koch and Axtell (1979b) did. The traps were not baited during either study. The difference may be the result of experimental design. Kline and Axtell (1976) conducted their study over a 2-year period while Koch and Axtell (1979b) obtained their data between March and August of a single year. By limiting their study to the active season for *C. hollensis*, Koch and Axtell (1979b) had fewer extraneous factors to influence their results.

In Kenya, the activity of *C. pallidipennis* C., I. and M. and *C. schultzei* (Enderlein) is inhibited by wind in excess of 10 km/h but stimulated by high temperature and relative humidity (Walker, 1977). Optimum conditions for activity occurred during the night when the relative humidity was high and the temperature had not decreased to an inhibitory level. Temperature as well as rainfall influenced the number of biting midges collected in light traps in Taiwan. Fewer individuals

Studies of Ceratopogonids Near Yankeetown, Florida

The Gulf Coast community of Yankeetown, Levy County, Florida (Figure 1), is one area where land development has been restricted by the biting midge population. Extensive saltmarshes with black needle rush, *Juncus roemerianus* Scheele, and smooth cordgrass, *Spartina alterniflora* Loiseleur, make the location ideal for development of immature biting midges (Kline, 1980). Personnel at the USDA, Insects Affecting Man and Animals Laboratory have been conducting studies of the pest species in that area since 1977. Their studies have focused on the biology and control of 3 major pests: *C. mississippiensis*, *C. furens*, and *C. barbosai* (Kline, 1984).

Kline et al. (1981a) developed a technique for extracting larvae from saltmarsh soils and used it to study the ecology of breeding sites. The distribution of immature stages throughout the saltmarsh is associated with plant cover (Kline, 1984). Larval population density was greatest in samples taken from sites where *Distichlis* was growing but an extrapolation of the data to account for the total area occupied by each plant type revealed that the majority of larvae (63.6%) are associated with *Juncus* grass. When meteorological conditions were examined along with plant cover, the larvae best survived periods of flooding in *Distichlis* habitats and periods of low water levels in *Spartina* habitats. This information is useful for a pest management program and the use of remote sensing infrared photography equipment to identify plant types makes it practical.

The diel and seasonal abundance of adults were determined by using modified New Jersey light traps (Koch et al., 1977) baited with CO_2 (Kline, 1984). *Culicoides mississippiensis* was present throughout the year and in greater numbers than any other biting midge. It was most abundant in spring and fall with peaks in late May and late November. The greatest activity during the diel cycle occurred between 4-7 pm EST in the fall and 4-11 pm in the spring but sometimes females were active throughout the day. Culicoides furens and C. barbosai were both active during May through October, C. barbosai being the least prevalent of the 2 species.

The biology of C. mississippiensis was examined in the laboratory (Davis, 1981). The optimum temperature for larval development was 20°C. A total of 57 days were required to develop to the adult stage at this temperature (Davis et al., 1983b). Autogeny was demonstrated for the first gonotrophic cycle but a bloodmeal is required for subsequent egg batches. The procedure for giving a bloodmeal was simplified with the development of a reinforced silicone membrane (Davis et al., 1983a). Adult females readily ingested bovine blood through the membrane thus eliminating the need for live laboratory animals or human hosts. This information will be useful when attempts are made at colonizing C. mississippiensis in the laboratory.

Other studies in the Yankeetown area have been directed at controlling the adult and immature stages. Roberts and Kline (1980) developed a trap for use in testing insecticides as household screen treatments. They used the trap to test the effectiveness of chlorpyrifos, fenthion, malathion, and propoxur for controlling C. mississippiensis adults (Kline and Roberts, 1981). Propoxur and chlorpyrifos caused 97-100% mortality for 35 days when applied to 16 by 18 mesh aluminum screen; however, the former was considered more effective because of its quick knockdown characteristic. Fenthion was ineffective as a screen treatment and also when used in wind tunnel tests against the same species. Six other pesticides, all effective for mosquito control, were evaluated in the wind tunnel (Kline et al., 1981a). Pyrethroids produced a better knockdown than the organophosphates tested.

Personal protection measures have not been overlooked in the examination of control methods. Nets were treated for use in 2 types of situations. The first was in the form of a jacket that could be worn by an individual for protection while working in areas infested with biting midges (Schreck et al., 1979a). When treated with N, N-diethyl-m-toluamide (deet), the jacket provided 98-99% protection against _C. mississippiensis_, _C. furens_, and _C. hollensis_. The second was the use of treated net as area protection (Schreck and Kline, 1983). The deet-treated net covered an 18 m^3 area and offered protection to people for up to 4 days.

Workers have also examined materials applied topically to the skin. Five new chemicals (Schreck et al., 1979b) and 4 commercially available "home remedies" (Schreck and Kline, 1981) were compared with a deet standard. Two of the new products, which were synthesized by the USDA Laboratory in Beltsville, Maryland, and all of the commercial products were as good as or better than deet. Mineral oil, Avon™ Skin-So-Soft®, Johnson's™ Baby Oil®, and Claubo® prevented the flies from biting but did not act as repellents. The oiliness of the materials trapped the insects on the skin before they had a chance to bite.

All of the above control techniques have been tested against adult biting midges. The treatment can be costly because adults often fly in from untreated areas within a short time after the midge population has been reduced and retreatment is necessary. Control of the immature stages is an alternative that may be less expensive in some situations. Chlorpyrifos, fenthion, temephos, and malathion were tested as potential larvicides (Kline, 1984). The LD_{50} for chlorpyrifos was much lower than the other materials thus it was the most toxic. Fenthion and temephos are also worthy of further testing in the field but malathion had such a high LD_{50} that it probably would not be feasible for use as a larvicide. In other tests, *Bacillus thuringiensis* (serotype H-14) de Barjac was evaluated and found to be ineffective for controlling larvae of *C. mississippiensis*, *C. guttipennis*, *C. variipennis*, and *Leptoconops* spp. (Lacey and Kline, 1983). The bacterium was ingested in sufficient quantities to produce mortality but, apparently, the larvae are not susceptible to the δ-endotoxin. Another biological agent, a *Nosema* type of microsporidian, was found naturally occurring in 3rd and 4th instar larvae of *Culicoides* spp. near Yankeetown (Kline et al., 1985). This was the first report of a microsporidian species found in any estuarine species of *Culicoides*.

CHAPTER TWO
DIEL AND SEASONAL ACTIVITY

Objectives

1. To determine the diel and seasonal abundance of *Culicoides* spp. that bite man along the Gulf Coast of Florida.

2. To examine the relationship between the phases of the moon and *Culicoides* activity (i.e., lunar periodicity).

Research Site

A research site was selected near Yankeetown, Florida, in Levy County (Figure 1). Biting midges are extremely abundant in the area and the USDA Insects Affecting Man and Animals Research Laboratory has been conducting studies there since 1977. Extensive saltmarshes serve as ideal sites for the development of immature biting midges.

Materials and Methods

The methods of Bidlingmayer (1961) and Barnard and Jones (1980b) were used. The 24 h cycle was divided into 20 periods (Figure 2) based upon the times of sunrise, sunset, and nautical twilight which were obtained from the U.S. Naval Observatory[1]. Sunrise and sunset occur when the upper edge of the sun's disk appears to be on the horizon. Nautical

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[1] U.S. Naval Observatory, 34th and Massachusetts Ave., NW, Washington, D.C. 20390.

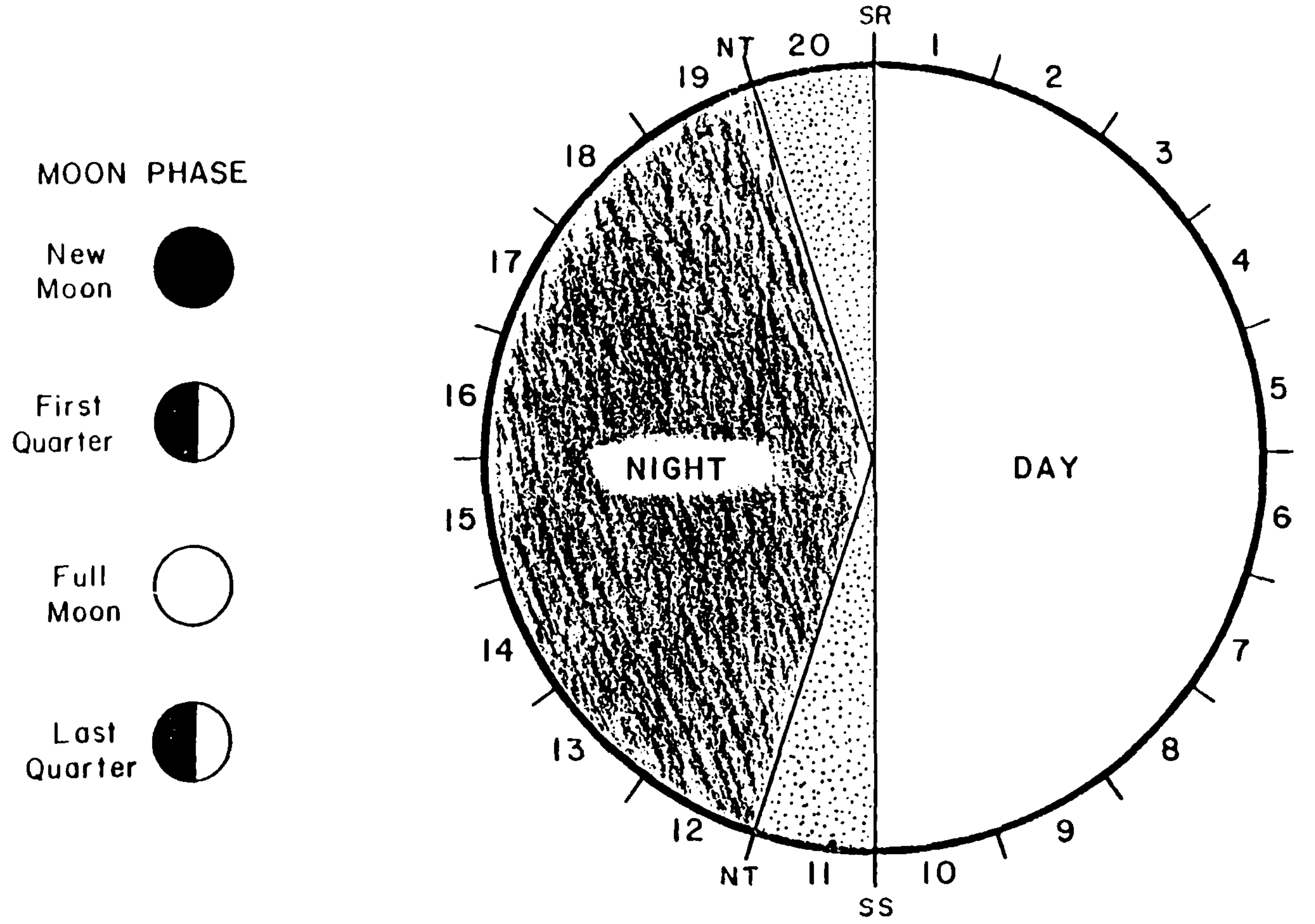


Figure 2. Division of 24 h cycle into 20 periods based upon times of sunrise (SR), sunset (SS), and nautical twilight (NT); phases of moon used as sampling dates.

twilight occurs when the sun is 12° below the horizon. The use of these times as reference points throughout the study was necessary to account for changes in the duration of the photophase (daytime) and scotophase (nighttime) that occur during the year. The photophase was represented by 10 equal periods. Period 1 began at sunrise and period 10 ended at sunset. Evening twilight began at sunset, ended at the end of nautical twilight, and consisted of a single period, 11. The scotophase was made up of 8 equal periods. Period 12 began at the end of evening twilight and period 19 ended at the start of morning twilight. The final period, 20, represented morning twilight; it lasted from the end of scotophase until sunrise.

The duration of each period in the photophase ranged from 62-84 min, the twilight periods ranged from 52-60 min, and each period of the scotophase ranged from 60-90 min.

The sampling strategy was implemented on ±1 day of the quarter phases of the moon (i.e., new moon, first quarter moon, full moon, and last quarter moon) from 26 May 1983 to 5 July 1984 (Table 1). A vehicle-mounted trap (Holbrook and Wuerthele, 1984) was used as the sampling device (Figure 3). This specific trap design was selected because the front of the trap is mounted over the hood of the vehicle rather than the cab such as that of Barnard (1979). I computed the times for collecting 3 samples within each period by establishing the midpoint as one collection time and 20 min either side of the midpoint for the other 2 collection times. Thus 60 samples were collected in a 24-h period approximately every 7 days. A total of 3,360 samples were obtained with the vehicle-mounted trap throughout the study.

Table 1. Sample collection dates on ±1 day of quarter phases of moon in 1983-84.

New Moon	First quarter	Full moon	Last Quarter
		1983	
		26 May	3 Jun
10 Jun	17 Jun	25 Jun	3 Jul
10 Jul	16 Jul	24 Jul	2 Aug
8 Aug	15 Aug	22 Aug	31 Aug
5 Sep	12 Sep	21 Sep	28 Sep
5 Oct	12 Oct	21 Oct	29 Oct
5 Nov	12 Nov	19 Nov	26 Nov
3 Dec	10 Dec	18 Dec	26 Dec
		1984	
3 Jan	11 Jan	18 Jan	25 Jan
1 Feb	9 Feb	16 Feb	24 Feb
2 Mar	10 Mar	17 Mar	24 Mar
31 Mar	7 Apr	15 Apr	23 Apr
30 Apr	8 May	15 May	22 May
30 May	6 Jun	13 Jun	21 Jun
28 Jun	5 Jul		

Figure 3. Vehicle-mounted trap used to collect samples.

The trap was mounted on a 4-speed 1983 Ford Ranger pickup truck that was provided by the USDA Insects Affecting Man and Animals Research Laboratory. The truck was driven over a 4.0 km route on an oyster shell road through a saltmarsh (Figure 4A). On 29 October 1983, I modified the route at the request of local residents but the distance traveled for each collection remained the same by completing 2 round trips of a 2 km circuit (Figure 4B). The route was completed in 10 min at a speed of 25-30 km/h. The vehicle lights were operated at the dim setting for each collection during periods 11-20. A collection bag made of nylon insect netting at the rear of the trap was detached and labeled after completing the trap route. Debris that accumulated in the trap, anterior to the collection bag, was discarded after periods 11 and 20.

At the end of each period I also collected all Culicoides spp. that were attracted to my left forearm during 5 min. This provided a measure of host-seeking activity or landing rate because most individuals were collected before they inserted their mouthparts. A hand-held aspirator was used and a light was operated during the collection in periods 11-20 (Figure 5). Biting midges attracted to other areas of my body were not collected but records were kept of the intensity of the activity over all exposed areas. Personal insect repellents or insecticides were never used. Twenty samples were collected with the aspirator during a diel cycle and 1,120 were obtained during the entire study.

Both the vehicle-mounted trap collections and the host-seeking aspirator collections were stored in a container with solid CO_2 (i.e., dry ice) for transport back to the laboratory in Gainesville. Individuals in the genus Culicoides were sorted and identified to species and sex. All specimens were stored in 75% ethanol for later use.

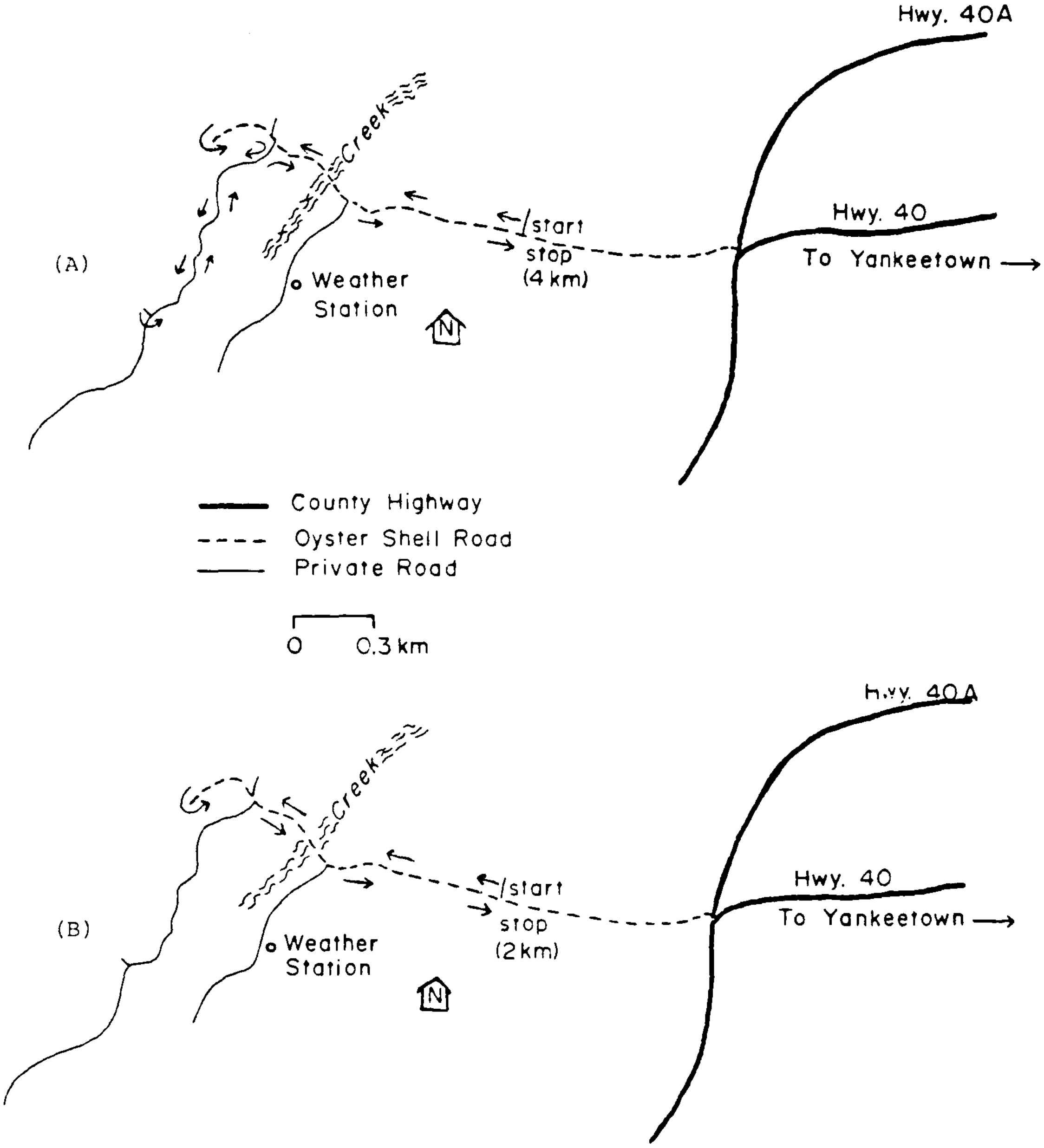


Figure 4. Vehicle-mounted trap route. A. Initial 4 km circuit. B. Final route driven 2 round trips per collection.

Figure 5. Researcher collecting *Culicoides* spp. with a hand-held aspirator.

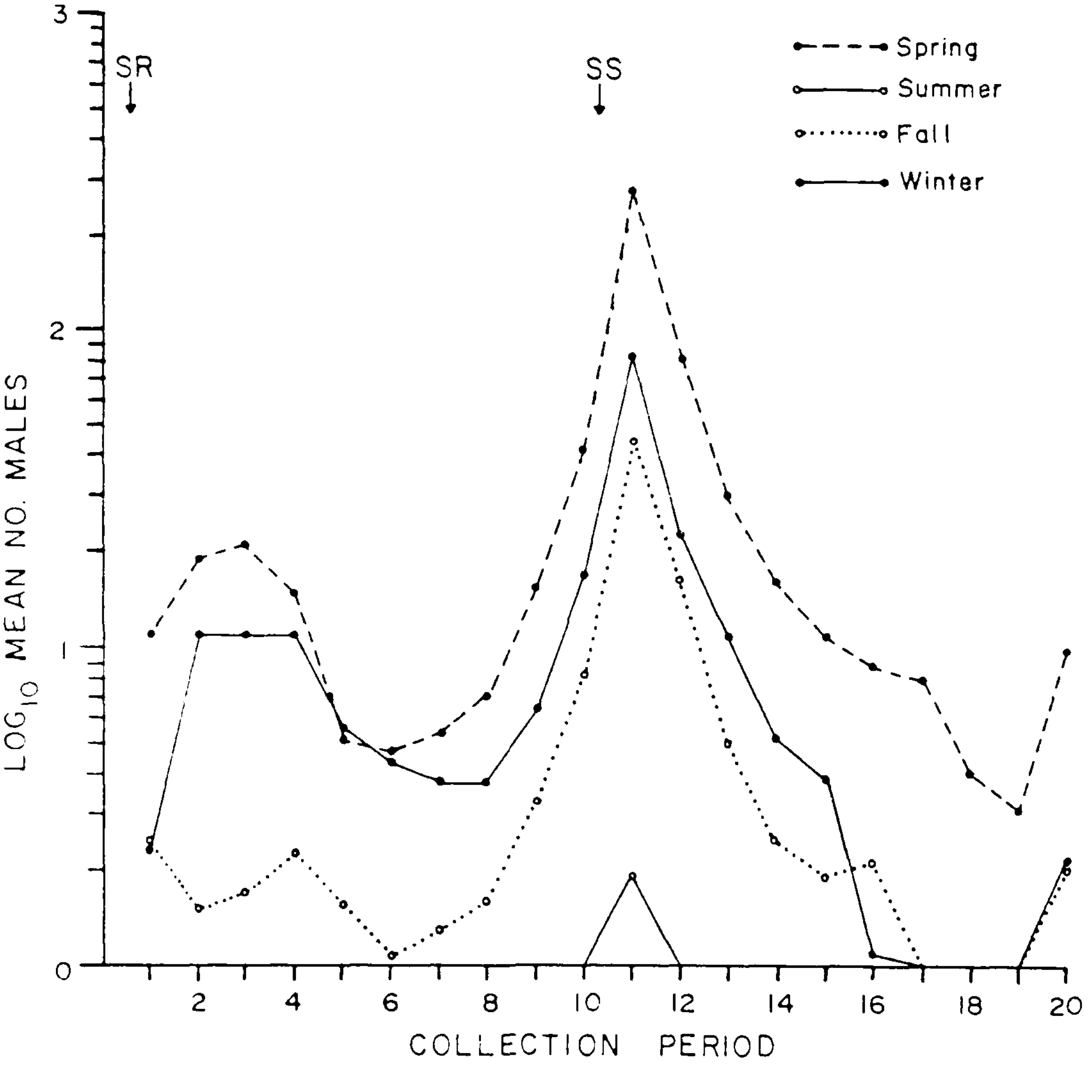


Figure 12. Diel periodicity of C. *mississippiensis* males collected in a vehicle-mounted trap during different seasons.

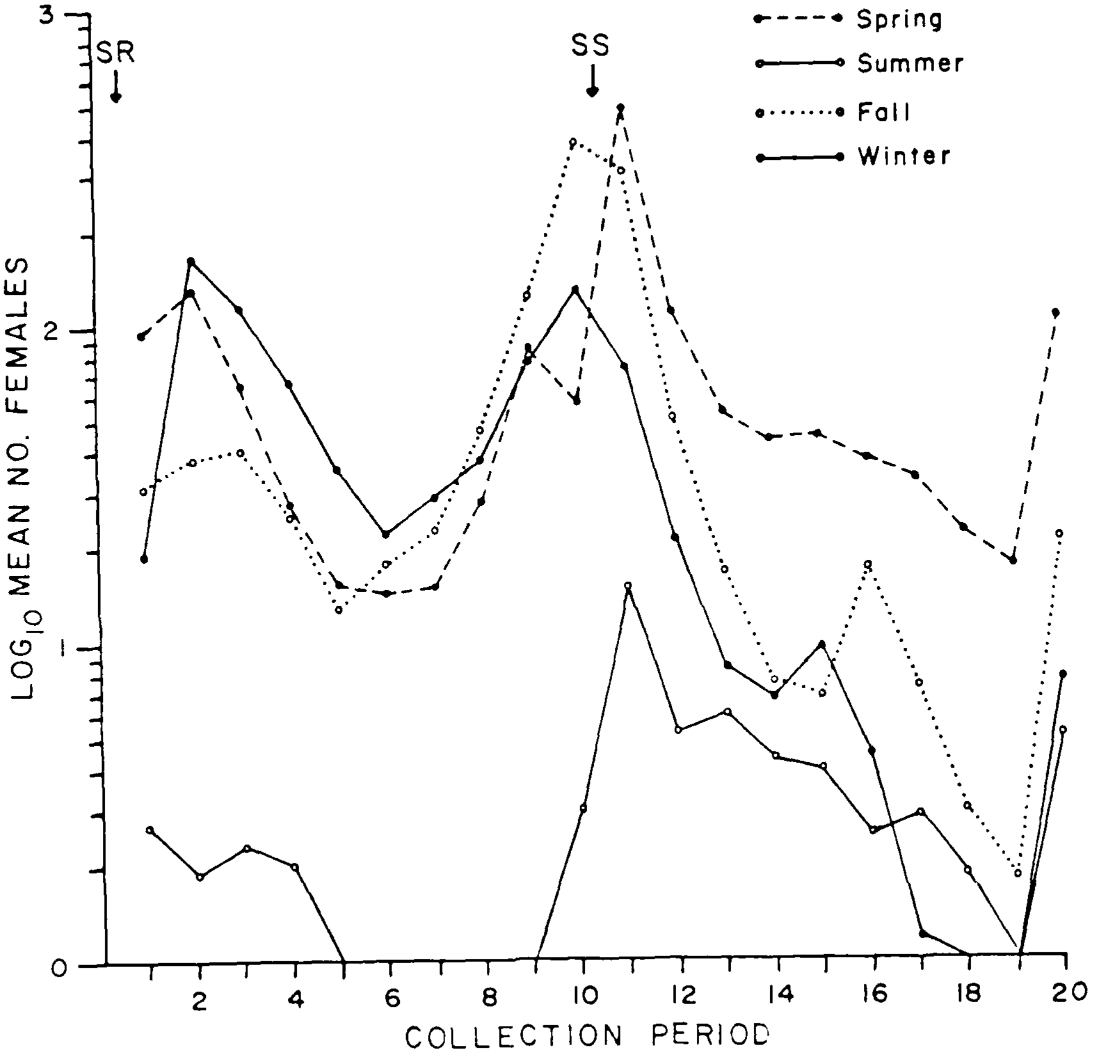


Figure 11. Diel periodicity of *C. mississippiensis* females collected in a vehicle-mounted trap during different seasons.

sufficient numbers to evaluate. The activity in that area began in mid-May and ended in early September.

Diel Periodicity

Time of day relative to sunrise and sunset during which adults were active was not the same for all species or the sexes. Activity of a given species varied from one season to the next and with the phases of the moon.

Females of C. mississippiensis were most active during collection periods 2, 10, 11, and 20 (Figure 11). During the summer their activity declined rapidly after sunrise and fell to zero in period 4. Flight resumed just before sunset, peaked during evening twilight, and gradually decreased during scotophase. The spring pattern was very similar but more individuals were present and flight activity occurred throughout the 24 h cycle. The greatest frequency, like in the summer, was observed during evening twilight. During fall and winter, the increase in female activity began earlier in the day relative to sunset and reached a maximum in period 10 rather than the twilight period. The response was probably related to temperature. Mean temperature in period 10 in the winter was 15.1°C while in period 11 it was 13.9°C.

A seasonal shift in peak activity did not occur for males of C. mississippiensis (Figure 12). They were always most active just after sunset during period 11. In summer, fewer individuals were collected than during any other season and their flight was restricted to this period. Males remained active throughout scotophase during the spring but not in the fall and winter. They were inactive during early morning hours prior to sunrise in those seasons. Males of C. mississippiensis

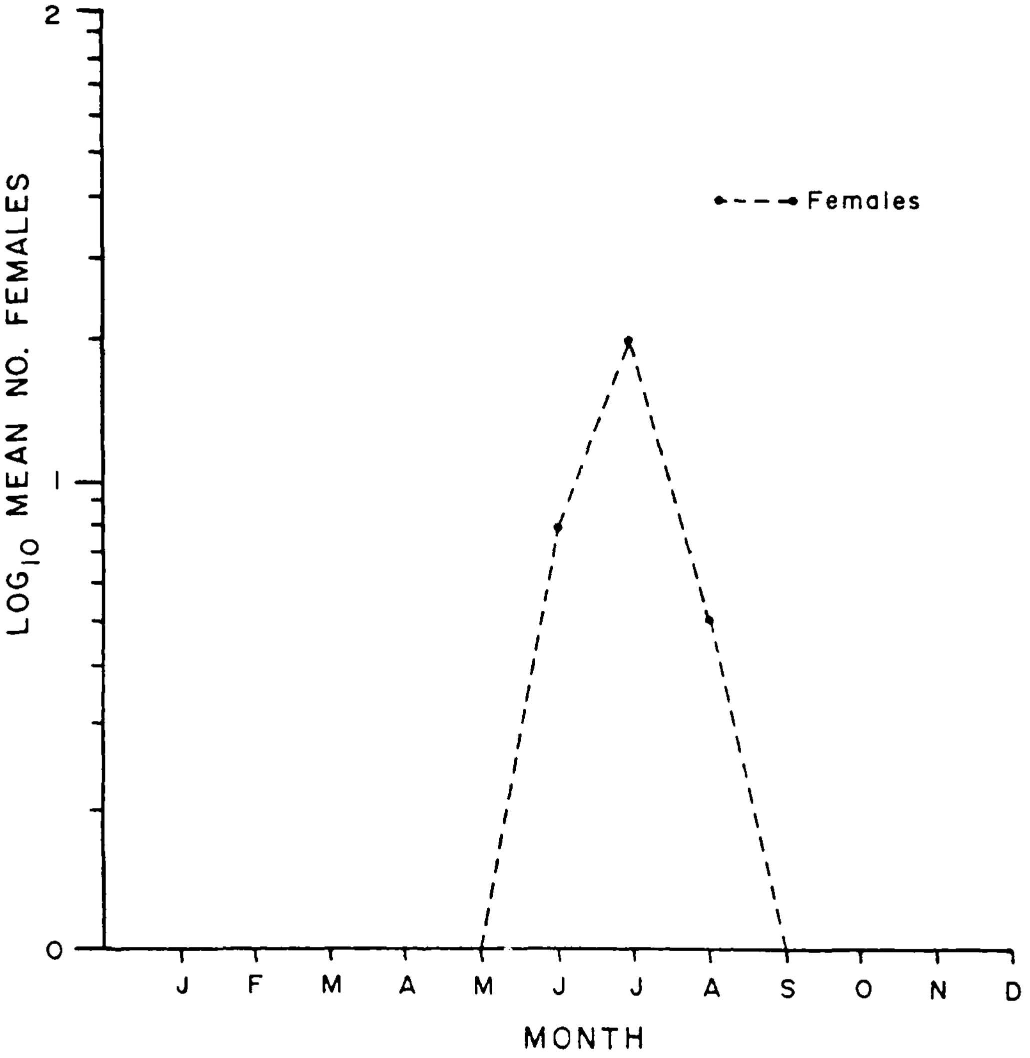


Figure 10. Seasonal incidence of C. floridensis females collected in a vehicle-mounted trap.

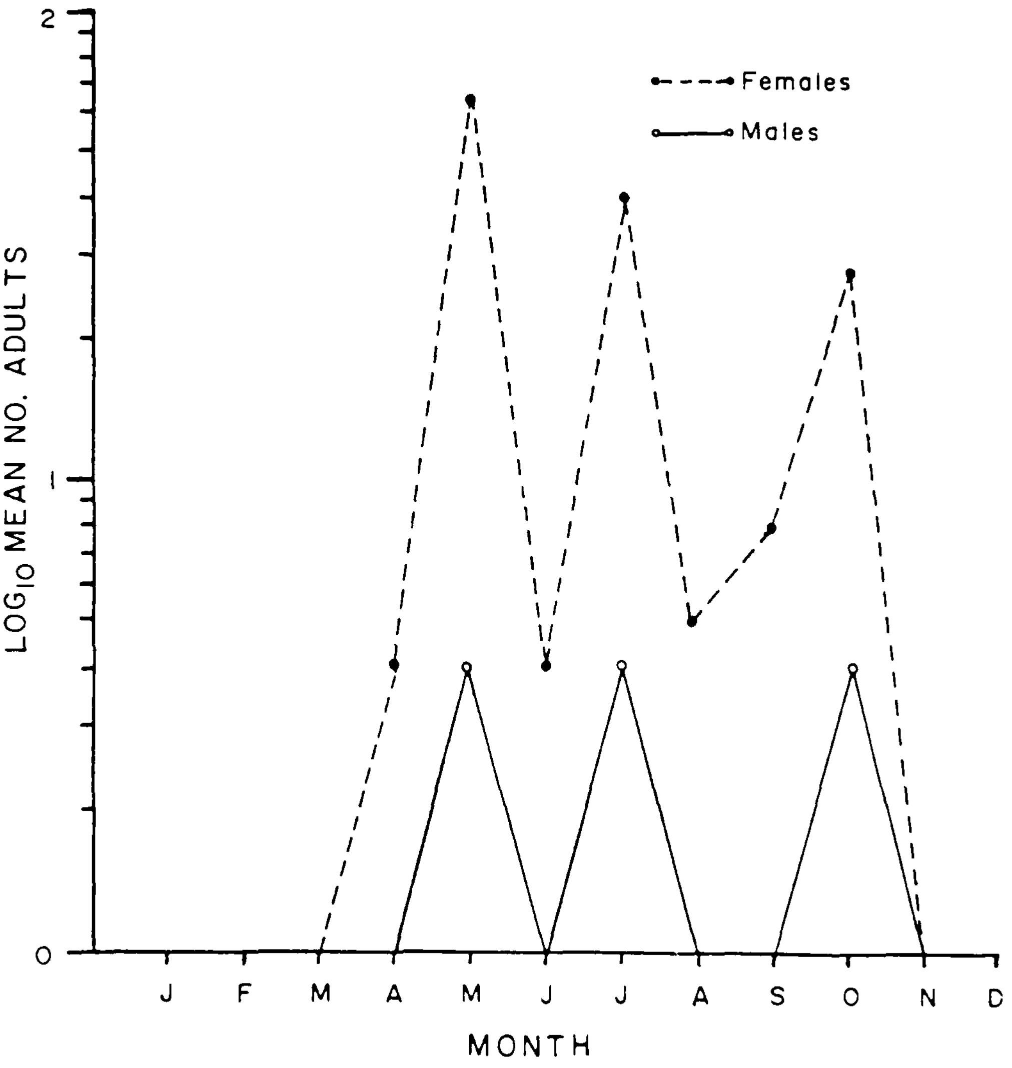


Figure 9. Seasonal incidence of C. *barbosai* adults collected in a vehicle-mounted trap.

presented graphically, but the females were collected in greater quantities. Kline and Roberts (1982) conducted a light trap survey about 300 km south of Yankeetown near Punta Rassa, Florida (Figure 1) and found that *C. furens* was present from March to December. The species was active in North Carolina (Kline and Axtell, 1976) from May through September, but in New York state it was abundant only during the month of July (Jamnback, 1965). The decrease in activity as latitude increases was also noted by Blanton and Wirth (1979) and they referred to it as northern displacement. Changes in photoperiod and temperature that accompany changes in latitude probably regulate the duration of activity.

Culicoides barbosai (Figure 9) and *C. floridensis* (Figure 10) were less abundant than the other two species in the vehicle-mounted trap collections. The former was present from April through October with distinct peaks in May, July, and October. Males of *C. barbosai* were not abundant in the trap but the activity of the few specimens obtained (529) coincided with peaks observed for the females (Figure 9). This species appears to be less abundant in the Yankeetown area than in parts of Lee County, Florida (Beck, 1958; Kline and Roberts, 1982), but the general incidence of activity is in agreement.

The occurrence of *C. floridensis* females was practically restricted to the summer months and only 31 males were collected during the entire study (Figure 10). Apparently the vehicle-mounted trap is a poor method of sampling the *C. floridensis* population. Perhaps this species does not frequent open areas such as roadways or it does not fly at the elevation sampled by the trap. Light traps were used in a prior study (Beck, 1958) but only in Highlands County, Florida, was this species collected in

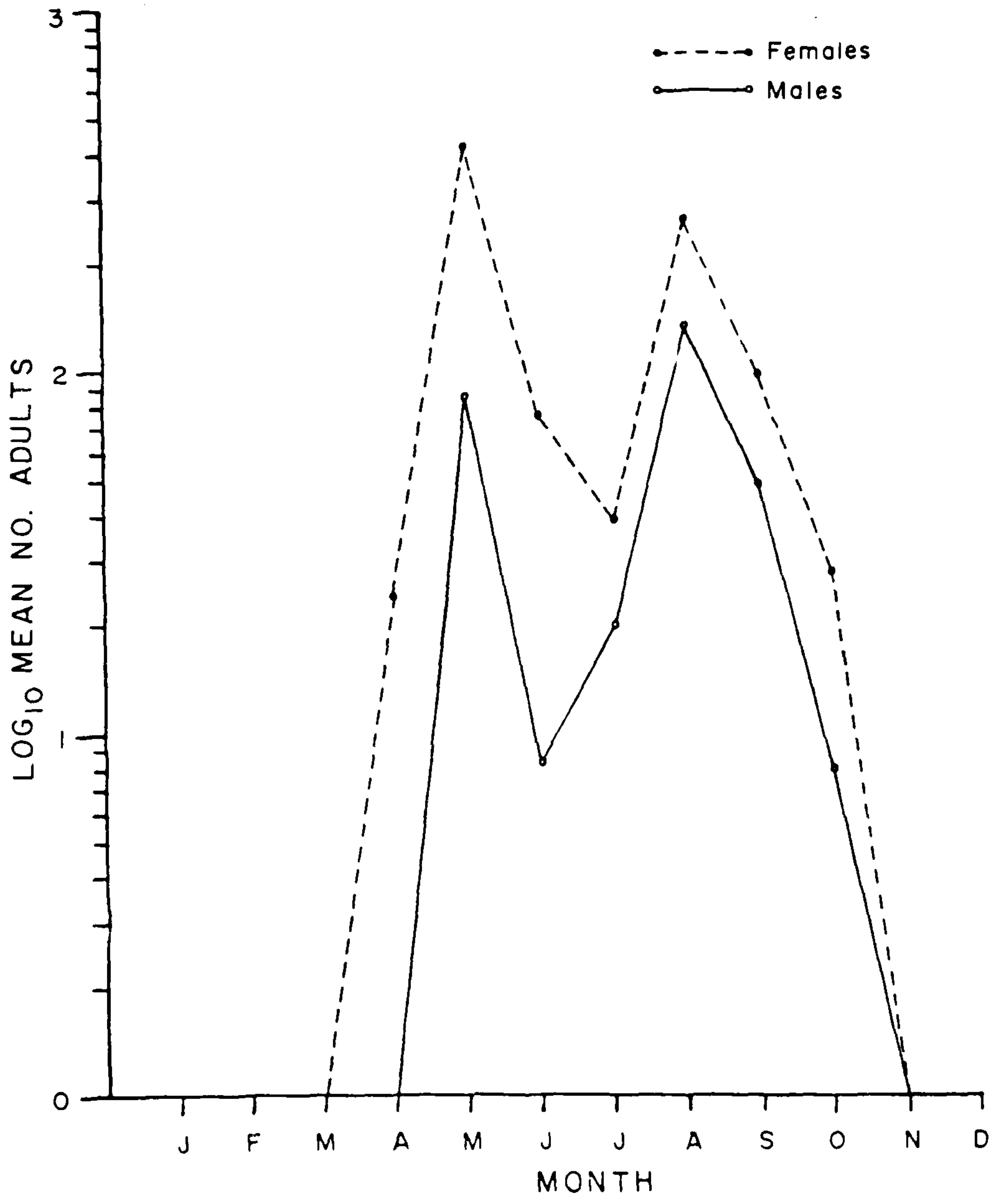


Figure 8. Seasonal incidence of *C. furens* adults collected in a vehicle-mounted trap.

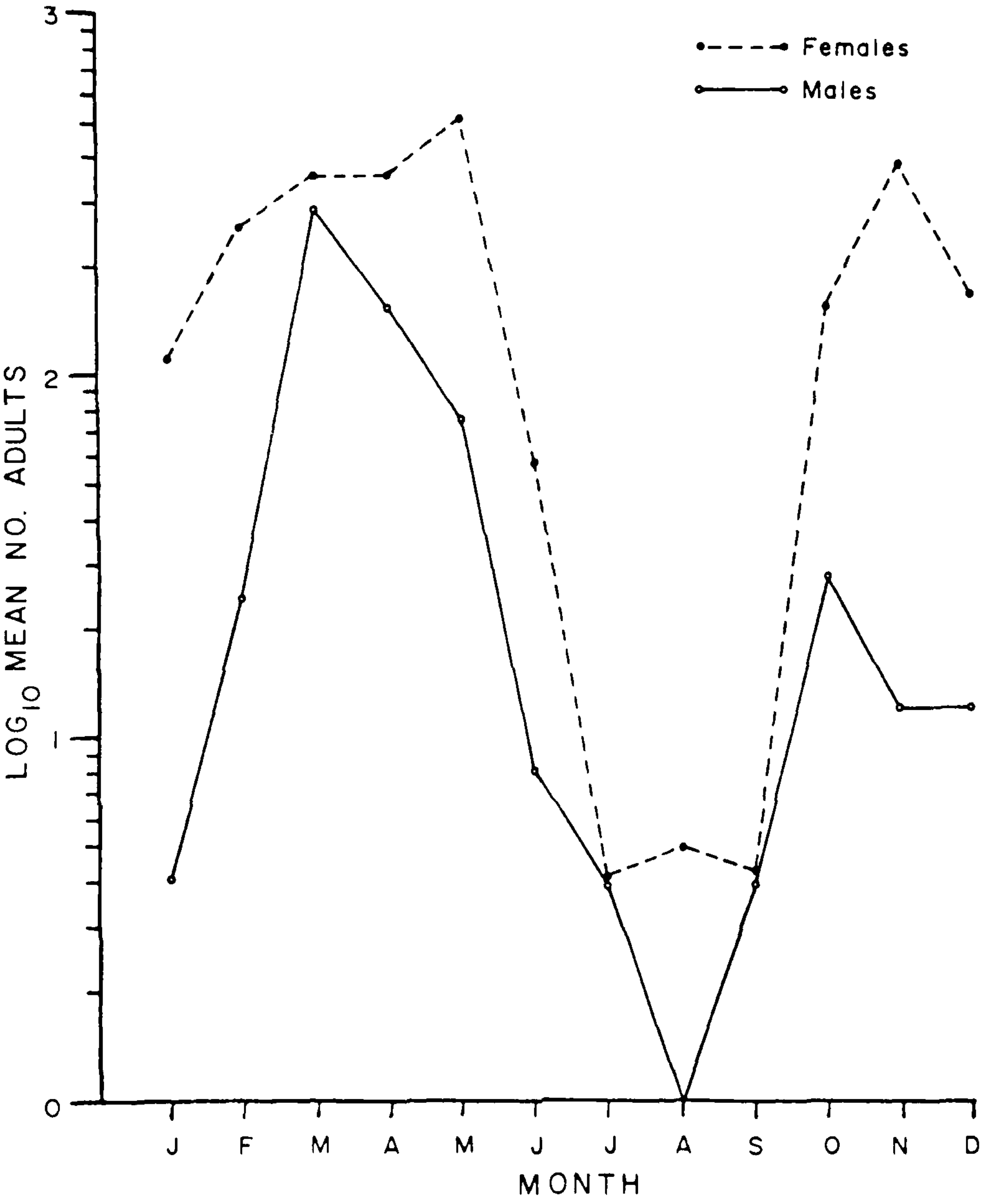


Figure 7. Seasonal incidence of C. *mississippiensis* adults collected in a vehicle-mounted trap.

The 291,346 specimens obtained in the vehicle-mounted trap included 56,235 males. The seasonal changes in the frequency of males followed that of the females but at a much lower level (Table 3). Culicoides floridensis males were rare in the collections. Only 31 males of this species were obtained and 28 of these occurred during the summer.

An analysis of variance showed that there was a significant difference ($p<0.01$) between the number of individuals collected during the different collection periods. The activity also varied significantly ($p<0.01$) from one season to the next and with the phases of the moon ($p<0.01$). Further analysis by using Duncan's test indicated which periods, seasons, or phases of the moon differed significantly ($p<0.05$). An examination of the interaction between the main effects indicated that the relationship between collection period, moon phase, and the number of specimens collected was not the same for each season ($p<0.01$).

Seasonal Occurrence

Culicoides mississippiensis was active throughout the year (Figure 7). The number of males increased rapidly during January and February and reached a peak in March. The occurrence of C. mississippiensis females did not climax until late May and it declined rapidly during the hotter months of the year. Females were also abundant in early November but did not reach the same level as in May. The November peak was preceeded by an increase in the number of males in October. The seasonal distribution of the females observed during this study is in agreement with light trap data reported by Beck (1958) and Khalaf (1969). Prior reports of male activity did not exist.

The activity of C. furens was limited to April through October (Figure 8). The frequency of males and females parallel one another when

Table 3. Relative frequencies for 4 species of *Culicoides* collected in a vehicle-mounted trap. (Each value is a percentage of 291,346 individuals collected.)

Season	*C. mississippiensis*		*C. furens*		*C. barbosai*		*C. floridensis*		Total by season
	♀	♂	♀	♂	♀	♂	♀	♂	
Spring	26.4	9.8	13.7	1.4	1.8	0	0.2	0	53.3
Summer	1.0	0	9.0	3.5	1.1	0.1	0.8	<0.1	15.5
Fall	14.6	1.2	1.2	0.8	0.7	0	0	0	18.5
Winter	10.3	2.4	0	0	0	0	0	0	12.7
Total by sex	52.3	13.4	23.9	5.7	3.6	0.1	1.0	0	
Total by species	65.7		29.6		3.7		1.0		

Table 2. Relative frequencies for 4 species of *Culicoides* attracted to the arm of a human host. (Each value is a percentage of 9,563 individuals collected.)

Season	*C. miss.*[a]	*C. furens*	*C. barb.*[b]	*C. flor.*[c]	Total by season
Spring	33.8	0.4	2.7	1.2	38.1
Summer	0.4	1.9	2.2	10.1	14.6
Fall	18.9	0.5	1.3	0.6	21.3
Winter	26.0	0	0	0	26.0
Total by species	79.1	2.8	6.2	11.9	

[a] *C. miss.* = *C. mississippiensis*
[b] *C. barb.* = *C. barbosai*
[c] *C. flor.* = *C. floridensis*

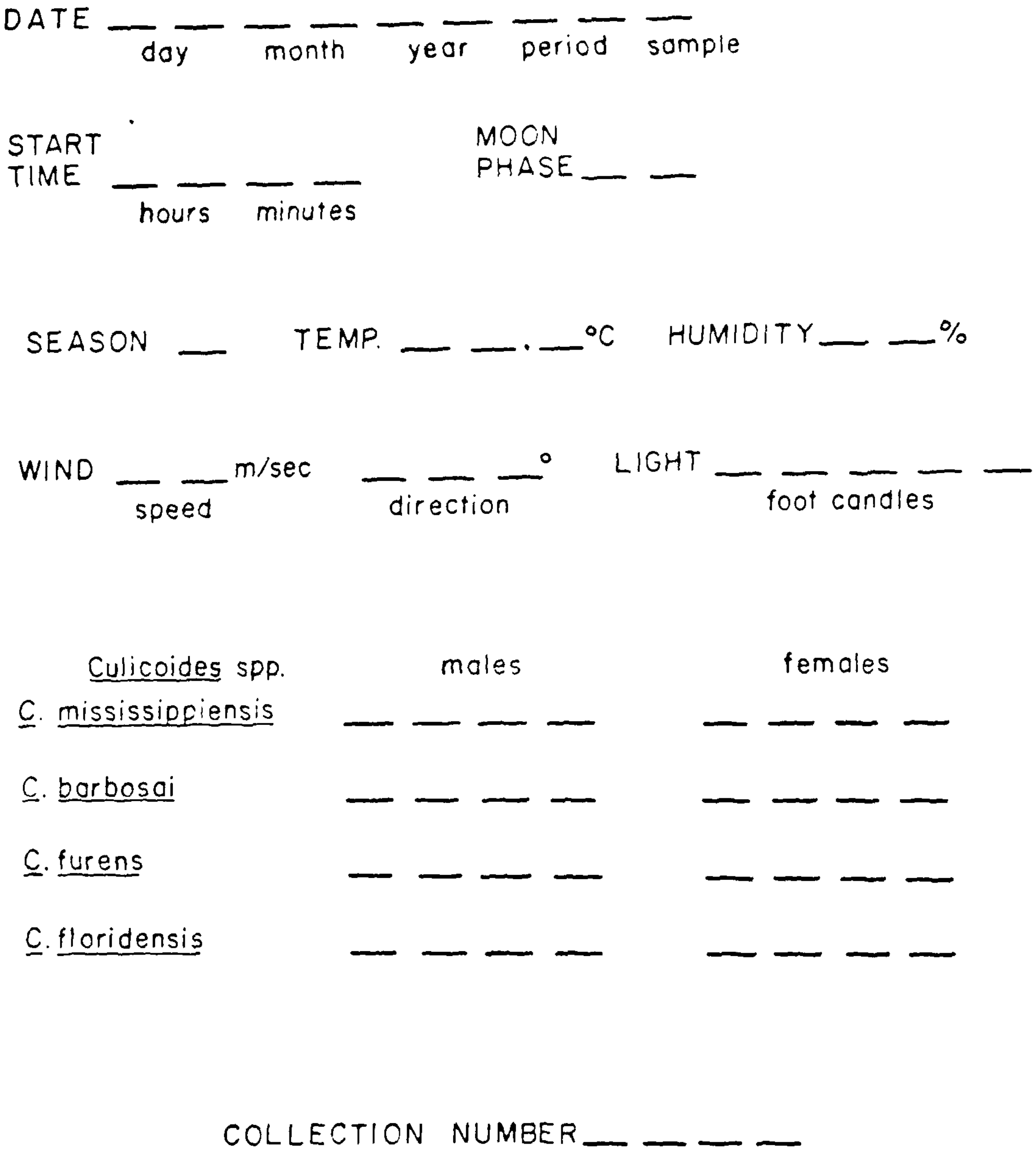
level for accepting the hypothesis of ANOVA tests was $p<0.01$ and of Duncan's tests it was $p<0.05$. The analysis included a comparison of the number of individuals of each species collected during different collection periods, phases of the moon, and seasons of the year. Distinction between the four seasons was based on dates of the summer and winter solstices and the spring and fall equinoxes.

Results and Discussion

Relative Abundance

Culicoides mississippiensis, *C. furens*, *C. barbosai*, and *C. floridensis* Beck were found to bite man near Yankeetown, Florida. A total of 9,563 specimens were obtained in the host-seeking collections. The majority of individuals (79.1%) were *C. mississippiensis* (Table 2). This species was most abundant in the spring (33.8%) when biting activity in general was greatest (38.1%). The least common host-seeking species was *C. furens* (2.8%).

The four species taken in the biting collections were represented in the vehicle-mounted trap collections but in different frequencies relative to one another (Table 3). *Culicoides mississippiensis* was the most common, represented by 65.7% of the 291,346 specimens collected. Kline (1984) also found it to be the most prevalent species of *Culicoides* in the Yankeetown area based on light trap collections. The least common biting species, *C. furens*, was the second most abundant (29.6%) in the trap. Only 1% of the individuals obtained in the trap was *C. floridensis*. The low percentage of this species does not accurately reflect its frequency as a pest of man in the area (Table 2).



DATE ___ ___ ___ ___ ___ ___ ___ ___ ___
day month year period sample

START TIME ___ ___ ___ ___
hours minutes

MOON PHASE ___ ___

SEASON ___ TEMP. ___ ___.___°C HUMIDITY ___ ___%

WIND ___ ___m/sec ___ ___ ___°
speed direction

LIGHT ___ ___ ___ ___ ___
foot candles

Culicoides spp.	males	females
C. mississippiensis	___ ___ ___ ___	___ ___ ___ ___
C. barbosai	___ ___ ___ ___	___ ___ ___ ___
C. furens	___ ___ ___ ___	___ ___ ___ ___
C. floridensis	___ ___ ___ ___	___ ___ ___ ___

COLLECTION NUMBER ___ ___ ___ ___

Figure 6. Data collection form used to record all data from vehicle-mounted trap program.

In addition to the above sampling program, environmental conditions were monitored. Temperature (°C) and wet bulb depression were measured after every vehicle-mounted trap collection by using a hand-held sling psychrometer. Light intensity (foot candles) was measured at the same time by using an International Light model IL710A Research Photometer®. A weather station was set up at the research site to monitor wind speed and wind direction throughout the study (Figure 4).

All data from the vehicle-mounted trap program were recorded on a data collection form (Figure 6) to aid in computer entry. Abbreviations were used for some information on the form to reduce the amount of material entered. The term 'period' referred to collection periods 1-20 and the term 'sample' referred to the 3 samples (A, B, or C) collected in each period. The time that a specific sample was collected was entered as start time. Moon phase was abbreviated as NM, FQ, FM, and LQ for new moon, first quarter, full moon, and last quarter, respectively. Abbreviations for seasons were P (spring), S (summer), F (fall), and W (winter). All samples were assigned a collection number in the order they were obtained. Each collection number was recorded on the collection bag, at the bottom of the collection form, and at the end of the specific line of computer entry for all data associated with a given sample. The collection number served as a means of cross-indexing these 3 aspects of record keeping. Data from the host-seeking study were recorded in a field notebook. Records were entered into the University of Florida computer system and analyzed by using the Statistical Analysis System (SAS) packaged programs. An analysis of variance (ANOVA) and Duncan's multiple range test were used as part of the General Linear Models procedure (Goodnight et al., 1982). The statistical significance were active for less of the diel cycle than females, especially during the summer.

The total number of females was usually greater than the number of males but at certain times of the day such as just after sunset the males actually outnumbered the females by as much as 3:1 (not illustrated). This was especially true in late March, early April, and late October when the males were most abundant. Mating swarms have been observed for *C. mississippiensis* around sunset (D.L. Kline, personal communication) and such activity may explain the change in sex ratio. Nathan (1981) observed swarms of *C. phlebotomus* being intercepted by a vehicle-mounted trap in north Trinidad and the male to female ratio changed drastically during such instances.

The ratio of male to female *C. furens* was also about 3:1 just after sunset during months of greatest male activity, May and August. In general, both sexes appeared together in large numbers during the evening twilight of spring, summer, and fall and during the morning twilight of summer (Figures 13 and 14). The evening peak was consistently greater than the morning peak. Females (Figure 13) did not undergo seasonal shifts in their bimodal periodicity but males did (Figure 14). In spring, the nighttime flight activity of the males declined through morning twilight and did not increase until period 1, immediately after sunrise.

Koch and Axtell (1979) also observed a larger evening peak for *C. furens* females in suction trap collections in coastal North Carolina but Bidlingmayer (1961) collected more females during the morning by using a vehicle-mounted trap near Vero Beach, Florida. Males were not collected in sufficient quantities to evaluate in either study. Kline and Roberts

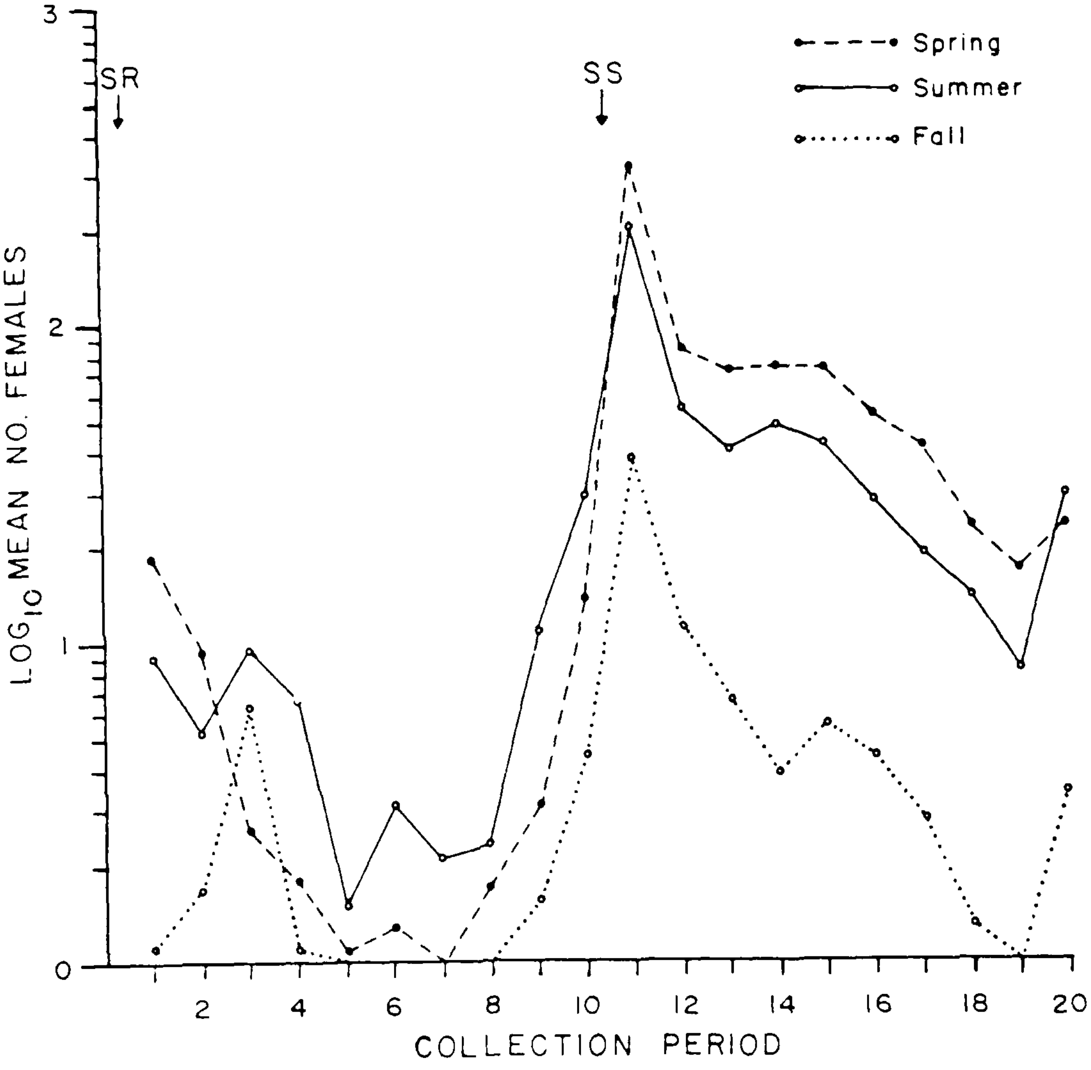


Figure 13. Diel periodicity of *C. furens* females collected in a vehicle-mounted trap during different seasons.

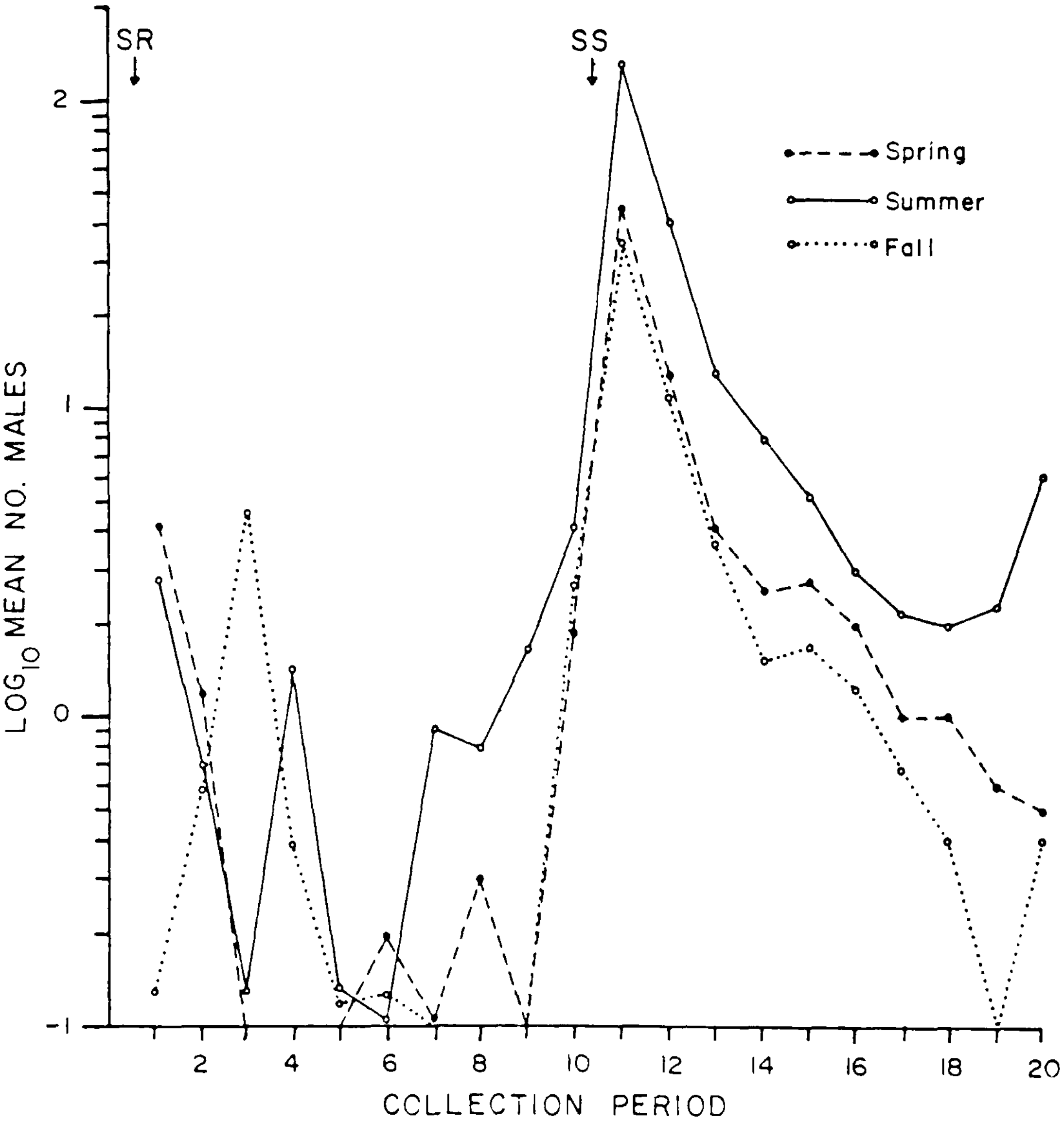


Figure 14. Diel periodicity of *C. furens* males collected in a vehicle-mounted trap during different seasons.

(1982) did not obtain enough of either sex of C. furens near Punta Rassa, Florida, to make an assessment of their diel periodicity.

Females of C. barbosai were active primarily during the twilight periods, but, unlike C. furens and C. mississippiensis, the morning peak was greater than that at dusk (Figure 15). Kline and Roberts (1982) also observed a higher level of activity in the morning for this species near Punta Rassa, Florida. They used a non-attractant device, a suction trap, to sample the population. Near Yankeetown, the females were active during scotophase in the spring only. Activity increased prior to sunrise and the females remained active for about 2-3 hours after that time. Additional activity during photophase occurred only in the fall. Major peaks of male flight activity (Figure 16) coincided with those of the females. Males were also more abundant at dawn than at dusk but only during the summer. Neither sex of C. barbosai was active during the winter.

Culicoides floridensis was collected in low numbers during late spring and early summer. Females were active during most of the diel cycle (Figure 17). In general, their activity was crepuscular and nocturnal with peaks of approximately equal amplitude immediately after sunset and just before sunrise. Too few males were taken to provide an accurate evaluation of their diel periodicity. Of the 31 males collected throughout the study, 10 were obtained during period 20, 5 during each of periods 1 and 10, and the remainder during scotophase.

Lunar Periodicity

Adult females of C. mississippiensis, C. furens, and C. barbosai were active for a greater duration of the scotophase and in greater numbers when the moon was full. The occurrence varied seasonally. Males

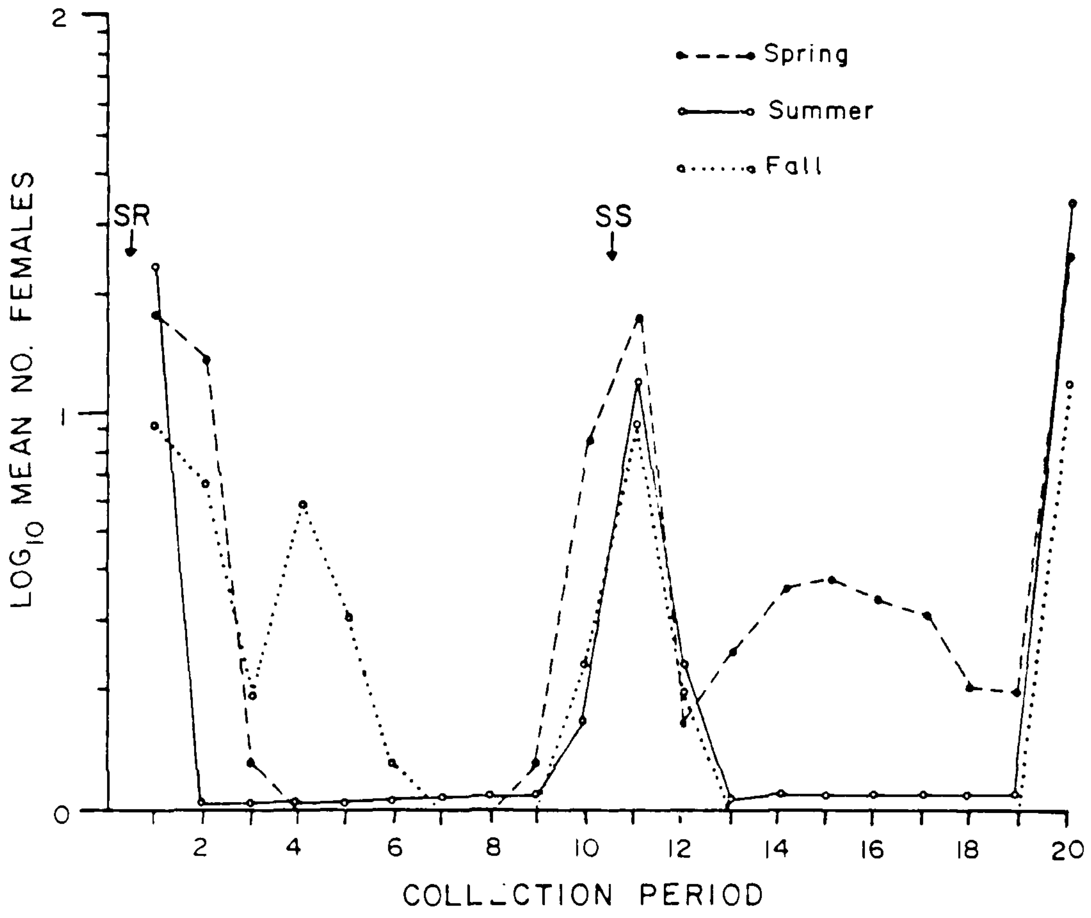


Figure 1[illegible]. Diel periodicity of *C. barbosai* females collected in a vehicle-mounted trap during different seasons.

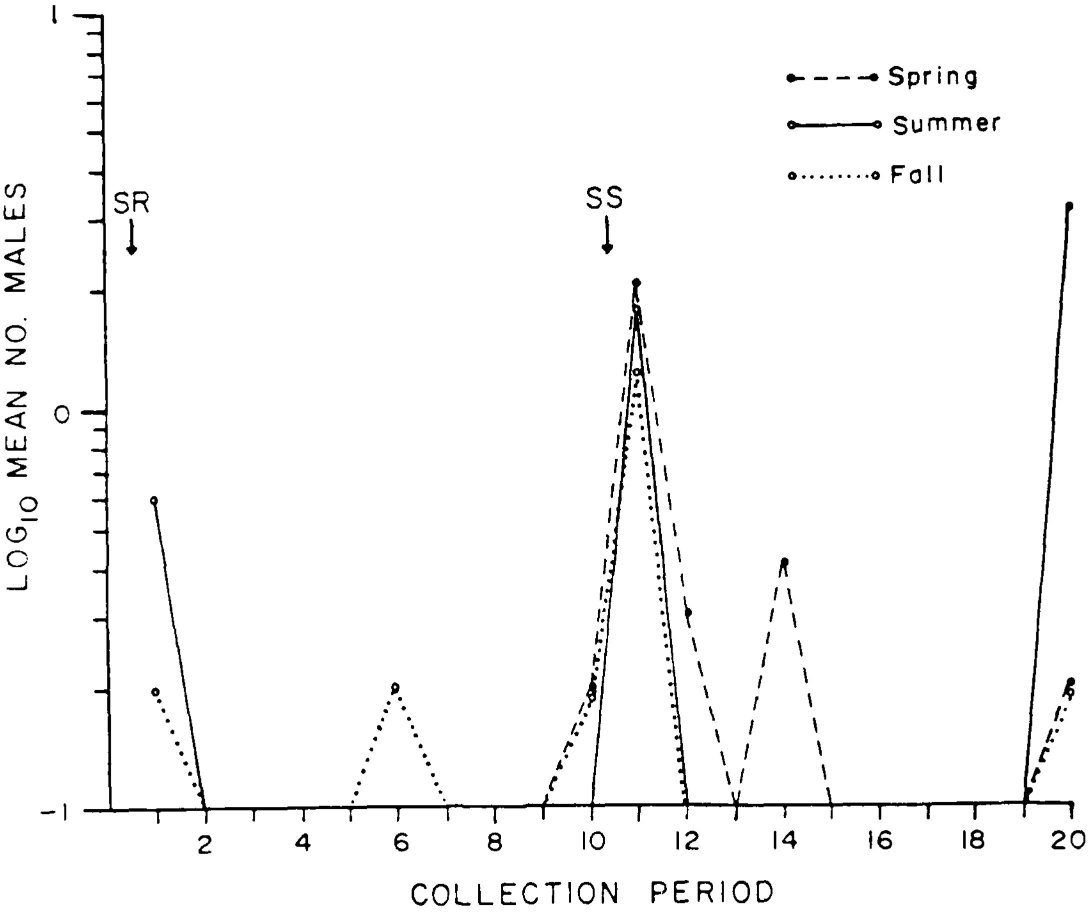


Figure 16. Diel periodicity of *C. barbosai* males collected in a vehicle-mounted trap during different seasons.

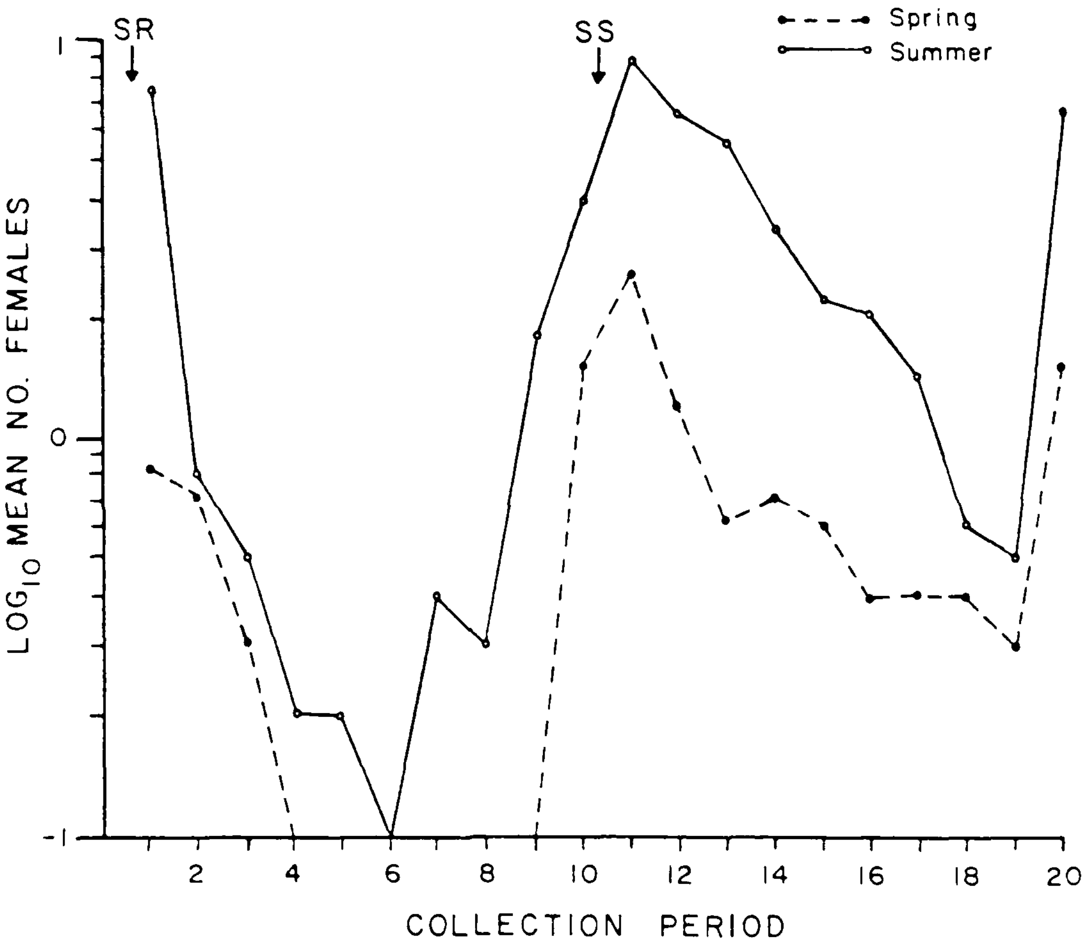


Figure 17. Diel periodicity of C. floridensis females collected in a vehicle-mounted trap during different seasons.

of these species did not show the same relationship (Figure 18). The combined data for the three species indicates that the diel periodicity of the males did not change significantly relative to the phase of the moon. This differed from C. crepuscularis and C. variipennis in northeastern Colorado where both sexes were active in greater numbers when the moon was full (Barnard and Jones, 1980b).

More C. mississippiensis females were caught when the moon was full in spring (Figure 19) and summer (Figure 20) than in fall (Figure 21) and winter (Figure 22). The number of individuals in flight peaked during period 11, declined in periods 12 and 13, but changed very little after that time when the moon was full (Figures 19 and 20). The amplitude continued to decrease after period 13 when the moon was in some other phase. During last quarter moon, female activity began to increase when the moon rose midway through scotophase (ca. period 16). Barnard and Jones (1980b) also reported an increase in the abundance of Culicoides spp. at the time of moonrise in northeastern Colorado. Bidlingmayer (1967) observed the same phenomenon for mosquitoes in Florida. Another noteworthy association with the moon was a rapid decrease in female activity at the time of moonset (ca. period 16) during the first quarter phase (Figure 19).

Light intensity may have regulated flight activity during scotophase; however, other factors were most likely important because new moon collections often surpassed those of first and last quarter phases (Figures 19 and 20). Temperature was probably a critical factor in the fall (Figure 21) and winter (Figure 22) because the activity of biting midges declined rapidly as the temperature decreased after sunset. The number of individuals often increased again during period 15, 16 or 17,

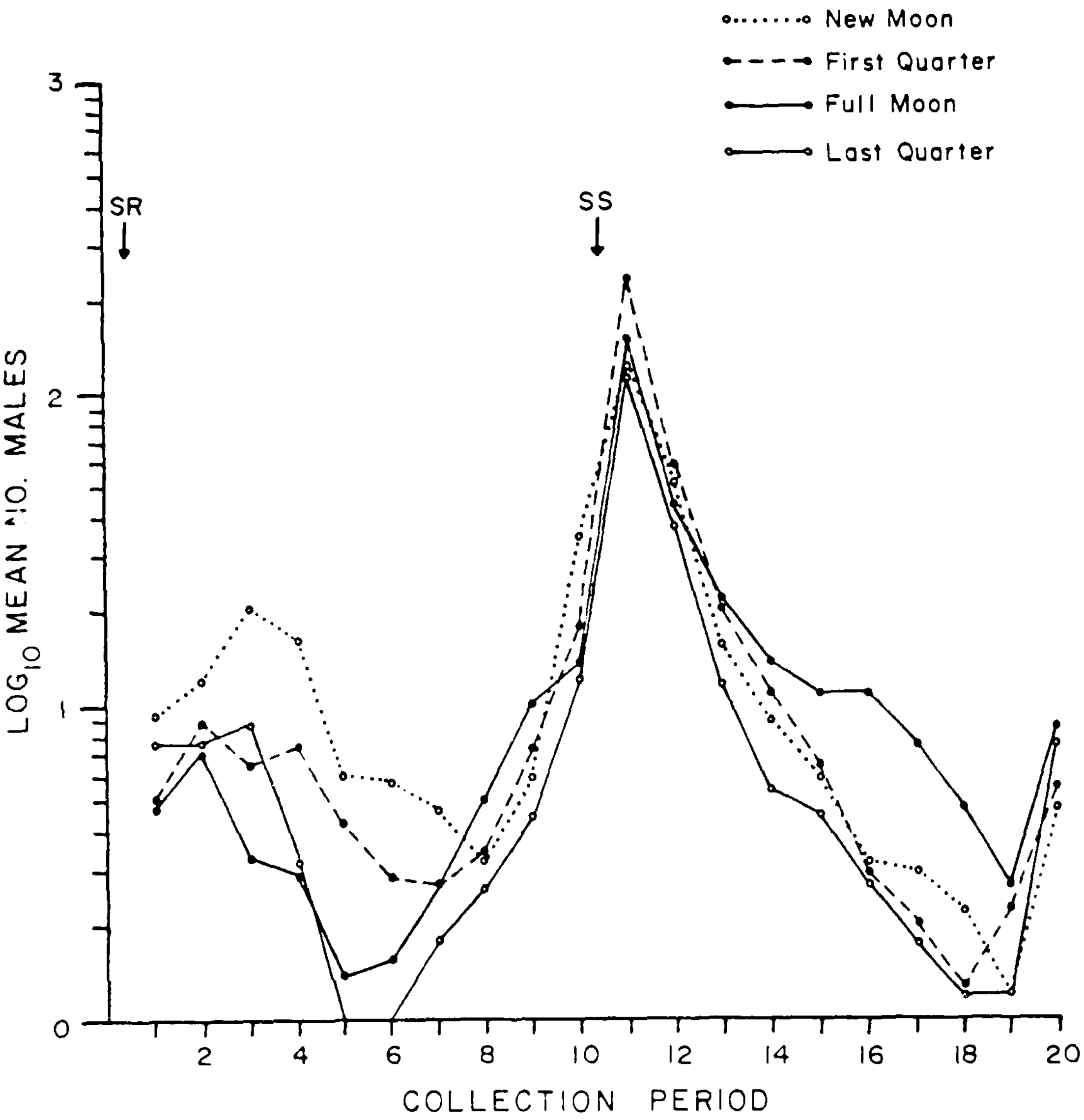


Figure 18. Diel periodicity of male Culicoides spp. collected in a vehicle-mounted trap on quarter phases of moon.

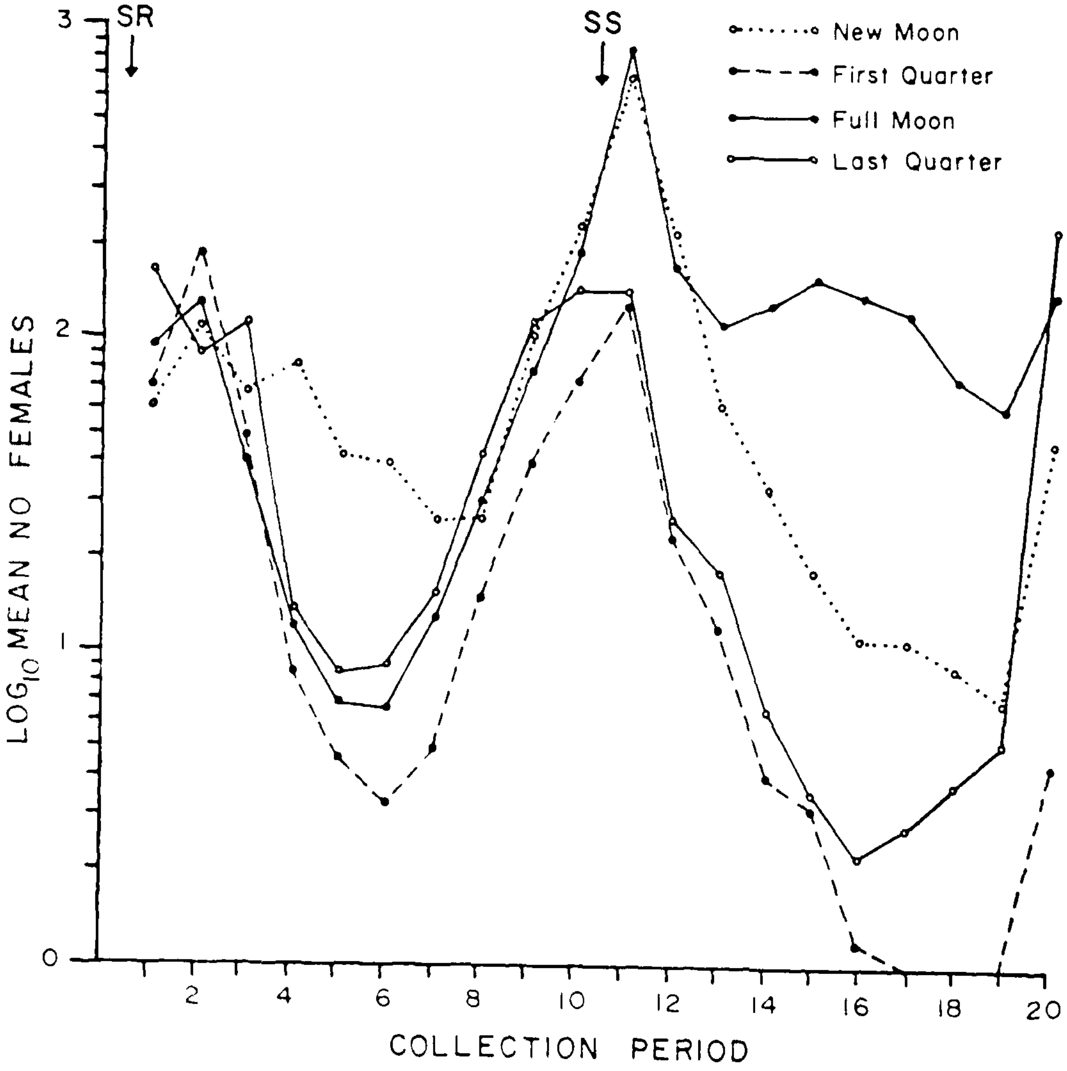


Figure 14. Diel periodicity of *C. mississippiensis* females collected in a vehicle-mounted trap on quarter phases of moon in the spring.

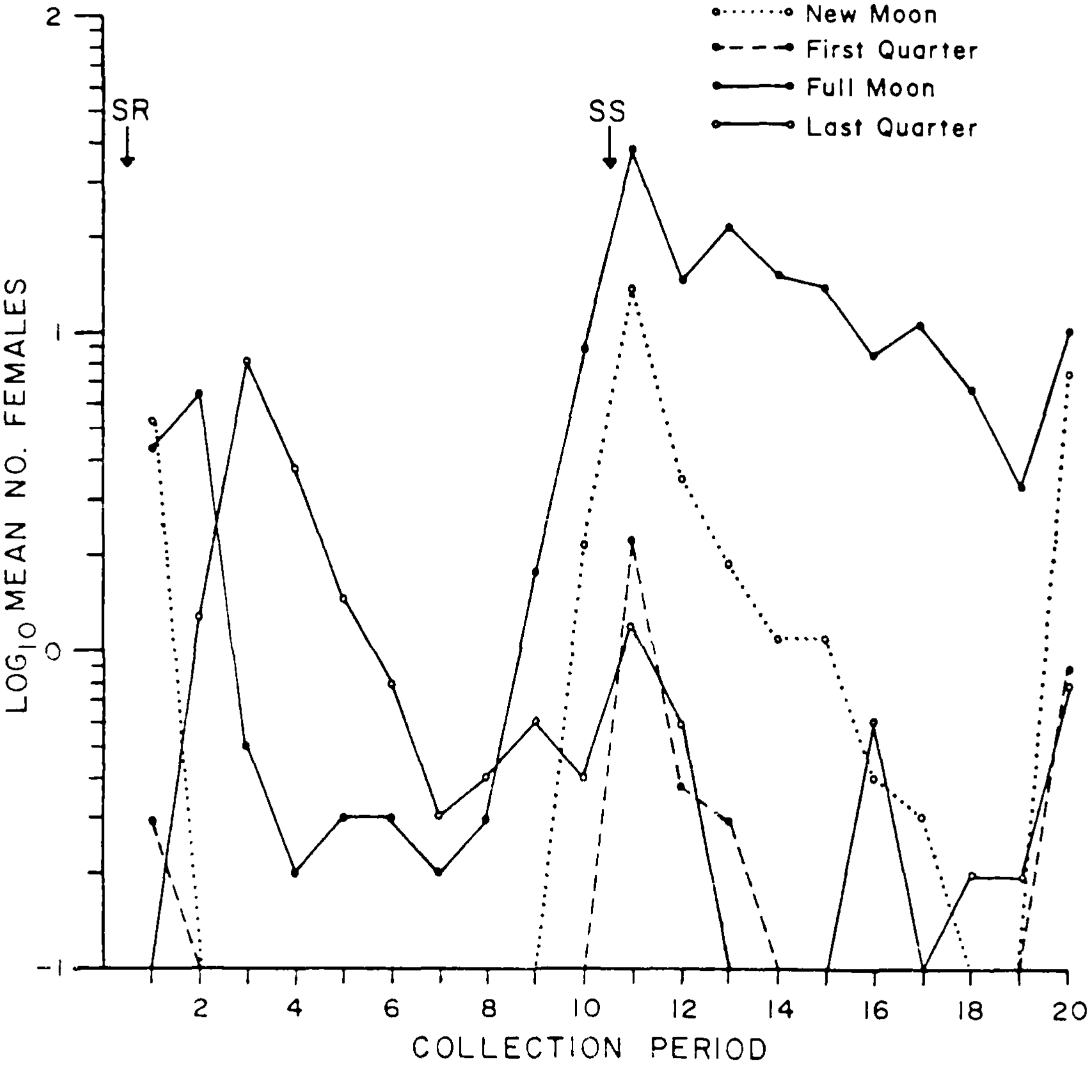


Figure 20. Diel periodicity of *C. mississippiensis* females collected in a vehicle-mounted trap on quarter phases of moon in the summer.

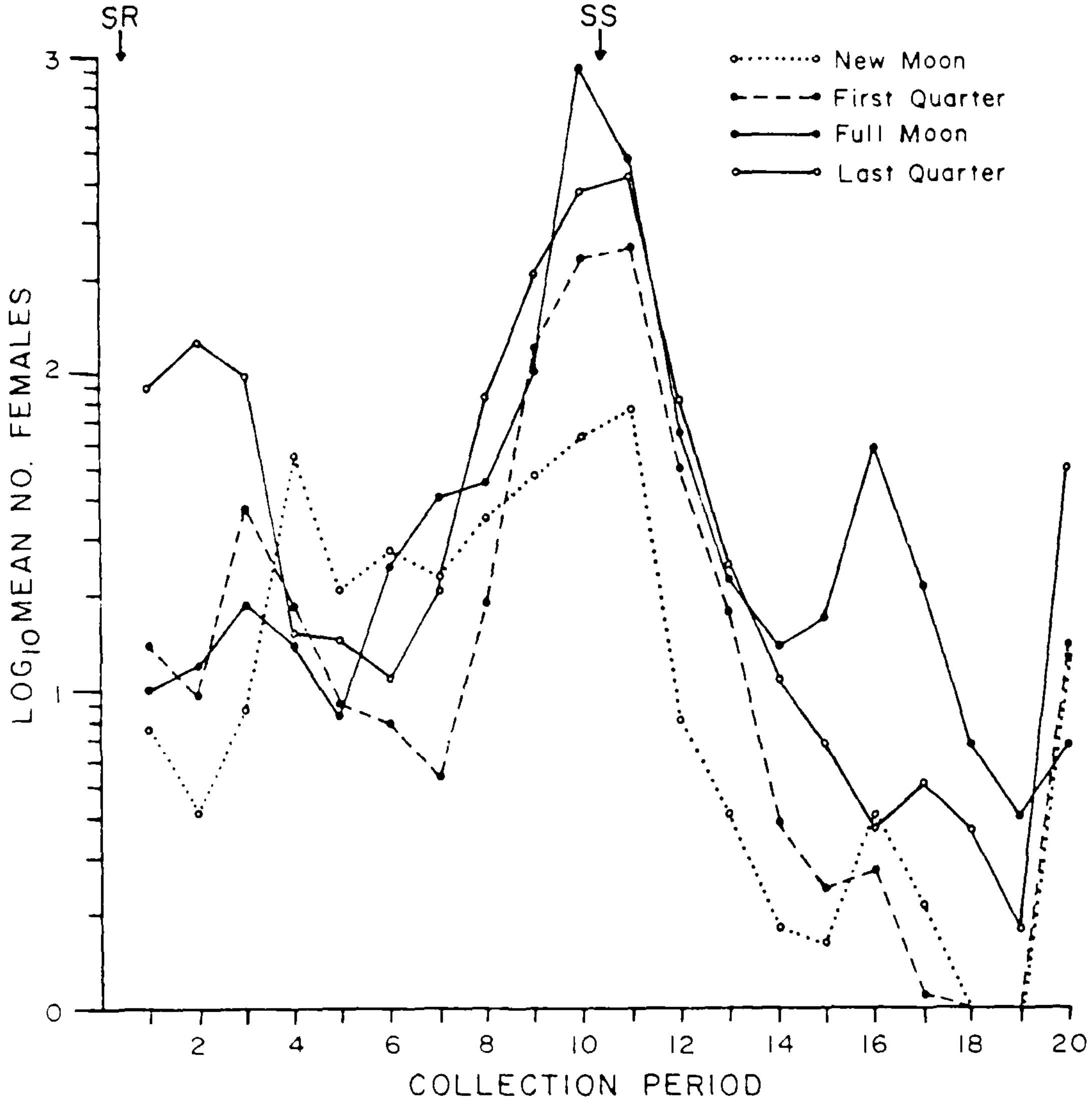


Figure 21. Diel periodicity of *C. mississippiensis* females collected in a vehicle-mounted trap on quarter phases of moon in the fall.

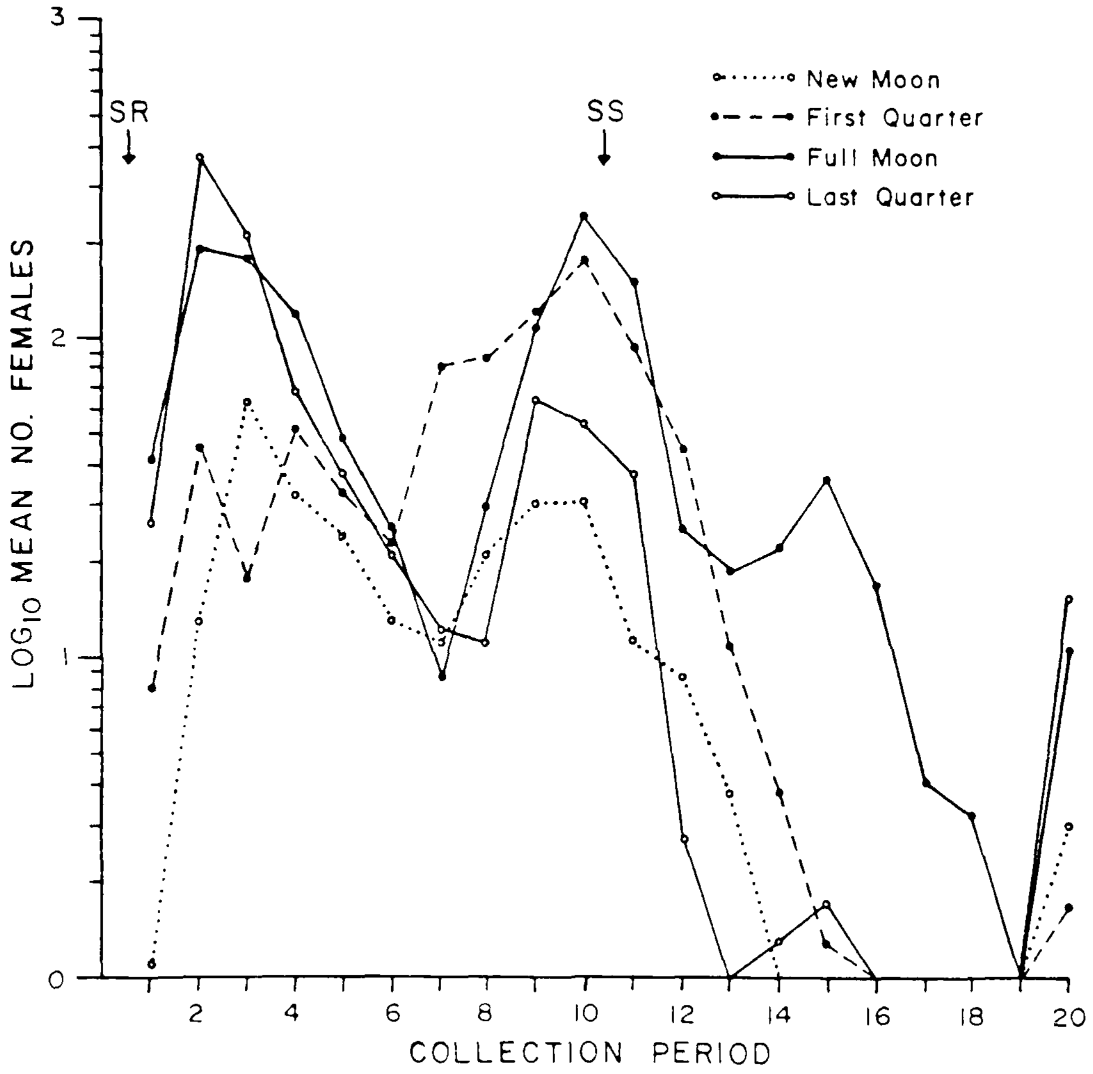


Figure 22. Diel periodicity of *C. mississippiensis* females collected in a vehicle-mounted trap on quarter phases of moon in the winter.

but it did not persist, even following moonrise, for the duration of the night. In winter, the adults were active for less of the scotophase than in other seasons (Figure 22).

The relationship between moon phase and C. furens activity was similar to that of C. mississippiensis during the spring (Figure 23) and summer (Figure 24) only. Specimens were collected in great quantity throughout the night when the moon was full. Also, an increase in activity followed moonrise while a decrease in activity followed moonset (ca. period 16). In fall, C. furens activity (Figure 25) was similar to that of C. mississippiensis (Figure 21) by showing an increase during period 15 or 16, but the 2 species differed in their relationship with full moon in that season. Culicoides furens were taken in large numbers on some occasions in the fall when the moon was full but not always. The mean for full moon collections was less than or equal to the means for new and last quarter (Figure 25). Bidlingmayer (1961) also reported an inconsistent pattern with respect to moon phase and C. furens activity in Florida. He conducted his study during the summer and fall of 1959 and found that the number of females collected during scotophase was not always greatest when the moon was full.

The association with full moon and female activity did not fluctuate with the seasons for C. barbosai. The number of individuals in flight was always highest when the moon was full, therefore, the data were combined for all seasons (Figure 26). The adults were virtually absent between periods 14 and 19 during new, first quarter, and last quarter moon. The amplitude of the nighttime collections of this species during full moon was approximately 20 times greater than during other phases.

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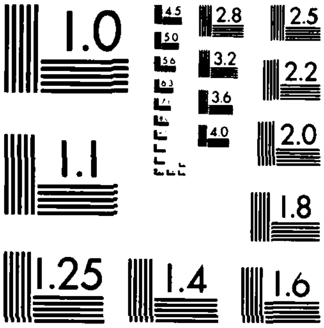

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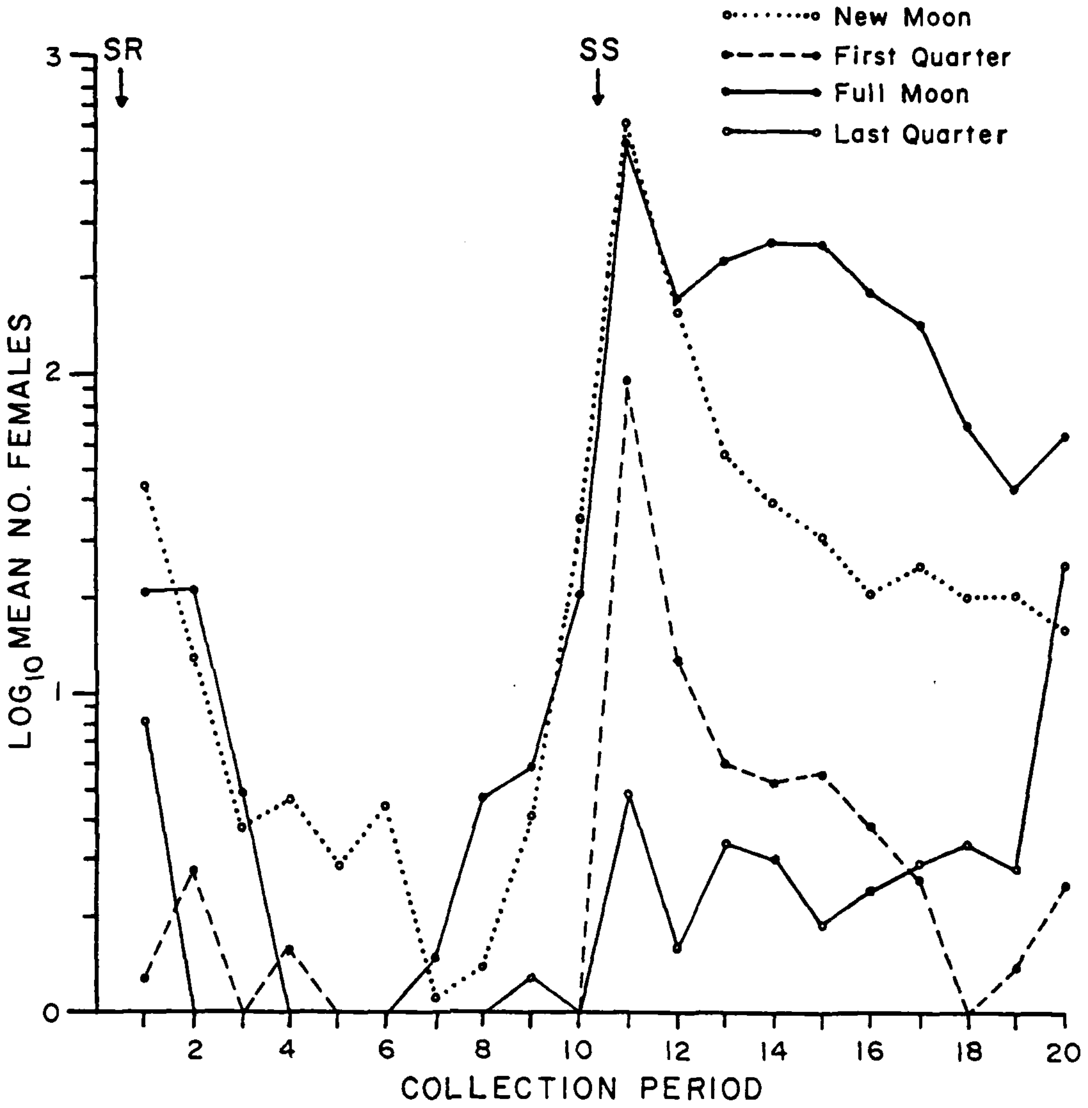

Figure 23. Diel periodicity of C. furens females collected in a vehicle-mounted trap on quarter phases of moon in the spring.

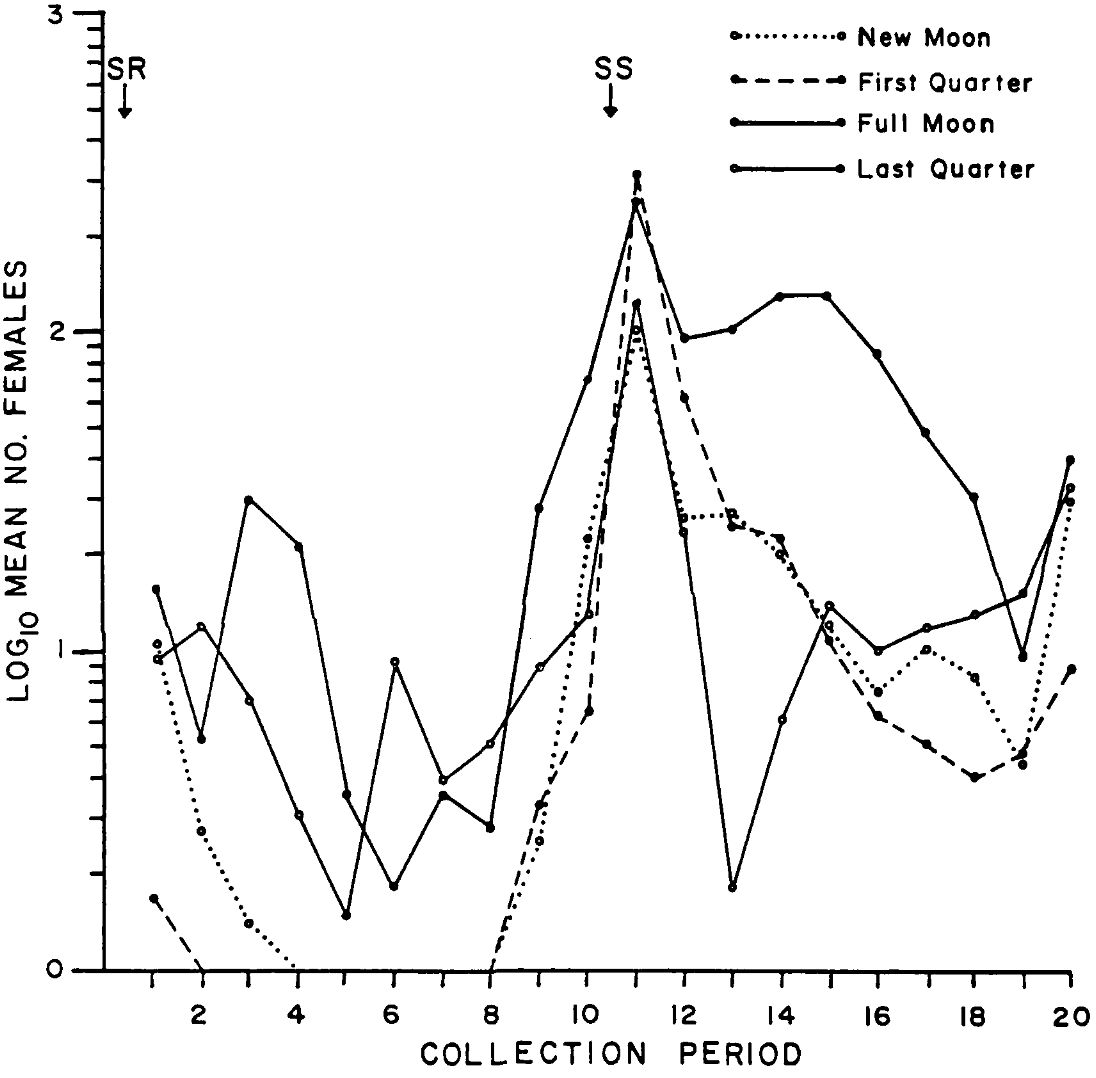

Figure 24. Diel periodicity of C. furens females collected in a vehicle-mounted trap on quarter phases of moon in the summer.

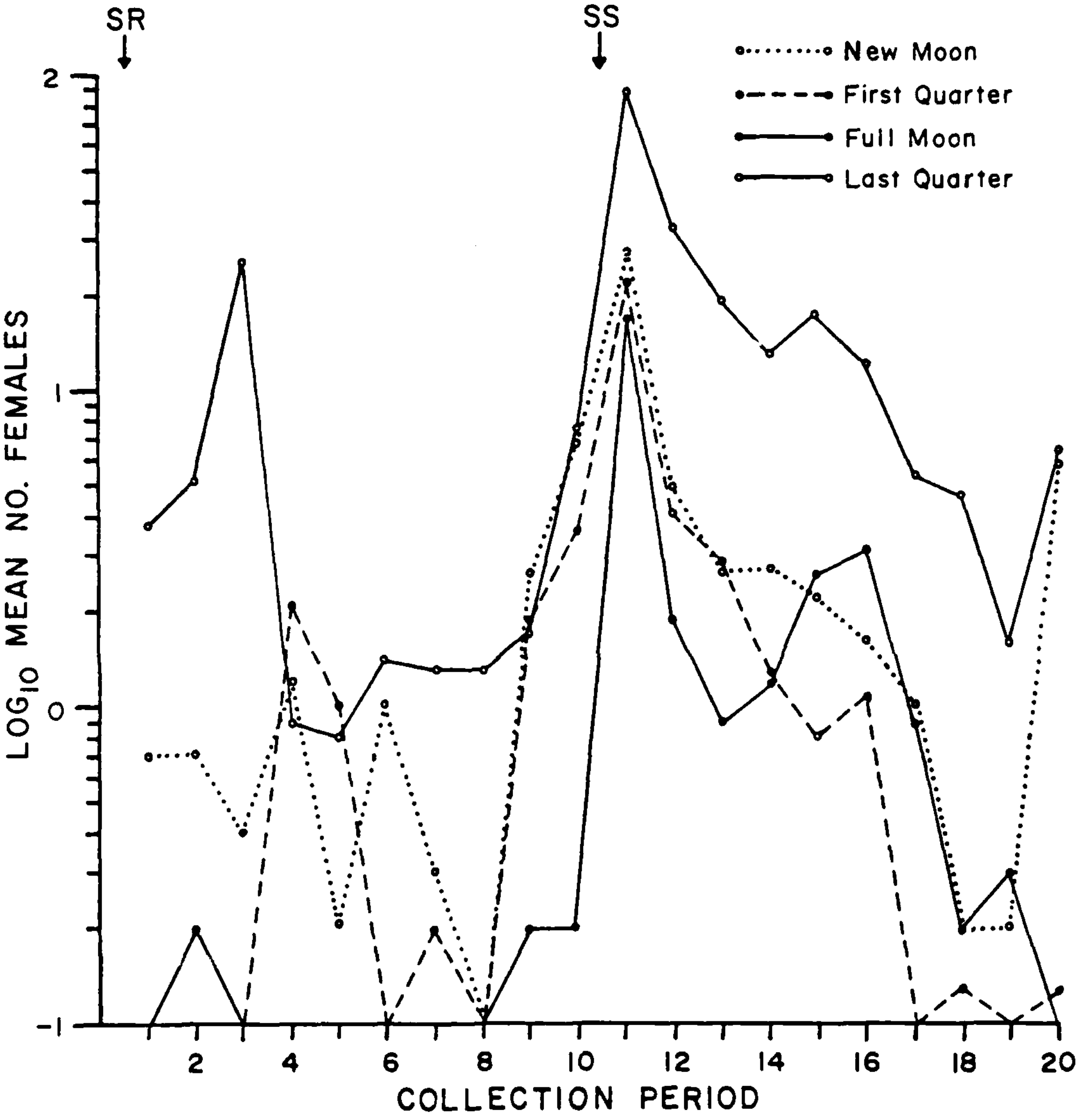

Figure 25. Diel periodicity of C. furens females collected in a vehicle-mounted trap on quarter phases of moon in the fall.

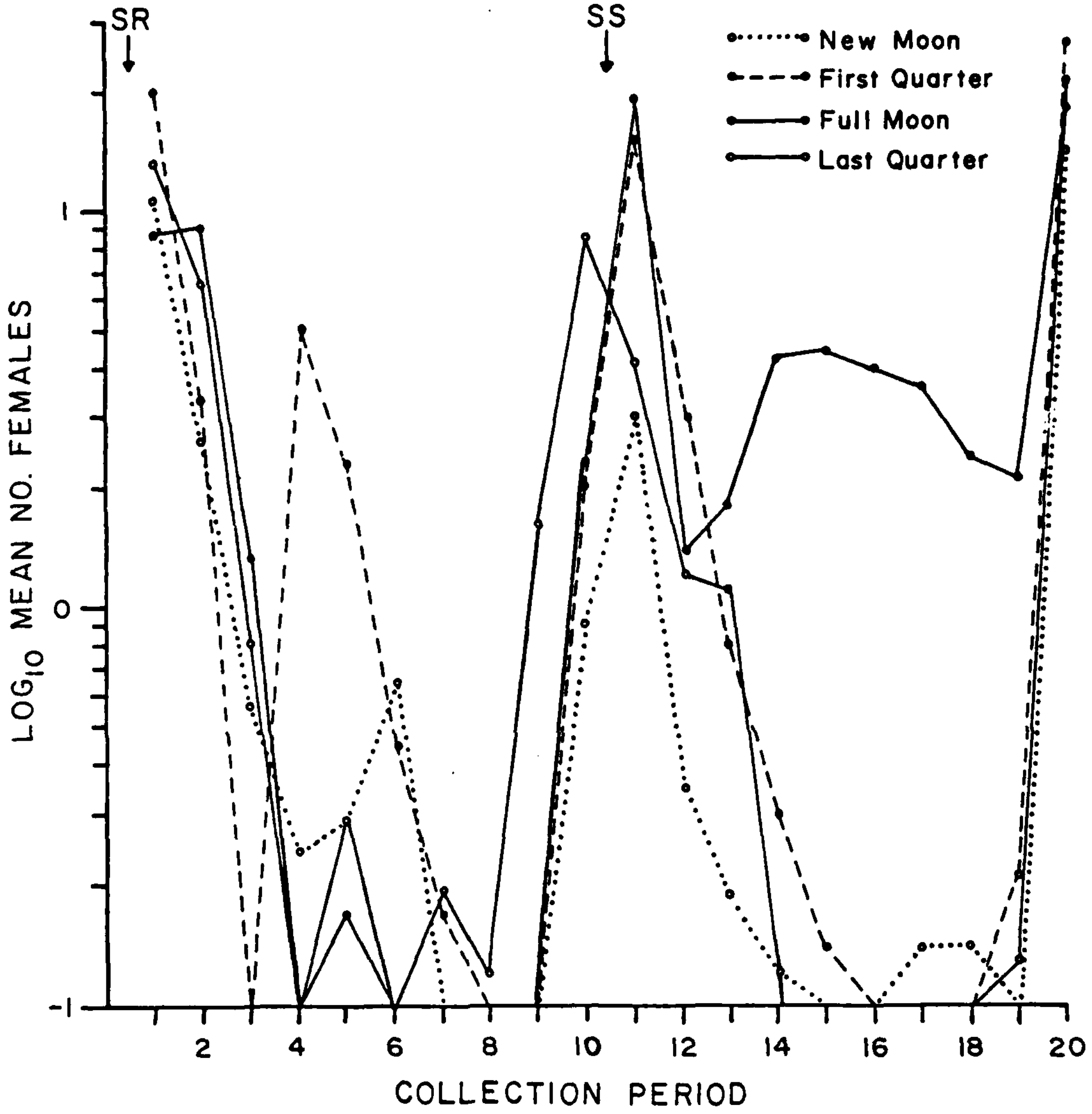

Figure 26. Diel periodicity of C. barbosai females collected in a vehicle-mounted trap on quarter phases of moon.

The occurrence of C. floridensis when the moon was full did not differ significantly ($p>0.05$) from new and first quarter moon collections (Figure 27). A possible reason for such behavior is that this species is active for only a brief portion of the year (Figure 10) compared to the other species. Individuals may cue on some environmental factor other than moonlight to regulate their nighttime activity. Another possible explanation is the bias of the vehicle-mounted trap as a collection device for C. floridensis. Fewer specimens were obtained in the trap (Table 3) than in aspirator collections (Table 2).

Diel and Seasonal Host-Seeking Activity

The diel periodicity of host-seeking specimens did not always agree with that of specimens collected in the vehicle-mounted trap. The most likely reason for this occurrence was that the two sampling methods procured different populations of a given species. Aspirator collections contained individuals seeking a bloodmeal. In contrast, the vehicle-mounted trap did not employ an attractant and was much less selective. The trap contained adults in search of an oviposition site or a mate as well as those seeking a host. The most apparent difference was that the vehicle-mounted trap collected both sexes while only females were obtained with the aspirator. Bidlingmayer (1961) encountered similar disparities in the two sampling techniques.

The number of C. mississippiensis attempting to engorge was greatest just before sunset (Figure 28). The rate of attack was unbearable at this time, particularly in the spring. As many as 190 specimens were collected from the forearm over a 5 min interval in late March. Biting was also intense on other exposed areas of the body especially the face and neck. Few specimens attacked during the first 2 min of exposure but

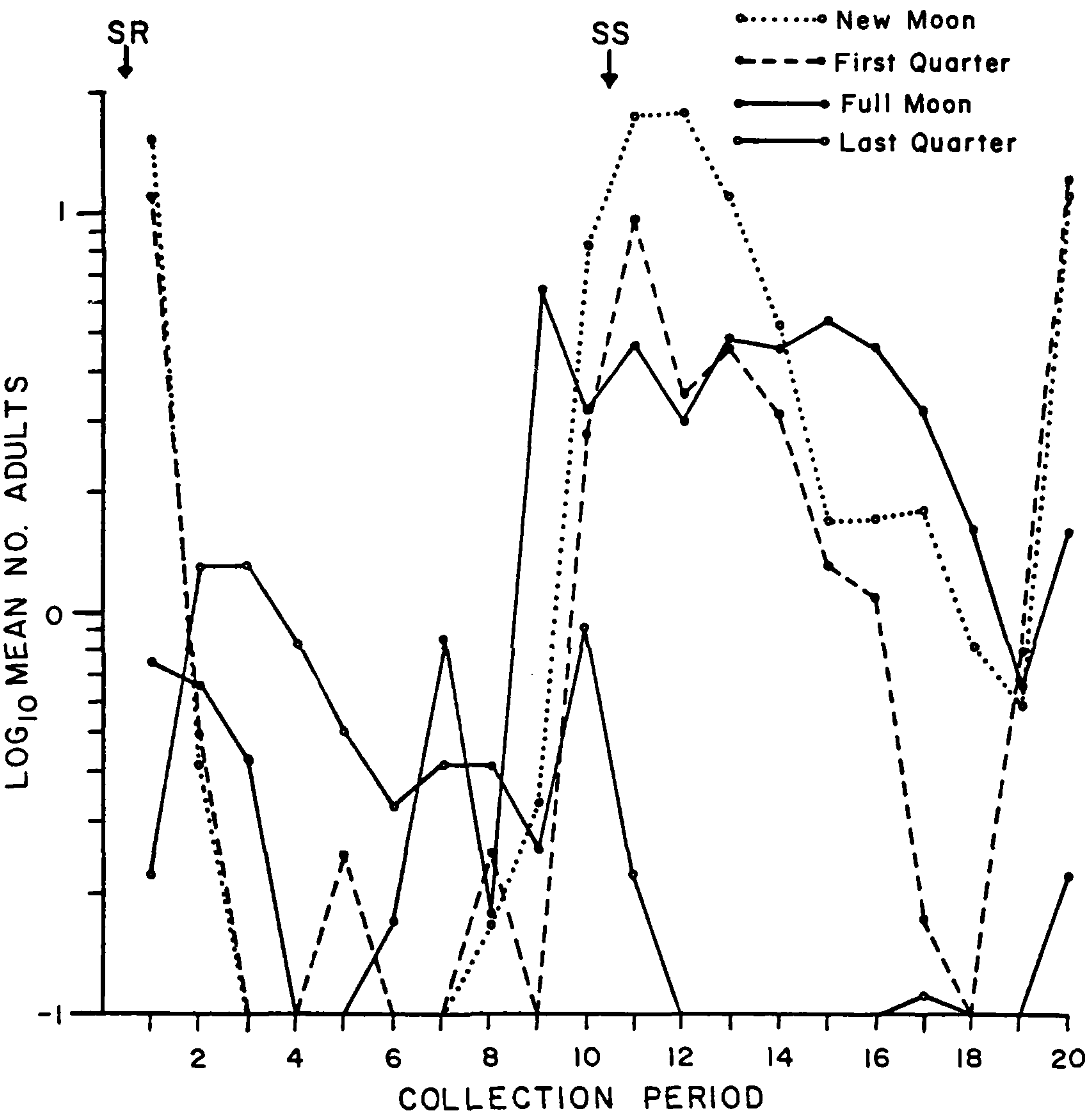

Figure 27. Diel periodicity of C. floridensis adults collected in a vehicle-mounted trap on quarter phases of moon.

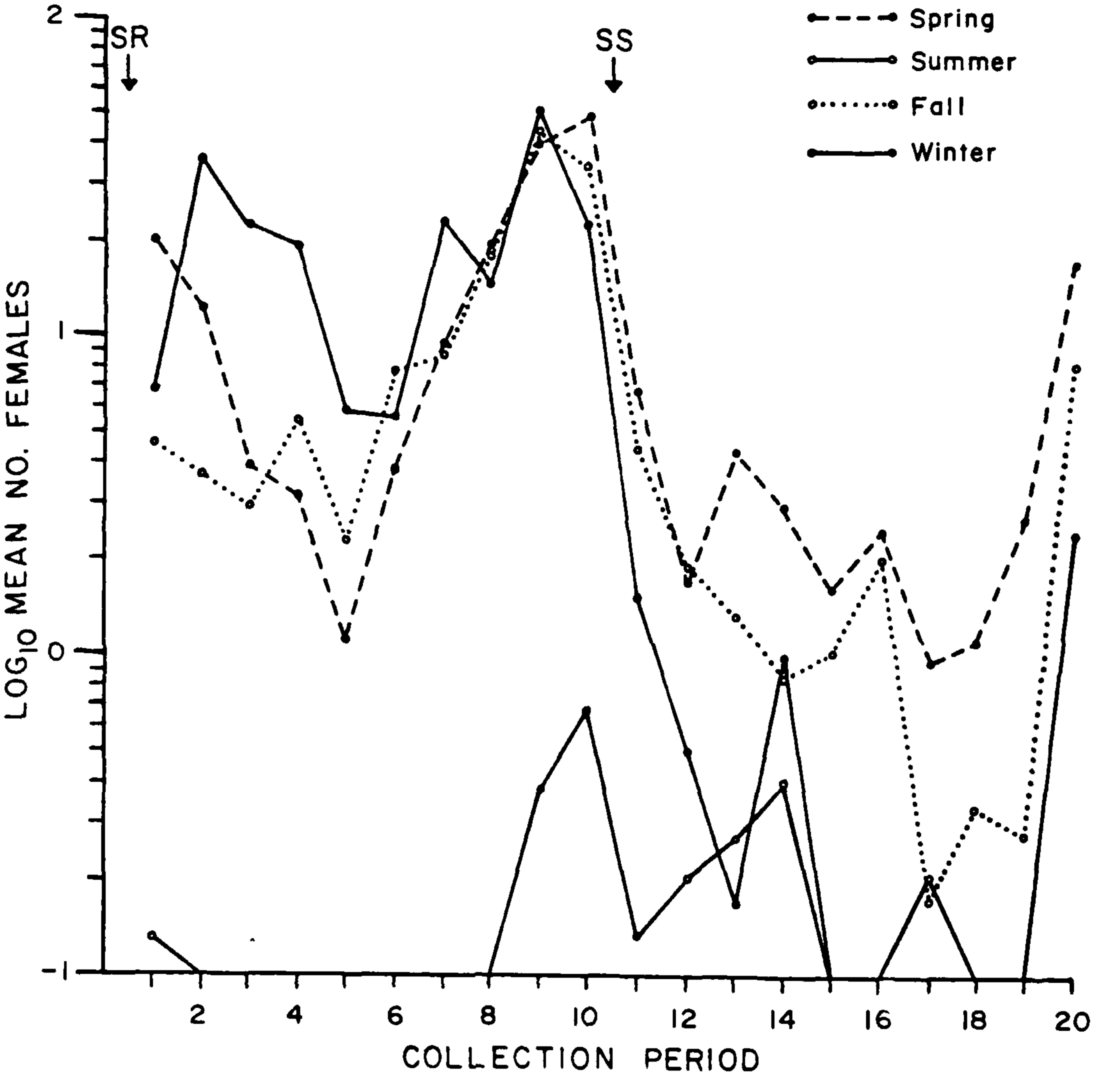

Figure 28. Diel host-seeking activity of C. *mississippiensis* during different seasons.

then they descended in waves approximately every 30-60 sec. Individuals usually crawled over the skin for a brief period before attempting to insert their mouthparts. It was during this crawling period that most specimens were taken with the aspirator.

The attack activity of *C. mississippiensis* dropped sharply after sunset but individuals continued to seek a bloodmeal throughout the scotophase in spring and fall (Figure 28). A second peak occurred during early morning hours. The increase began just before sunrise (period 20) and continued for 2-3 hours after that time during all seasons except summer. Attack by this species persisted throughout photophase in spring, fall, and winter. Only in summer was the host-seeking activity below the threshold, 5 bites/h (Linley and Davies, 1971), for comfortable human activity during most of the diel cycle. The attack rate was also low during portions of the night in fall and winter.

Culicoides floridensis was the next most abundant species taken in host-seeking collections. Individuals were attracted to a human host during spring, fall, and summer (Figure 29), although they were collected in the vehicle-mounted trap during spring and summer only (Table 3). This species was a vicious biter in the Yankeetown area. Attacks to the scalp were most annoying. The pale yellow color and small size hampered the collection process. Up to 81 specimens were collected over a 5-min period. Individuals crawled around for a longer time than other species before attempting to feed. They often flew around the host in a swarm, landed briefly, flew off a few centimeters, and finally alighted to crawl around and feed.

Blanton and Wirth (1979) reported that *C. floridensis* attacked man during the daytime in Florida. Contrary to their statement, biting

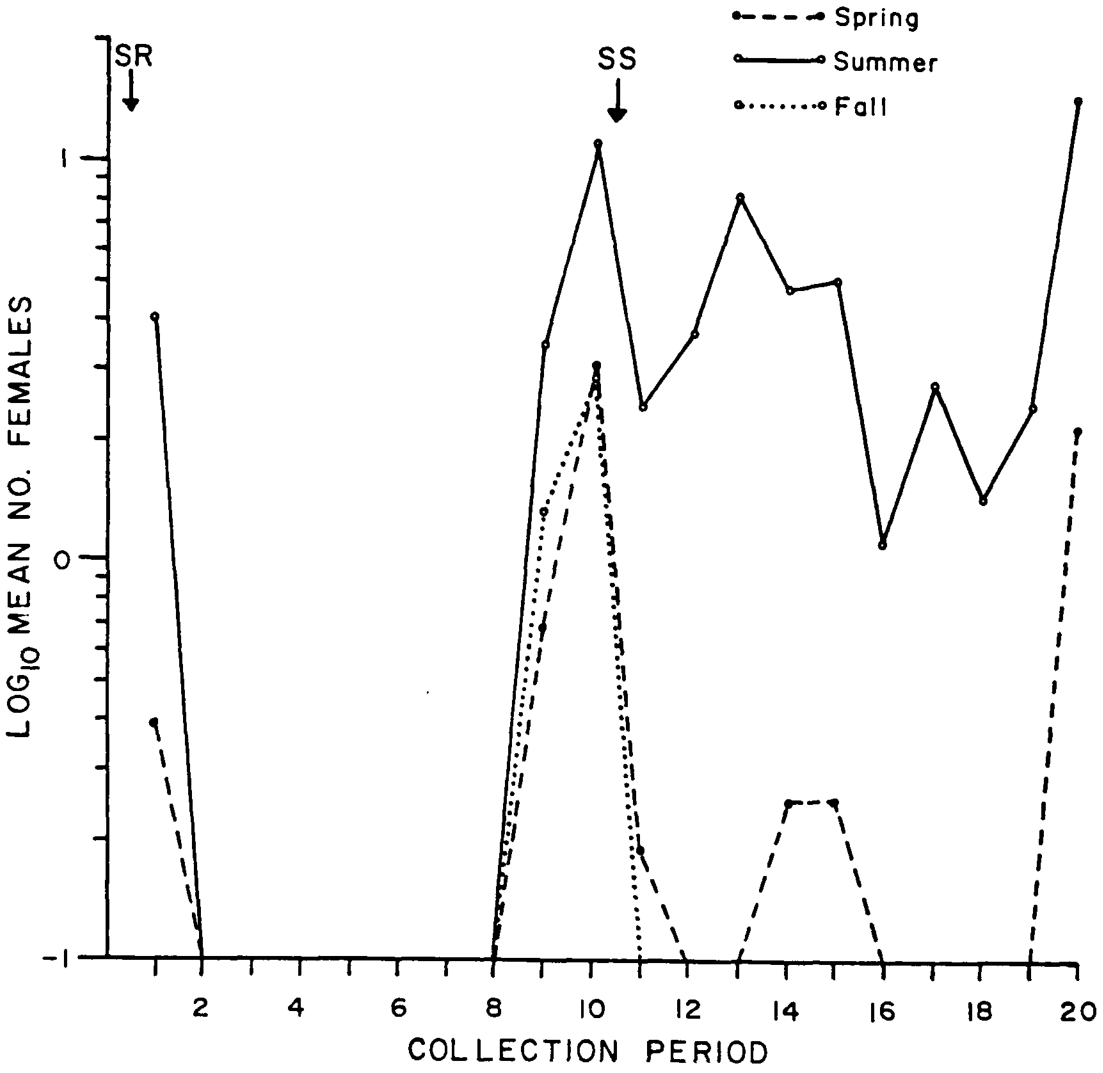

Figure 29. Diel host-seeking activity of C. floridensis during different seasons.

during the day was rare near Yankeetown (Figure 29). The number of host-seeking females declined to zero in the hour following sunrise and did not increase again, regardless of the season, until shortly before sunset. They remained active throughout the scotophase in summer only. Host-seeking activity during the fall was restricted to a brief time before sunset.

Culicoides barbosai was also actively seeking a host during all seasons except winter (Figure 30). Individuals would crawl over the skin for less time than other species prior to feeding. They often attempted to feed on the fingers and the palm of the hand. Kettle (1969a, b) found that *C. barbosai* preferred to feed on the legs rather than the arms of a human host at night but that was not examined in the Yankeetown study. The largest 5 min collection of this species was taken in late July and contained 80 specimens. The host-seeking activity was greatest at dusk and dawn with the dawn peak being more pronounced of the two. Biting occurred throughout the scotophase in spring and summer and usually declined rapidly after sunrise. Only during the fall did the biting activity of *C. barbosai* persist beyond the middle of the photophase.

Colicoides furens was absent from collections of host-seeking specimens during much of the daylight hours (Figure 31). Little was noted about the behavior of this species on the host because it was taken in such low frequency. Attack activity peaked shortly before sunset in the fall and subsequent peaks occurred with irregular periodicity during the night. Flies continued to seek a host for the entire scotophase during the summer and their activity at night was occasionally greater than at dusk.

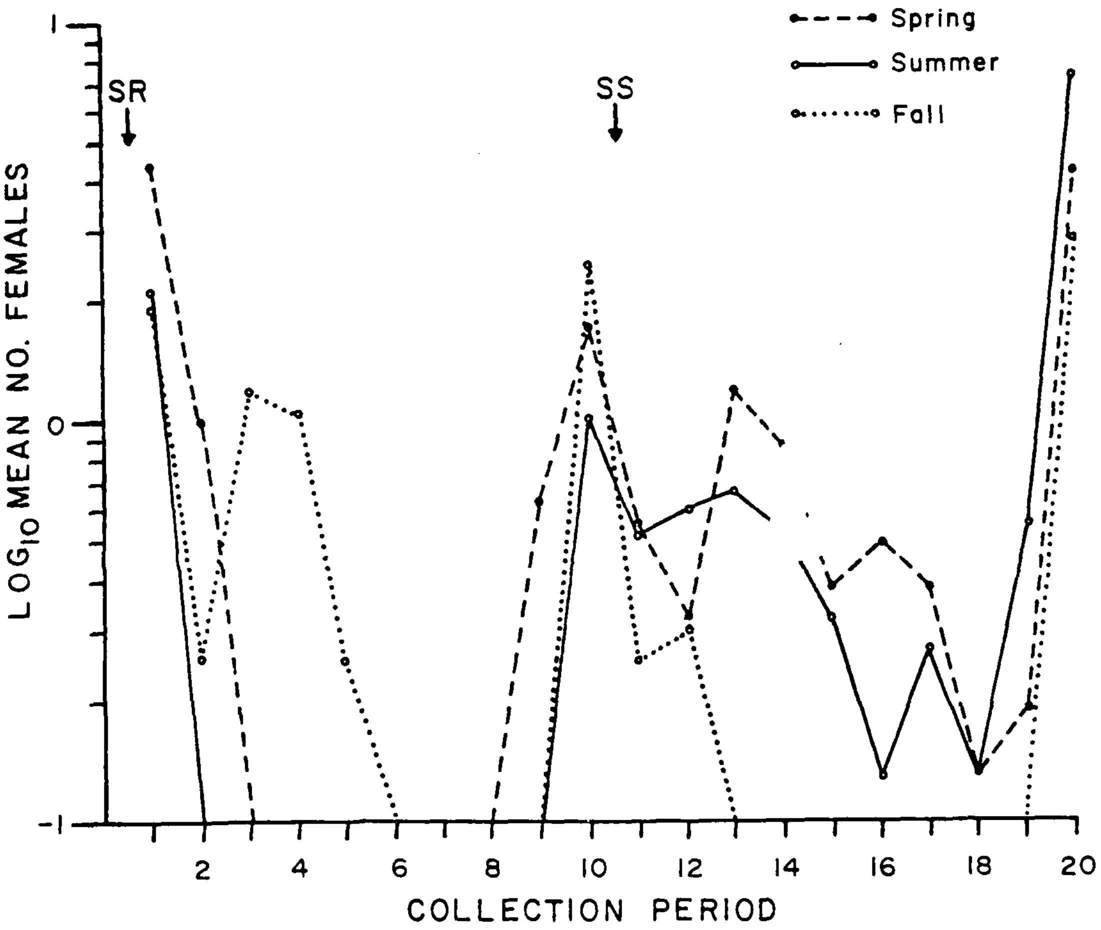

Figure 30. Diel host-seeking activity of C. barbosai during different seasons.

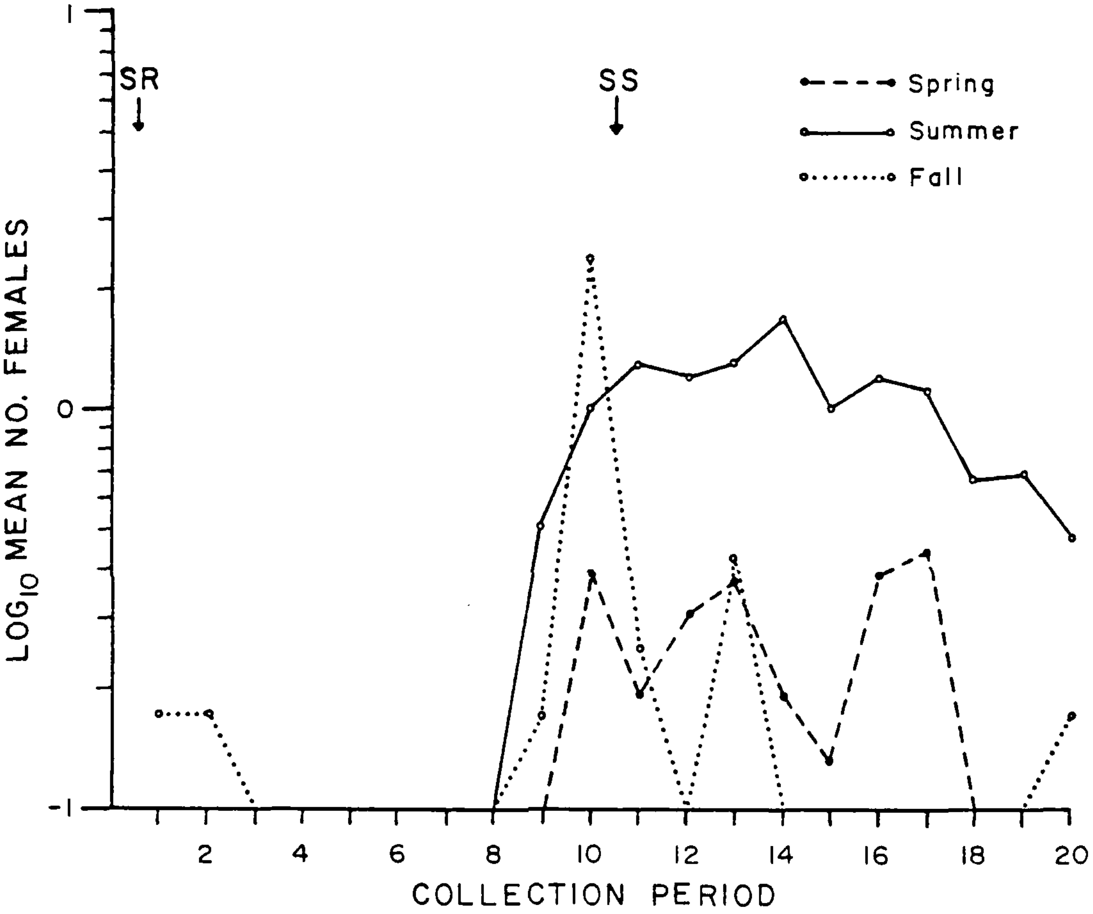

Figure 31. Diel host-seeking activity of _C_. _furens_ during different seasons.

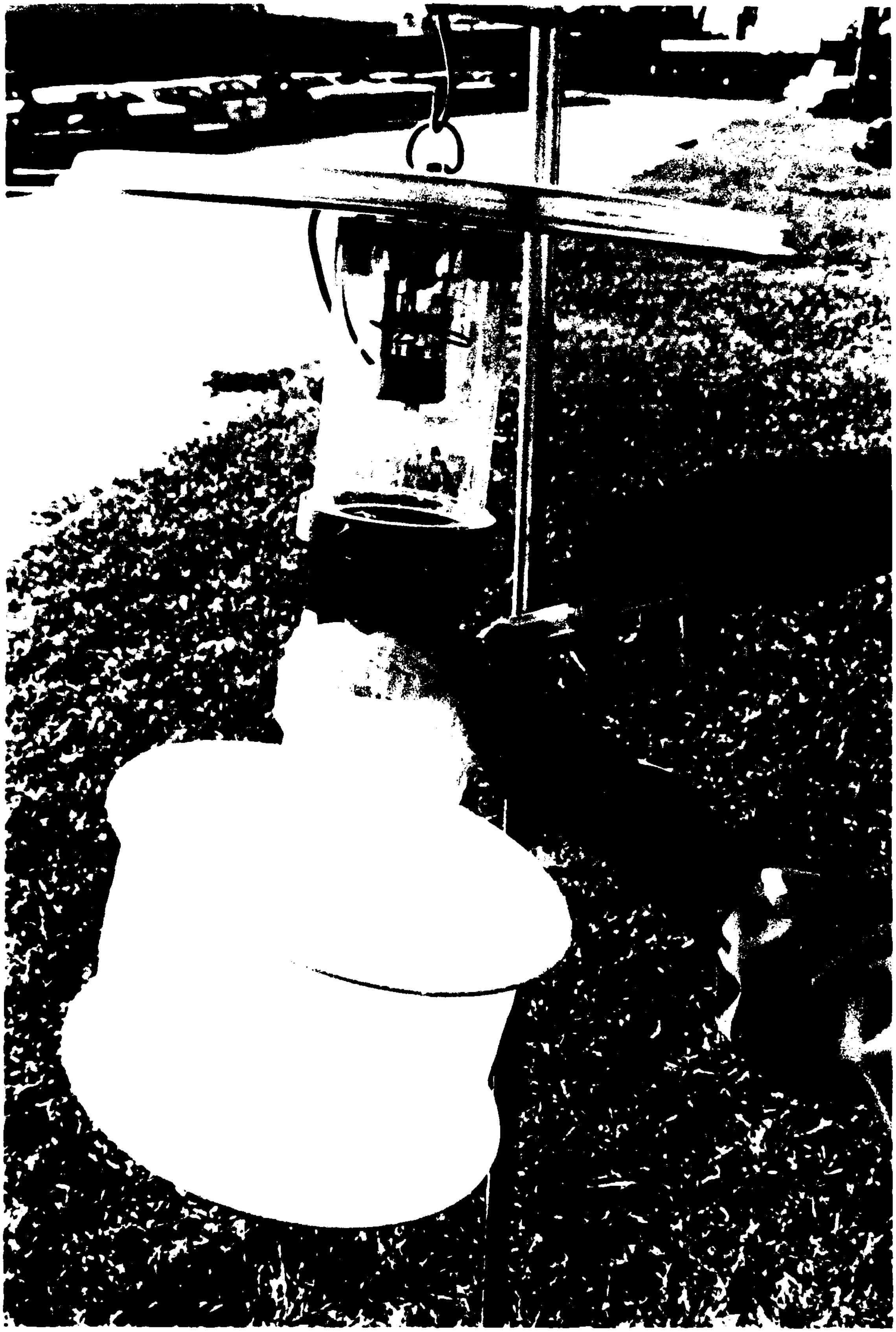

Figure 38. Researcher injecting fluorescent dust through brass screen of CDC trap to mark *C. mississippiensis* adults.

CHAPTER THREE
DISPERSAL OF *CULICOIDES MISSISSIPPIENSIS*

Objectives

1. To determine the mean distance traveled (MDT) by *C. mississippiensis* females following their release in a saltmarsh.

2. To determine if *C. mississippiensis* females developing in a saltmarsh near Yankeetown, Florida, are capable of dispersing from the breeding site to a nearby residential community.

Materials and Methods

A mark, release, and recapture procedure was used to accomplish the above objectives. Specimens for marking were live-trapped in 6 CDC portable light traps positioned in a saltmarsh near Yankeetown, Florida. Each trap was baited with 2.2 kg of solid CO_2 and operated for 24 hours. The collections were marked at 1030 h on 2 April 1984 by inserting the needle of a 5 ml syringe through the screen adaptor on the lower portion of a trap (Figure 38). Approximately 0.4 ml of micronized fluorescent dust (U.S. Radium[1] Color 1953, green) was injected into the collection bag. The fan on the trap was operated during the marking process to circulate the dust. Preliminary tests indicated that 100% of the specimens in the collection bag could be marked by using this technique. In a prior study of *C. variipennis*, the dust remained on marked adults

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[1] U.S. Radium Corp., P.O. Box 409, Hackettstown, N.J. 07840.

by saltmarsh on both sides and the site of the biting collections was adjacent to the marsh explains such results. An individual residing on the Gulf of Mexico, however, stated that biting midges were not a problem when the wind was blowing inland from the Gulf, but the annoyance was extremely great when it was from the opposite direction (D.D. Allen, personal communication). Studies were not performed to confirm his observation or to determine the species involved.

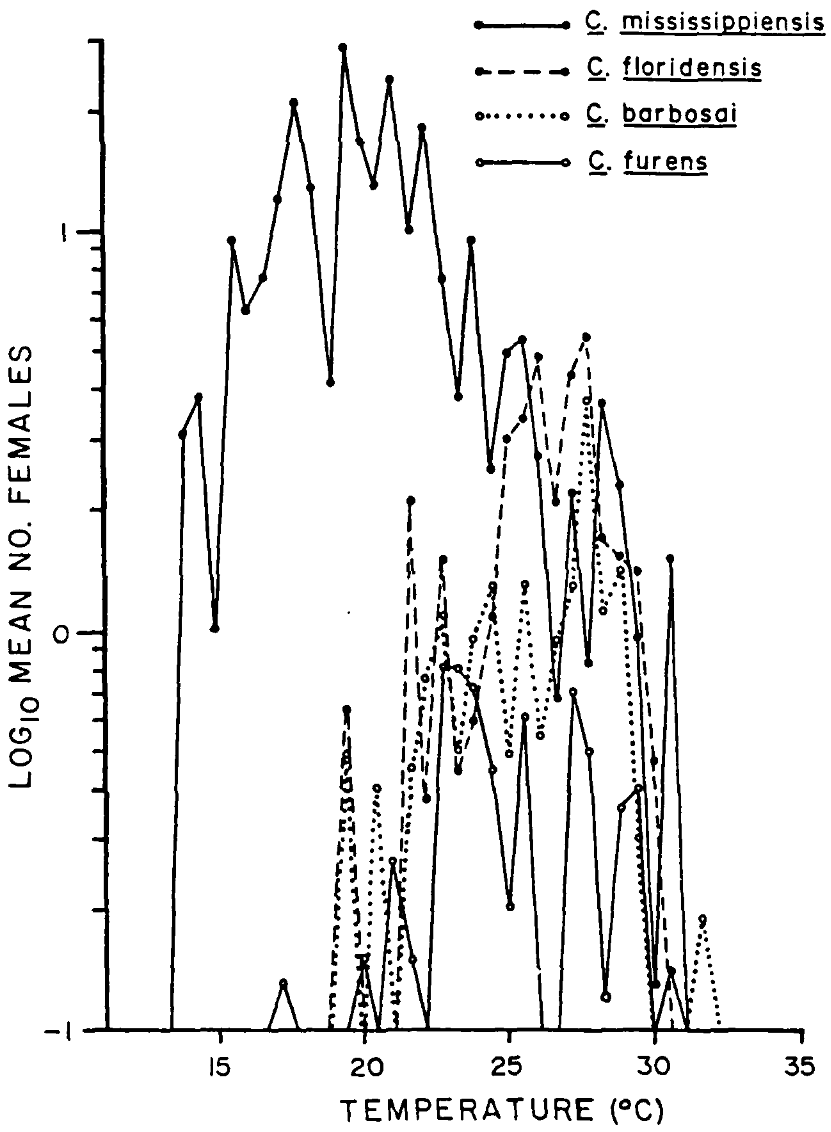

Figure 37. Relationship between ambient temperature and host-seeking activity of 4 species of Culicoides.

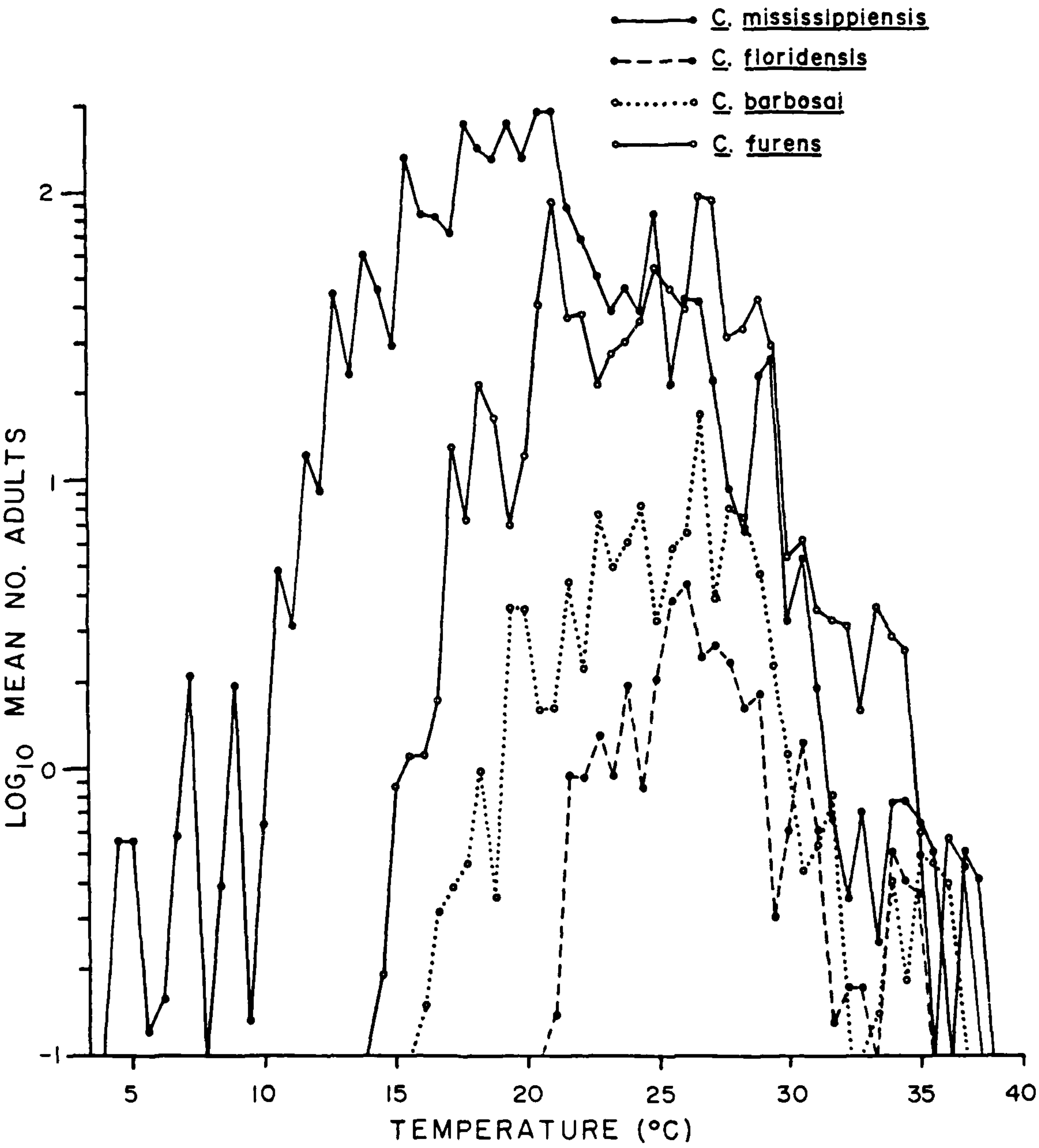

Figure 36. Relationship between ambient temperature and flight activity of 4 species of Culicoides collected in a vehicle-mounted trap.

than the other species. It was detected in the vehicle-mounted trap at temperatures ranging from 4 to 37°C and was most abundant at 21°C (Figure 36). This species did not begin biting, however, until the temperature reached 14°C (Figure 37). The other species were not present in the vehicle-mounted trap at temperatures below 14°C and did not begin biting until the temperature exceeded 16°C. Culicoides barbosai and C. floridensis were most abundant at 26°C in both types of collections.

The relationship with light intensity was much more variable. Specimens were collected during new moon when the only detectable light measured 1×10^{-4} fc and during bright sunlight when a measurement of 12,500 fc was recorded. Only C. mississippiensis and C. furens were collected at the extremes. Culicoides floridensis was virtually absent when the light intensity exceeded 1,000 fc and the upper limit for C. barbosai was approximately 2,000 fc. The majority of all specimens were obtained at light intensities ranging from 1 to 1,000 fc. Light intensity during the crepuscular periods, 11 and 20, changed rapidly and ranged from 1 to 200 fc. On full moon nights, a measurement of approximately 0.01 fc was recorded and the value was slightly lower during first and last quarter phases of the moon.

An interaction between light and temperature was observed. Ceratopogonids were not active during bright sunlight in the summer when the temperature exceeded 30°C; however, they were active during daylight hours in the winter when the temperature remained below that level. The situation was different during scotophase. Biting occurred on full moon nights during the summer but not during the winter.

Wind direction had no influence on the biting or flight activity of the 4 species. The fact that the vehicle-mounted trap route was bordered found to substantiate this claim. My observation may be biased because I could see more clearly when the moon was full. Were the vertebrates really more active or were they observed with greater frequency on full moon because I could see better? These questions should be addressed in a later study.

Meteorological Conditions

Kettle (1972) discussed the relative importance of 10 factors that might influence the biting activity of *C. furens*, *C. barbosai*, and *L. becquaerti* in Jamaica. Of the meteorological conditions he considered, wind speed was the most important, temperature was of less importance, and light intensity and relative humidity were the least important. The ranking of these factors in the Yankeetown area was similar.

The wind speed ranged from 0 to 24 km/h. Specimens were collected in the vehicle-mounted trap at all wind speeds observed but biting was inhibited at the higher velocities. *Culicoides floridensis* was the most sensitive. Biting by this species did not occur when the wind exceeded 9 km/h. *Culicoides barbosai* and *C. furens* were absent from host-seeking collections when the wind speed was greater than 13 km/h. The threshold for *C. mississippiensis* was 17 km/h. A ranking of these species from smallest to largest based on female winglength (Blanton and Wirth, 1979) is identical to their ranking based on the effects of wind speed. The largest species, *C. mississippiensis*, was collected in the vehicle-mounted trap when the wind was blowing at 24 km/h.

Temperature thresholds for the 4 species of *Culicoides* in the Yankeetown area were also established. The temperature varied from -1.6 to 37.2°C during the study. *Culicoides mississippiensis*, the only species active during the winter, was more tolerant of cold temperatures

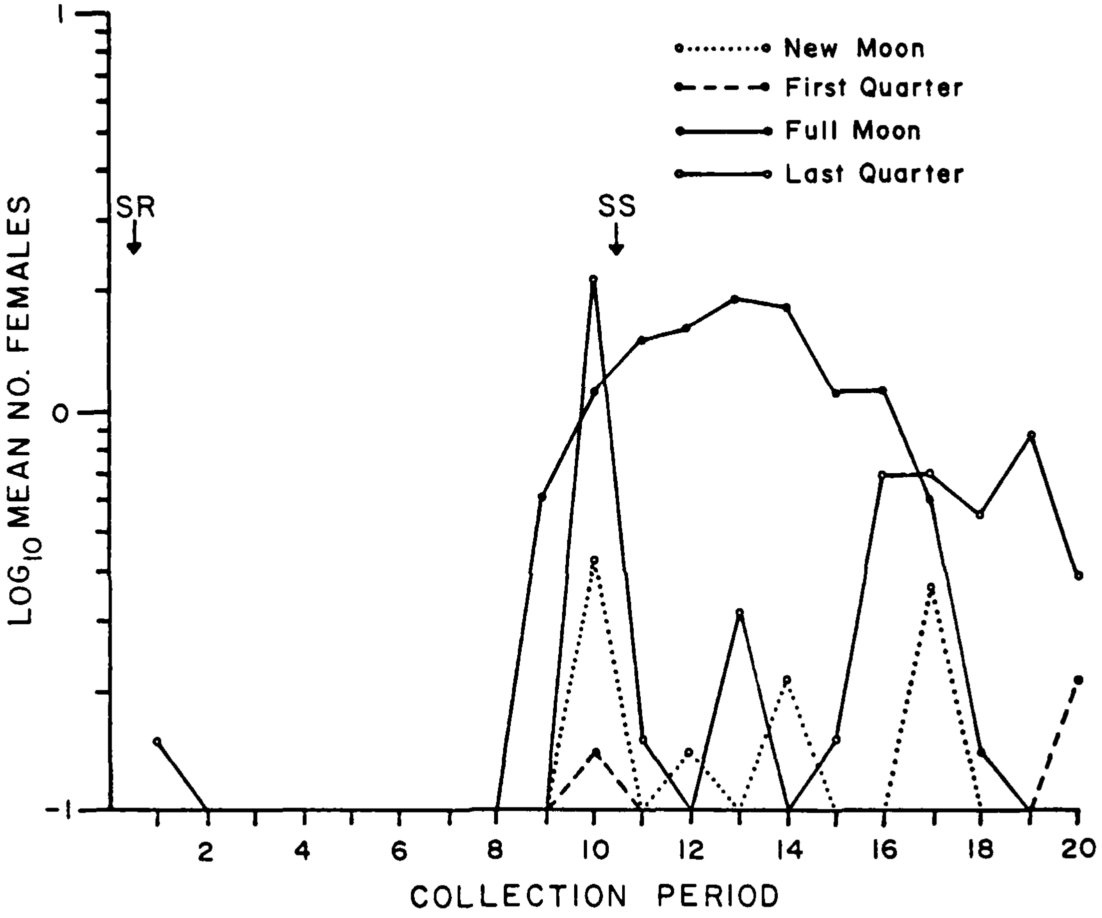

Figure 35. Diel host-seeking activity of C. furens collected on quarter phases of moon.

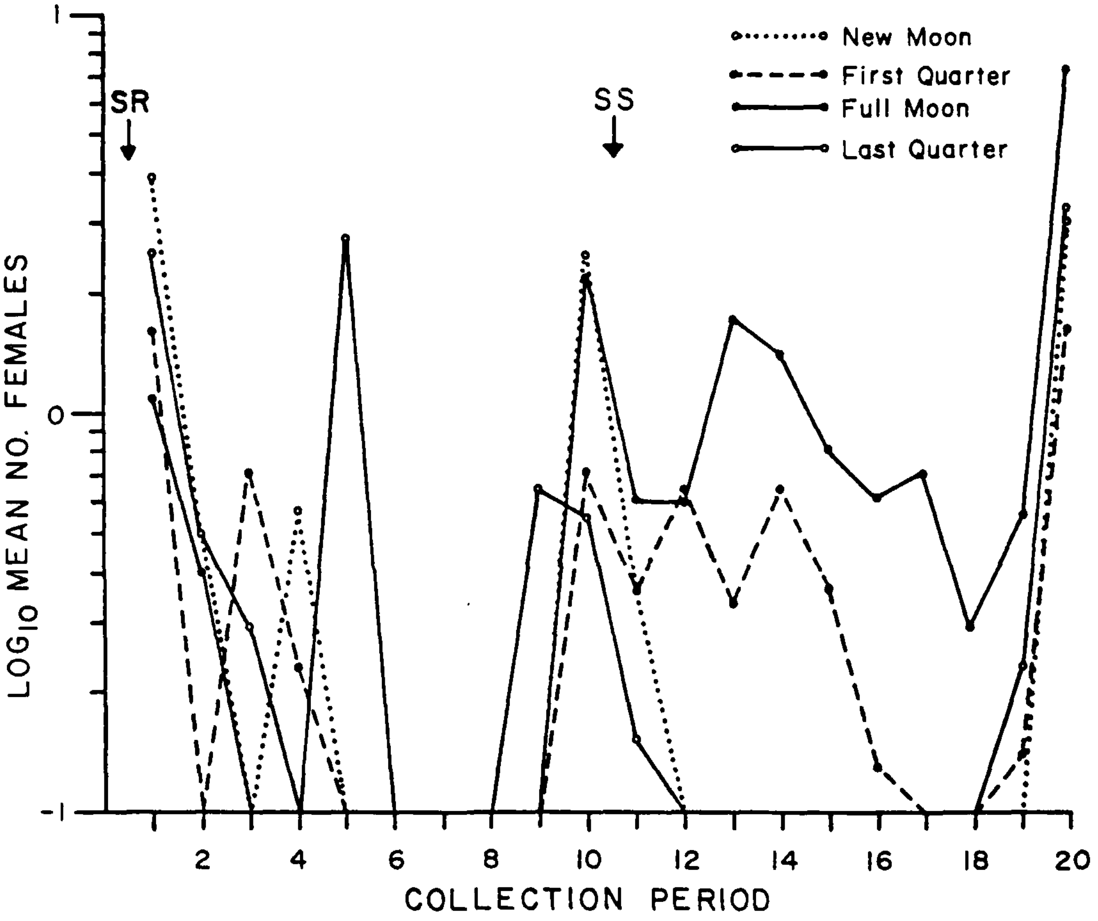

Figure 34. Diel host-seeking activity of C. barbosai collected on quarter phases of moon.

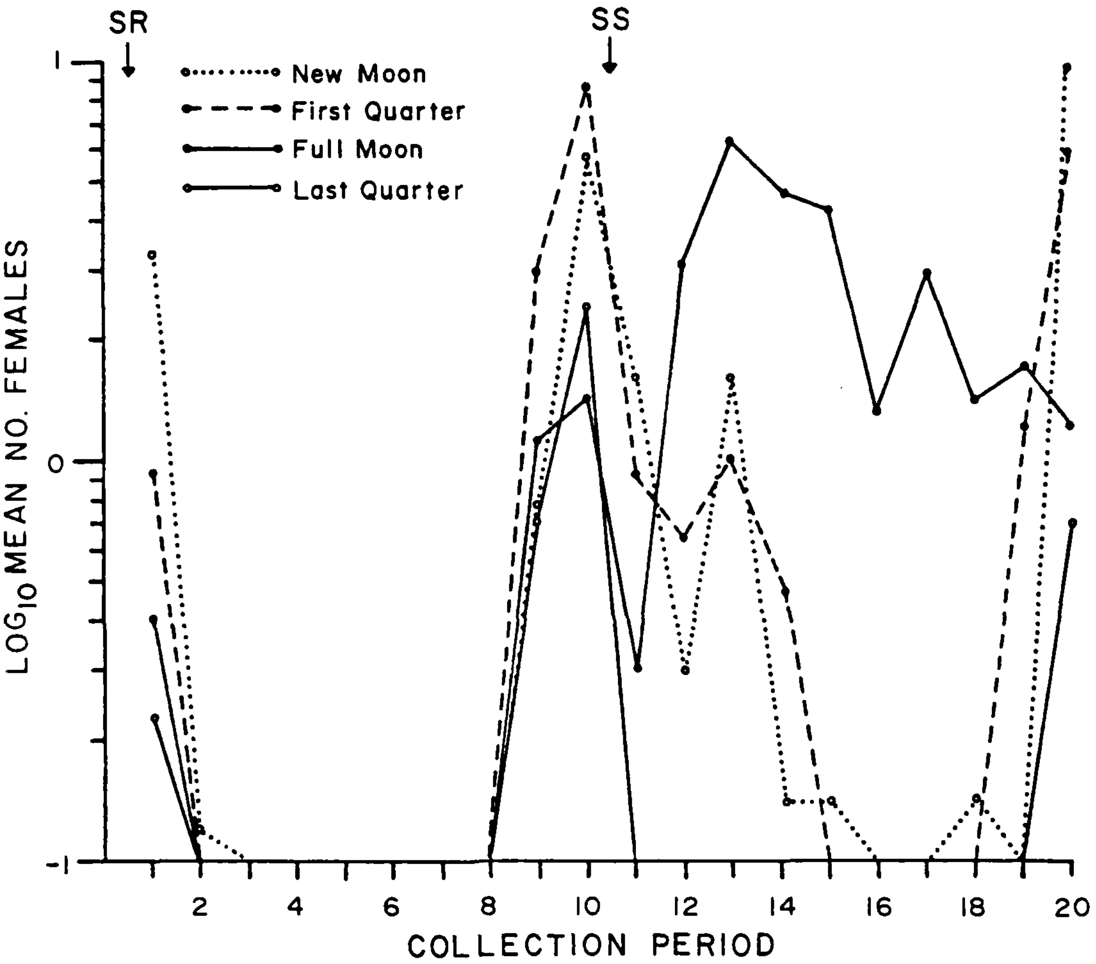

Figure 33. Diel host-seeking activity of *C. floridensis* collected on quarter phases of moon.

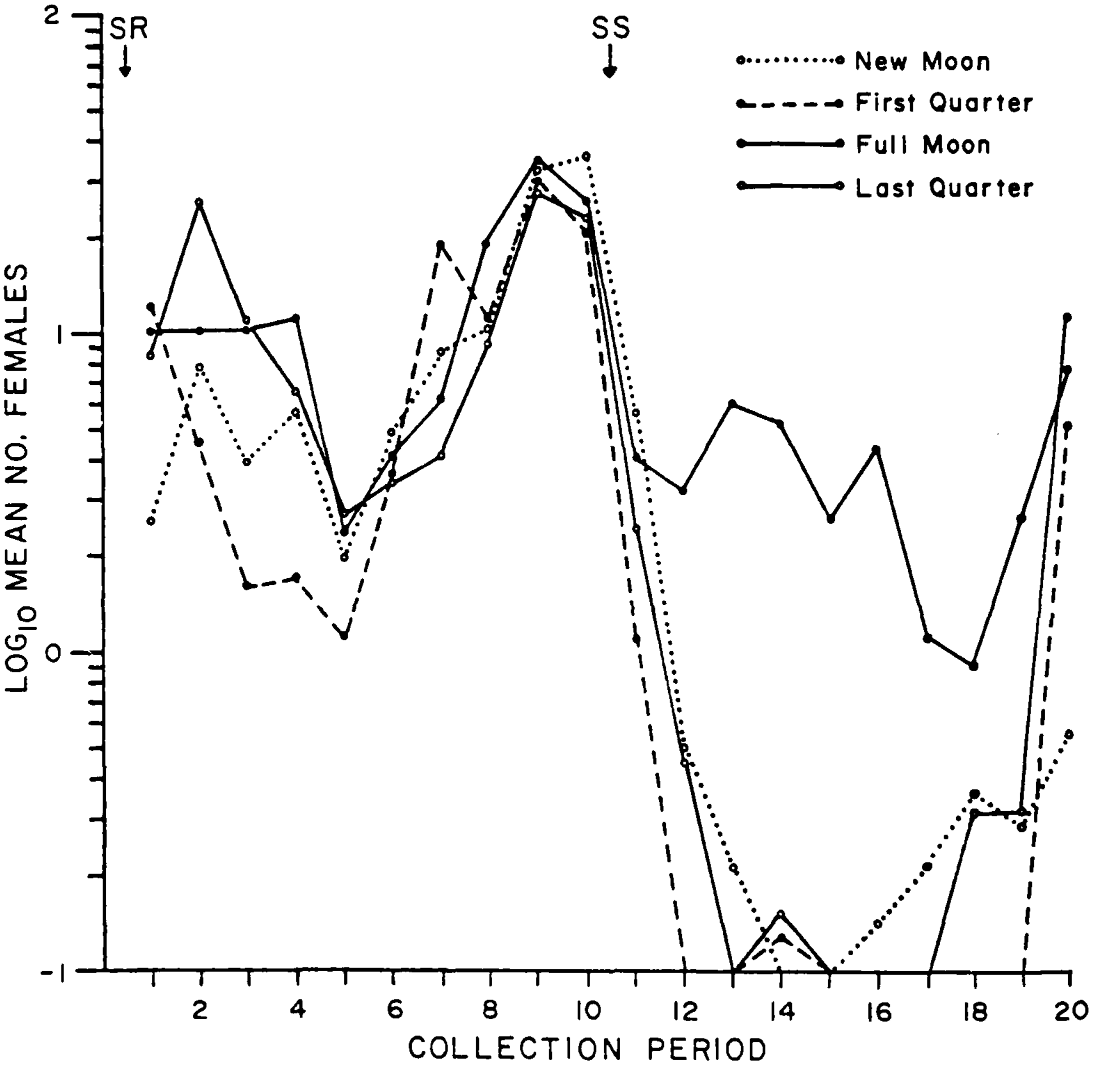

Figure 32. Diel host-seeking activity of _C. mississippiensis_ collected on quarter phases of moon.

and the number of biting midges obtained in the aspirator did not correlate.

Culicoides mississippiensis was collected in large numbers during daylight hours on all phases of the moon but the host-seeking individuals remained active at night only when the moon was full (Figure 32). The moon had no influence, however, during the winter. Attack activity in that season declined to zero around period 15 and did not resume until sunrise (Figure 28). The mean temperature was below 15°C during that time.

Culicoides floridensis was inactive as a biting species during the day but it also showed increased activity during full moon nights (Figure 33). The difference was significant ($p<0.05$) when compared with the other phases. In contrast, C. barbosai collections were greatest when the moon was full but they did not differ significantly ($p>0.05$) from collections taken on first quarter moon (Figure 34). Culicoides furens, the least abundant host-seeking species, also showed increased activity during full moon (Figure 35). It differed from the other species in that the amplitude on full moon was greater during early periods of the scotophase but dropped below the level of activity observed after moonrise (ca. period 16) on last quarter moon.

The relationship between the phase of the moon and host-seeking activity may be related to tides, as previously mentioned, or to host activity. Several vertebrate species including opossums (Didelphis virginiana), racoons (Procyon lotor), and chuck-will's widows (Caprimulgus carolinensis) were observed with greater frequency when the moon was full. Perhaps these vertebrates were more accessible to biting midges during that time. Literature on vertebrate activity could not be

Disparity between the vehicle-mounted trap results (Figure 13) and host-seeking collections (Figure 31) was obvious for C. furens. Based on the former method, the species was most abundant in spring but the aspirator technique indicated that its occurrence was greatest in summer. Possibly, the spring population was composed primarily of individuals developing an autogenous egg batch and the physiological requirements for a bloodmeal did not occur until early summer. Other possible sources of bias are the host and collection site (Kettle, 1969a, b). Only a single host was used for all collections near Yankeetown and the specific collection site never changed. The results may have been biased if the host was inadequate or C. furens did not frequent that particular site. The latter undoubtedly had more influence because the host was definitely suitable for other species and C. furens is known to feed on man. However, Kettle (1969a, b) reported that C. furens preferred to feed on the leg of a human rather than the arm. All samples were taken from the arm, approximately 1.2 m above ground level, in this study and the leg was never exposed.

Lunar Host-Seeking Periodicity

The frequency of attack by individuals seeking a bloodmeal during the night was greatest when the moon was full. In some instances the amplitude of full moon collections was approximately 100 times greater than when the moon was in some other phase. The study of Kettle (1972) in Jamaica found that the biting rates of C. barbosai and C. furens were maximal at new moon and not full moon. He speculated that the lunar effect was a result of tides influencing the time of emergence rather than the nighttime illumination stimulating adults. Tides may have been the regulating factor near Yankeetown as well, because light intensity their entire life and was not transferred from marked to unmarked flies during copulation (Lillie et al., 1981a).

An estimated 25,000 marked specimens were released at 1200 h on 2 April. This estimate was based on the mean number of *C. mississippiensis* collected over a 4-day period at the trapping sites used to obtain specimens for marking. Twenty traps were positioned within a 3.2 km radius of the release point to recapture marked individuals (Figure 39). Each trap was baited with 2.2 kg of solid CO_2 and equipped with a 1-liter jar of 70% ethanol as a collection receptacle. The marking material did not wash off in ethanol during a prior experiment (Lillie et al., 1981a). Every 24 hours for 4 days following the release, the collection jars were changed and a new supply of solid CO_2 was added to each trap. Most of the traps were located east of the release point because saltmarsh, tidal creeks, and the Gulf of Mexico were to the west. Equipment was available for servicing traps positioned on land or in shallow water only. The entire procedure was repeated for a second release on 16 April 1984. Approximately 15,000 flies were marked with the same color of fluorescent dust used on 2 April. There was little likelihood of confusing these with specimens released on 2 April because recapture in prior studies was not observed beyond 8 days postrelease (Lillie et al., 1981b).

The collections were examined by using a dissecting microscope (X 12 magnification) and a Black Ray™ model UVL 56 longwave ultraviolet lamp to detect marked specimens. All marked individuals were counted and the recapture location was recorded. The number and sex of unmarked *C. mississippiensis* were also determined by removing and counting the

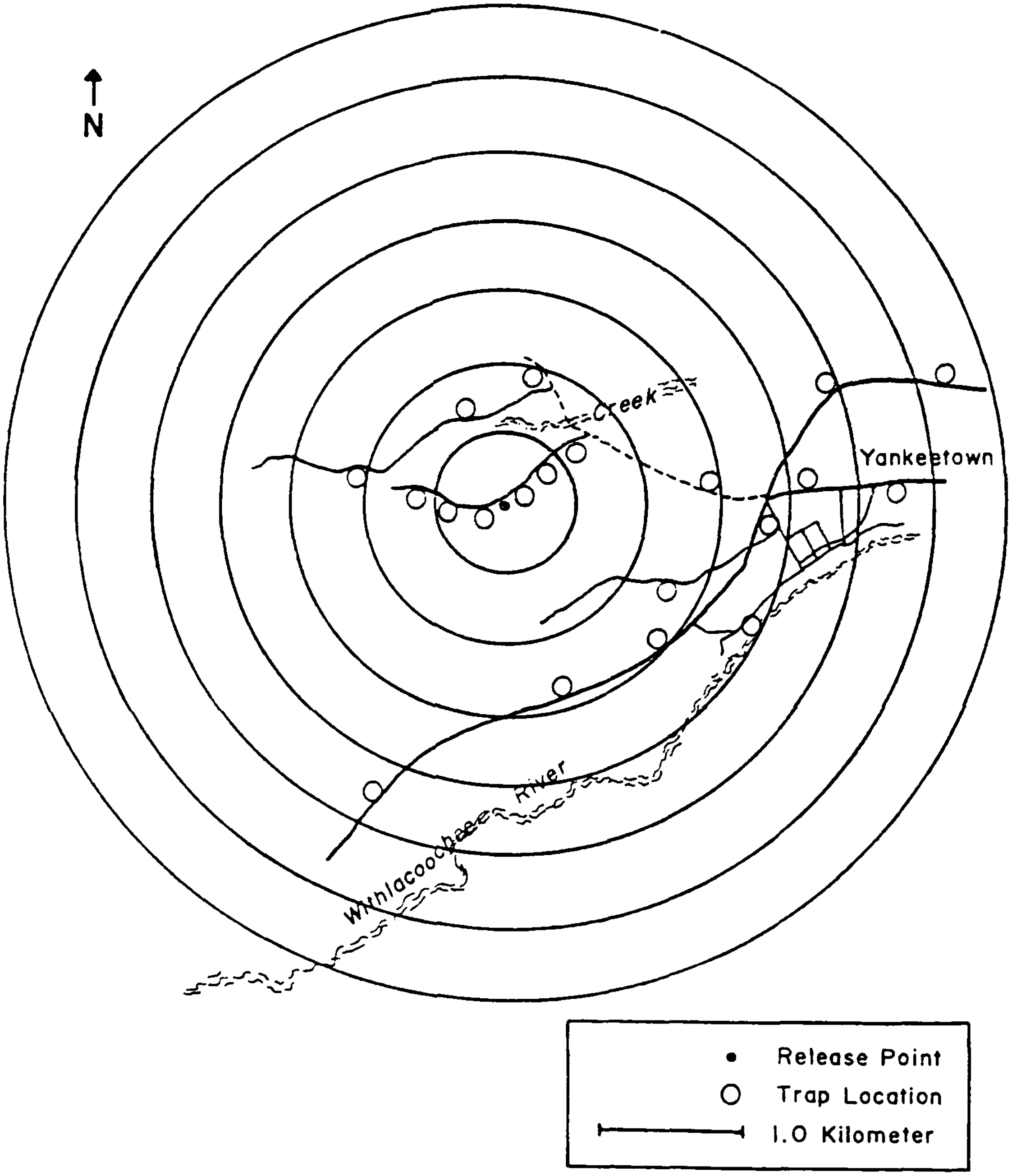

Figure 39. Trap locations and release point for mark-release-recapture studies near Yankeetown, Levy County, Florida.

individuals in a subsample. A petri dish with a grid system was used for the subsample procedure.

The number and location of recaptured specimens were used to determine the mean distance traveled (MDT) by _C. mississippiensis_ females. Data transformation prior to determining the MDT was necessary to account for an uneven number of traps/unit area. This method was used previously by Lillie et al. (1981b) and Brenner et al. (1984b). The recapture site was divided into subunits to calculate correction factors for transforming data. There were 7 subunits radiating out in concentric rings every 0.5 km from the release point to 3.5 km (Figure 39). The proportion of the total area occupied by each subunit was calculated and the number of traps, based on a total of 20, required for equal trap density throughout the recapture area was determined. A correction factor for each subunit was obtained in this manner which is summarized in the following formula:

$$CF = A_S/A_T \times 20$$

where A_S = subunit area and A_T = total trapping area (Table 4).

The number of specimens recaptured/trap in each subunit was multiplied by the corresponding correction factor to derive the number of individuals I would have expected to recover if equal trap density was employed.

Transformed data were used to calculate the MDT for the release day and each day postrelease by using the following formula:

$$MDT = \frac{\Sigma(\text{Expected No. Recovered x Distance})}{\Sigma\text{Expected No. Recovered}}$$

Table 4. Data used to calculate correction factors.

Trapping subunit (km)	Number of traps	Area (km^2)	Correction factor
0.5	4	0.8	0.42
1.0	4	2.3	1.19
1.5	5	4.0	2.08
2.0	2	5.5	2.86
2.5	3	7.0	3.64
3.0	1	8.6	4.47
3.5	1	10.3	5.38
TOTAL	20	38.5	20

Results and Discussion

Of the 25,000 specimens released on 2 April, 498 females (≈2%) were recaptured (Table 5). A lower percentage (≈0.5%) of the 15,000 biting midges released on 16 April were recovered (Table 6). Overall, approximately 1.5% (567) of the marked individuals were recaptured during the entire study. The percentage of flies recovered was less than that observed in a study of *C. mojave* (13%) in southern California (Brenner et al., 1984b) and more than the percentage of *C. variipennis* (0.5%) recaptured in Colorado (Lillie et al., 1981b). Usually less than 1% of the number of insects released in a mark-release-recapture study are recovered (Johnson, 1969). *Culicoides mojave* was an exception to this generality because of the desert environment in which it occurs (Brenner et al., 1984b). The population density of warm-blooded animals is lower in the desert than in a saltmarsh habitat, therefore, host-seeking females would cover a greater area in search of a bloodmeal and have a greater probability of recapture.

Movement of *C. mississippiensis* was neither unidirectional nor limited to a specific distance. Most individuals were taken within 1.5 km of the release point during the first 24 h, but a single specimen traveled 3.2 km over that period (Tables 5 and 6). The number of individuals recovered beyond 1.5 km increased during the second 24-h period (i.e., 1 day postrelease) and they were scattered throughout the trapping area. Heavy rainfall on 4 April (2 days postrelease) prevented trapping on that day and most likely influenced recapture on the following day. Also, data from 1 trap had to be deleted after the first release because it was contaminated with marking material during preliminary tests and inadvertently placed at a trapping site. Problems

Table 5. Number of specimens recaptured, transformed data, and mean distance traveled by *Culicoides mississippiensis* released on 2 April 1984.

Trapping subunit (km)	Day postrelease				Total by distance	Day postrelease				Total by distance
	0	1	2*	3		0	1	2*	3	
	number recaptured					transformed data				
0.5	29	14		3	46	4	2		1	7
1.0	5	11		1	17	2	4		1	7
1.5	27	123		5	155	12	52		2	66
2.0	0	11		0	11	0	16		0	16
2.5	0	257		0	257	0	312		0	312
3.0	0	12		0	12	0	54		0	54
3.5	0	0		0	0	0	0		0	0
Total by day	61	428		9	498	18	440		4	462
Mean distance traveled (km)						1.2	2.0		1.1	2.0

*Inclement weather prevented trapping on 2 days postrelease.

Table 6. Number of specimens recaptured, transformed data, and mean distance traveled by *Culicoides mississippiensis* released on 16 April 1984.

Trapping subunit (km)	Day postrelease				Total by distance	Day postrelease				Total by distance
	0	1	2	3		0	1	2	3	
	number recaptured					transformed data				
0.5	0	5	5	2	12	0	1	1	1	3
1.0	0	7	6	0	13	0	2	2	0	4
1.5	21	3	6	3	33	9	2	4	2	17
2.0	0	0	1	2	3	0	0	2	3	5
2.5	1	1	1	1	4	2	2	2	2	8
3.0	0	3	0	0	3	0	14	0	0	14
3.5	1	0	0	0	1	6	0	0	0	6
Total by day	23	19	19	8	69	17	21	11	8	57
Mean distance traveled (km)						2.3	2.5	1.6	1.8	2.2

relating to weather or equipment were not encountered for the second release.

The dispersal of C. mississippiensis did not appear to be aided by wind. The wind was blowing towards the east for about 6 h following the release on 2 April and gradually shifted to the south. Twenty-five of 61 individuals were recovered east of the release point but 29 individuals were obtained in traps located to the west. Wind speed during that time did not exceed 2 m/sec. A single individual traveling 3.2 km to the east in a 24-h period did so while the wind was blowing south. Brenner et al. (1984b) recaptured most marked C. mojave in the direction of the prevailing wind; however, 1 individual dispersed 6.0 km against the wind in 30 h. The movement of C. furens was most likely aided by wind in Panama (Breeland and Smith, 1962) and the Virgin Islands (Williams, 1962).

Observed data for C. mississippiensis females were transformed to account for unequal trap density and used to calculate an MDT of 2.0 km following the first release and 2.2 km for the second release. These distances are comparable to an MDT of 1.94 km for C. mojave and 1.89 km for C. variipennis (Brenner et al., 1984b; Lillie et al., 1981b). Yankeetown, Florida, was 2.0-2.5 km east of the release point (Figure 39). Data obtained from both releases indicate that a sufficient number of biting midges could travel from breeding sites in the saltmarsh to cause a nuisance in the residential area. Alternate breeding sites closer to Yankeetown are not a prerequisite for adult activity in that area and probably do not exist.

A change in the rate of dispersal was noted as the time postrelease increased. The MDT for the first 24 h after the release on 2 April was

1.2 km (Table 5). In the second 24 h, the MDT increased by only 0.8 km. The change was even less following the release on 16 April (Table 6). The MDT declined on 2 days postrelease and changed little after that time. This pattern of behavior could be an artifact of experimental design because of low trap density in peripheral areas or it is a realistic view of *C. mississippiensis* movement caused by omnidirectional flight habits and physiological changes.

Equal trap density would not have been practical because of the large number of traps needed to meet such a prerequisite. If 1 trap had been placed near the release point (Figure 39) then 13 traps would have been needed in the outermost subunit and 50 traps over the entire study area. The logistics of such an endeavor could not have been adequately handled in the field; therefore, data transformations were performed to correct for trap density.

The change in MDT was probably realistic. Individuals dispersed outward from the release point initially, but their movement was more likely to be towards or tangential to the release point as time elapsed. Such movement contributed little towards increasing the MDT. Also, as time elapsed the requirement for a bloodmeal was satisfied. Individuals were no longer attracted to CO_2 and therefore would not be trapped because of physiological changes that occur after ingesting a bloodmeal.

A few marked individuals other than *C. mississippiensis* females were also recaptured. Two males of *C. mississippiensis* were obtained 0.5 km west of the release point within 24 h of the release on 2 April. No other males were recovered. Male recapture was also rare in prior studies of *Culicoides* spp. The maximum distance traveled by male *C. variipennis* in Colorado was only 0.8 km (Lillie et al., 1981b). Males of

C. mojave were not recaptured in a dispersal study in southern California (Brenner et al., 1984b). Poor attraction of males to CO_2 and light traps in general explain these results.

Other marked species represented in the recapture data included: 1 *Chrysops fuliginosus* Wied., 4 *Anopheles quadrimaculatus* Say, 2 *Aedes sollicitans* (Walker), 1 *Ae. triseriatus* (Say), and 1 *Culex* sp. All were females. They were available for recapture because no attempt was made to separate *C. mississippiensis* from other species collected in the live-trapping operation prior to marking and release. *Chrysops fuliginosus* was captured approximately 0.8 km northwest of the release point following the first release. The culicids were obtained after both releases and traveled 0.5 to 2.0 km from the release point. These species were not collected in sufficient numbers to calculate MDT values.

Unmarked *C. mississippiensis* collected throughout the dispersal study were counted and the sexes were separated. A total of 627,560 females and 7,379 males was trapped. The trap index for this species in 140 trap nights (Note: a trap night is equal to 1 trap operated for 1 night) was 4,535. A single trap located in Yankeetown collected approximately 31,565 females during a 24-h period on 19 April. Another trap located adjacent to a breeding site also collected over 30,000 females on that day. In addition to these specimens, 7 gynandromorphs were attracted to light traps. Such a genetic condition produces individuals with male genitalia and a female head. This type of antero-posterior dimorphism in *C. mississippiensis* was also observed by Khalaf (1965). They are attracted to CO_2 because of female sense organs anteriorly. Based on CO_2-baited light trap data, the probability of a gynandromorph forming in a *C. mississippiensis* population is 1×10^{-5}.

CHAPTER FOUR
CONCLUSIONS

Pest Management Applications

Information about the seasonal, diel, and lunar periodicity of *Culicoides* spp. near Yankeetown, Florida, is important for planning a pest management program. Based on the data collected, adulticiding activities should begin 1 h before sunset and end 1 h after sunset in fall and winter. Treatment can be delayed until sunset in spring and summer. The lunar cycle should also be considered. Adulticides will have a greater probability of contacting midges in flight if applied on full moon.

The mean distance traveled (MDT) by individuals of a pest population can be used to determine the area that must be treated. Hocking (1953) recommended treating an area equivalent to the square of the MDT for effective control following a single application. Treatment of 4 km^2 surrounding each breeding site would be needed to suppress the *C. mississippiensis* population. Large expanses of saltmarsh available for larval development near Yankeetown make such an undertaking costly. Therefore, attempting to control the adult population by broadcasting pesticide over a large area should not be performed unless disease transmission is imminent or the quality of human life is deteriorating because of a pest's habits.

Alternative methods such as personal protection or scheduling outdoor activities so as to avoid peak periods of biting midge activity are recommended. Host-seeking data provide useful information for planning outdoor activities. Biting was intolerable near sunrise and sunset, during the night when the moon was full, and during afternoon hours in the winter and early spring. Outdoor events should be curtailed approximately 1 h before sunrise, particularly in winter and spring, when _C_. _mississippiensis_ is most abundant.

Such scheduling may be impractical for persons whose livelihood requires work outdoors during peak periods of biting midge activity. Campers and sportsmen may also be outdoors when biting midges are intolerable. Personal protection in the form of repellents (Schreck and Kline, 1981), treated jackets (Schreck et al., 1979a), treated netting (Schreck and Kline, 1983), or treated screens (Kline and Roberts, 1981) should be employed. Seasonal, diel, and lunar cycles determined in this study can be used to advise individuals of times when personal protection is required.

Adaptive Significance

Seasonal, diel, and lunar periodicities were determined for 4 species of _Culicoides_, but I can only speculate as to the proximate cues responsible for such activity and its adaptive significance. Only adults were collected but all stages of development should be considered as the 4 species interact as part of a saltmarsh community.

Interspecific competition exists between females that pursue a bloodmeal. This would not be sufficient to separate them temporally, however, because a diverse group of warm-blooded animals are plentiful in the area. Oviposition sites and larval competition for food are more likely to be limiting factors, especially for _C_. _mississippiensis_ and _C_.

furens which both develop in saltmarsh soil (Kline, 1984). Adult activity for these 2 species overlapped in late spring and early fall but the *C. mississippiensis* population declined when *C. furens* became more plentiful and vice versa. Photoperiod and temperature most likely regulate immature development and adult eclosion which in turn account for the seasonal occurrence of adults. Precipitation probably has less effect on these 2 species because the saltmarsh is periodically inundated during high tide. Precipitation may be more important for *C. barbosai* and *C. floridensis* but this cannot be determined until their breeding sites are found in the area.

The diel periodicity observed is probably under the control of physiological processes and a biological clock. Biting midges are small (1-4 mm) and therefore have a high surface to volume ratio. Changes in temperature have a profound effect on an individual's metabolic rate. More energy would be required for flight activity during hot, daylight hours than during cooler twilight and dark periods. This would explain the reduction in activity during photophase in summer. An activity threshold also exists at the opposite end of the temperature scale. *Culicoides mississippiensis* was the only species collected in the adult stage during winter and its nighttime activity was reduced because of cool temperatures. A crepuscular activity pattern most likely evolved because, on the average, environmental conditions are optimum during twilight periods for physiological processes to occur. A biological clock is also involved because the number of individuals did not suddenly increase in trap collections when the sun was blocked by a dark cloud and the temperature dropped. Biting midges obviously have some mechanism for keeping track of time, possibly cuing on sunrise, sunset, or photoperiod.

The biological clock, however, is not too rigid to allow exceptions because adults were active throughout the night when the moon was full but not during other phases. A lunar cycle exists in addition to the seasonal and diel cycles, but its adaptive significance is much more difficult to explain at the individual level. Possibly attractants or other stimuli produced by hosts are greater during full moon. This would explain why females are active during full moon and males are not.

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BIOGRAPHICAL SKETCH

Thomas Henry Lillie, the son of Charles and Antonia Lillie, was born in Lafayette, Louisiana, on 2 April 1954. He grew up in Breaux Bridge, a small community in south Louisiana, where he attended Saint Bernard Catholic School for 8 years (1960-1968). He received his high school education at Teurlings Catholic High School in Lafayette (1968-1972).

T.H. Lillie attended the University of Southwestern Louisiana (USL), Lafayette, Louisiana, as a wildlife management major (1972-1976). He was chosen for membership in Phi Eta Sigma honor society, served as secretary-treasurer for the USL Biological Society, and made the Dean's List every semester. In 1975, he enrolled in the Air Force ROTC two-year program with hopes of serving active duty as a medical entomologist. Prior to graduation he married Michelle J. Hamblet, the daughter of Albert and Dolores Hamblet, on 29 May 1976.

He graduated from USL with high distinction in August, 1976. He enrolled in the graduate school of Colorado State University, Fort Collins, Colorado, to pursue a Master of Science degree in medical entomology. He studied the dispersal of Culicoides variipennis as a graduate research assistant on the Culicoides/Bluetongue virus project. He was commissioned a second lieutenant in the USAF in May, 1977, and received his M.S. degree in May, 1978.

Lieutenant Lillie entered active duty in the Air Force medical entomology career field as a first lieutenant in June, 1978. He was assigned to the USAF, Occupational and Environmental Health Laboratory, Brooks AFB, Texas, where he worked as a consultant in environmental entomology and a liaison to the Armed Forces Pest Management Board. He worked on a variety of pesticide toxicology projects including the evaluation of chlordane contamination in USAF Family Housing.

He was promoted to captain and received a Regular Air Force commission in June, 1980. Captain Lillie was selected by the Air Force Institute of Technology to attend the University of Florida (UF) for attainment of a Doctor of Philosophy degree in entomology. He was accompanied by his wife and two daughters, Carrie and Carla. While at UF, Captain Lillie was chosen for membership in and served as chancellor of the Florida Chapter of the Fraternity of Alpha Zeta.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Donald W. Hall

Donald W. Hall, Chairman
Professor of Entomology and
Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Daniel L. Kline

Daniel L. Kline
Assistant Professor of
Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Ellis C. Greiner
Associate Professor of
Veterinary Medicine

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May, 1985

Jack L. Fry

Dean, College of Agriculture

Dean for Graduate Studies and
Research