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SALT MARSH *CULICOIDES* (DIPTERA: CERATOPOGONIDAE): SPECIES, SEASONAL ABUNDANCE AND COMPARISONS OF TRAPPING METHODS[1]

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ABSTRACT. The spatial and seasonal abundance of adult *Culicoides* in a coastal *Spartina* salt marsh in North Carolina was determined during a 2-yr period by a light trap, emergence traps, and sticky cylinder traps.

Twelve species were collected, the greatest number (11) by light trap. *C. hollensis* (Melander and Brues) and *C. furens* (Poey) were the most abundant. *C. hollensis* was abundant in spring and fall, and *C. furens* from late spring to early fall. Emergence traps provided more precise data on the seasonal occurrence of both species than sticky cylinder and light traps. The light trap and sticky cylinder traps were less effective in attracting *C. hollensis* than *C. furens*. A lower percentage of males of both species were obtained by the light trap and sticky cylinder traps than by emergence traps. Adult catches were correlated with temperature, rainfall and the percentage of time the marsh was flooded.

INTRODUCTION

Culicoides, commonly referred to as biting gnats, no-see-ums or sand flies are blood-sucking pests of people and livestock with some species being especially annoying along the Atlantic and Gulf Coasts of the United States. In a survey of public opinion, Gerhardt et al. (1973) concluded that biting gnats are one of the most important pests in the coastal areas of North Carolina and in some places are more annoying than mosquitoes and tabanids.

This investigation was undertaken in a coastal salt marsh of North Carolina to determine the species present and their seasonal abundance, and to compare 3 trapping methods for monitoring population levels and relating catch data to environmental variables.

Several trapping techniques have been used to study *Culicoides.* Three of the most important are light traps, emergence traps, and sticky cylinder traps. Many workers have used light traps for taxonomic and seasonal variation studies (James 1943, Wirth 1951, Beck 1958, Khalaf 1952, Williams 1955, Wirth and Bottimer 1956, Jamnback and Watthews 1963, Khalaf 1967, 1969, Service 1969, Linley et al. 1970). Other workers have relied on various forms of emergence traps (Dove et al. 1932, Williams 1955, Davies 1966, Linley et al. 1970). Sticky cylinder traps have been used by several workers (Hill 1947, Kettle 1951, Castle 1965, Nielson 1963, Onyiah 1971). Kettle (1962) reviewed sampling methods for *Culicoides* and the advantages and disadvantages of light traps were discussed by Beck (1958) and Provost (1959). There are very few reported comparisons of the results obtained by these trapping methods. Linley et al. (1970) compared catches of *C. furens* from light traps and catches from emergence traps.

MATERIALS AND METHODS

This study was conducted in 1972 and 1973 in and adjacent to a salt marsh along the Newport River, Morehead City, Car-

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teret County, North Carolina. The salt marsh vegetation was composed of relatively pure stands of *Spartina alterniflora* Loisel., *Distichlis spicata* (L.) Greene, and *Juncus roemerianus* Scheele which were separated by transition zones of mixed vegetation. *S. alterniflora* (short, intermediate, and tall forms) covered the greatest amount of the marsh. The study area is the same as shown in Figure 2 of Dukes et al. (1974) and described in Kline and Axtell (1976).

Light trap. A New Jersey light trap, equipped with an automatic timer and modified by replacing the regular screen of the delivery cone with 40-mesh brass screen, was operated in the upland adjacent to the marsh. The trap was operated from 1800–0600 hours in spring and fall, and from 2000 to 0600 hours in the summer. Collections were removed at least 3 times a week in 1972 and daily in 1973.

The number of *Culicoides* in a collection was estimated by the method described by Jamnback and Watthews (1963) except that the collections were processed through a series of screens in a dry state rather than washed through in 80% ethyl alcohol. The average daily catch was calculated from the combined data for 1 week.

Emergence traps. Conical emergence traps (Davies 1966 and Linley et al. 1970) consisted of a cone of galvanized metal sheeting 63.5 cm diameter at the base and 48.3 cm high, with a 5.1 cm diameter hole at the top. Around this hole a 2.5 cm band of galvanized metal sheeting was soldered in place to hold a polypropylene plastic pint jar lid (5.1 cm diam hole in the center) which was attached by sheet metal screws. Plastic lids were used because the standard metal ones deteriorated rapidly in the marsh environment. The threaded plastic lids held standard glass pint jars in the inverted position over the opening at the apex of the cone.

The inside of each jar was lined with a strip (10.2 cm x 20.3 cm) of transparent cellulose acetate which was thinly coated (with a small paint roller) with Bird Tanglefoot© (Tanglefoot Co., Grand Rapids, Michigan). A thin layer of Tanglefoot was also brushed onto the inner lip of the jar and onto the inside bottom of the jar. Adult *Culicoides,* attracted upwards by light, adhered to the sticky surfaces. Specimens were removed from the cellulose strips and the inside bottom and lip of the jar by rinsing in Varsol. The gnats were identified and counted under a dissecting microscope. Specimens were preserved for future reference by rinsing in naphtha solvent, transferring to small vials, allowing the naphtha to evaporate, and adding 70% ethyl alcohol.

The traps were arranged in the various vegetative zones (Kline and Axtell 1976): tall form *S. alterniflora* (>1.2 m height) near the river's edge and drainage ditches, intermediate form *S. alterniflora* (0.3—1.2 m), short form *S. alterniflora* (<0.3 m), *D. spicata, J. roemerianus,* and shrubs-woods area. There were 16–20 traps operated in 1972 and 32 in 1973. In addition, "control" traps (1 in 1972; 2 in 1973) were used to determine whether or not the traps were catching some adults entering from outside, rather than only those emerging from the soil beneath the trap. Each control consisted of a trap placed on a sheet of plywood with the rim raised 2.5 cm above the plywood sheet by small blocks of wood.

At each site, the trap was rotated twice weekly among 4 adjacent positions, in a clockwise fashion, to avoid the possibility that larvae are attracted beneath the trap from the surrounding area as suggested by Davies (1966). Jars were changed twice weekly in 1972 and once a week in 1973. No problems were encountered with deterioration of the specimens.

Sticky cylinder traps. Sticky cylinder traps similar to those described by Kettle (1951) were used. Sections (30.5 cm high x 10.1 cm diam.) of black plastic sewer pipe were attached by braces to wooden posts (2.4 m x 2.5 cm x 2.5 cm) so that their centers were located 1.8 m above the marsh ground level. Black pipe was used because Hill (1947) and Castle (1965) found greater numbers on black than other colors. Adult *Culicoides* were captured on a sheet of cellulose acetate, thinly coated with Tanglefoot wrapped around the outside of the cylinder and attached with metal clips. The acetate sheets were replaced weekly.

In 1972, 18 sticky cylinder traps were arranged in parallel transects ca. 30 meters apart with 3 traps in each of the following vegetative zones (Kline and Axtell 1976): intermediate form *S. alterniflora*, short form *S. alterniflora*, *D. spicata*, *J. roemerianus*, and shrub-woods. In 1973, 3 traps were in short *S. alterniflora* along a man-made drainage ditch. This arrangement was based on results of the emergence trap data for 1972 which indicated that the short and tall forms of *S. alterniflora* were the major breeding sites of *C. furens* (Poey) and *C. hollensis* (Melander and Brues), respectively.

ENVIRONMENTAL MEASUREMENTS. A recorder in the marsh was used to make continuous measurements of air temperature, and the hourly readings were used to calculate daily and weekly averages. Rainfall was recorded weekly or more often during periods of rainstorms. The percentage of time the marsh was flooded was calculated by relating the marsh level, measured with a transit, to data from a tide gauge recorder (Leopold-Stevens, Type F).

The degree of correlation by multiple regression analysis of these environmental factors and data with catches of adult *C. hollensis* and *C. furens* from light trap (1973), emergence traps (1973), and sticky cylinder traps (1972) were determined separately. Light trap catches were compared to moon phase (an average value calculated for each week that the light trap was in operation). Analyses of emergence trap and sticky cylinder trap data included vegetation and trap location.

RESULTS

From all the traps in both years, 12 species of *Culicoides* were collected (Table 1). The greatest diversity of species (11) was obtained by the light trap, followed by sticky cylinder traps (5) and emergence traps (3). This is the first record of the following species in North Carolina: *C. arboricola* Root and Hoffman, *C. bermudensis* Williams, *C. niger* Root and Hoffman, and *C. piliferus* Root and Hoffman. The most abundant species in the collections were *C. hollensis* (Melander and Brues) and *C. furens* (Poey) and only data on those species were used to compare seasonal abundance and trapping methods.

C. hollensis. Fig. 1 shows the seasonal catches of *C. hollensis* by the 3 trapping methods and the sex ratios are given in Table 2. Light trap collections showed the same seasonal trends for both years. This species first appeared in the collections in early April and disappeared by mid-October. Two peaks of abundance occurred with the spring peak much greater than the fall one. The spring collections were greatest during the second and third weeks of April in 1972, and in the first 2 weeks of May in 1973. Both years, very few gnats were caught from July thorugh most of August. In September there was a peak which was much

Table 1. Species of *Culicoides* collected by light traps (LT), emergence traps (ET), and sticky cylinder traps (SCT) in and adjacent to a *Spartina* salt marsh, Morehead City, Carteret County, North Carolina (1972 and 1973).[a]

C. arboricola Root and Hoffman (LT)
C. baueri Hoffman (LT)
C. bermudensis Williams (ET)
C. furens (Poey) (LT, ET, SCT)
C. haematopotus Malloch (LT), SCT)
C. hollensis (Melander & Brues) (LT, ET, SCT)
C. melleus (Coquillett) (LT, SCT)
C. niger Root and Hoffman (LT)
C. piliferus Root and Hoffman (LT)
C. spinosus Root and Hoffman (LT)
C. stellifer (Coquillett) (LT, SCT)
C. venustus Hoffman (LT)

[a] The assistance of Dr. W. W. Wirth in providing species determinations is gratefully acknowledged.

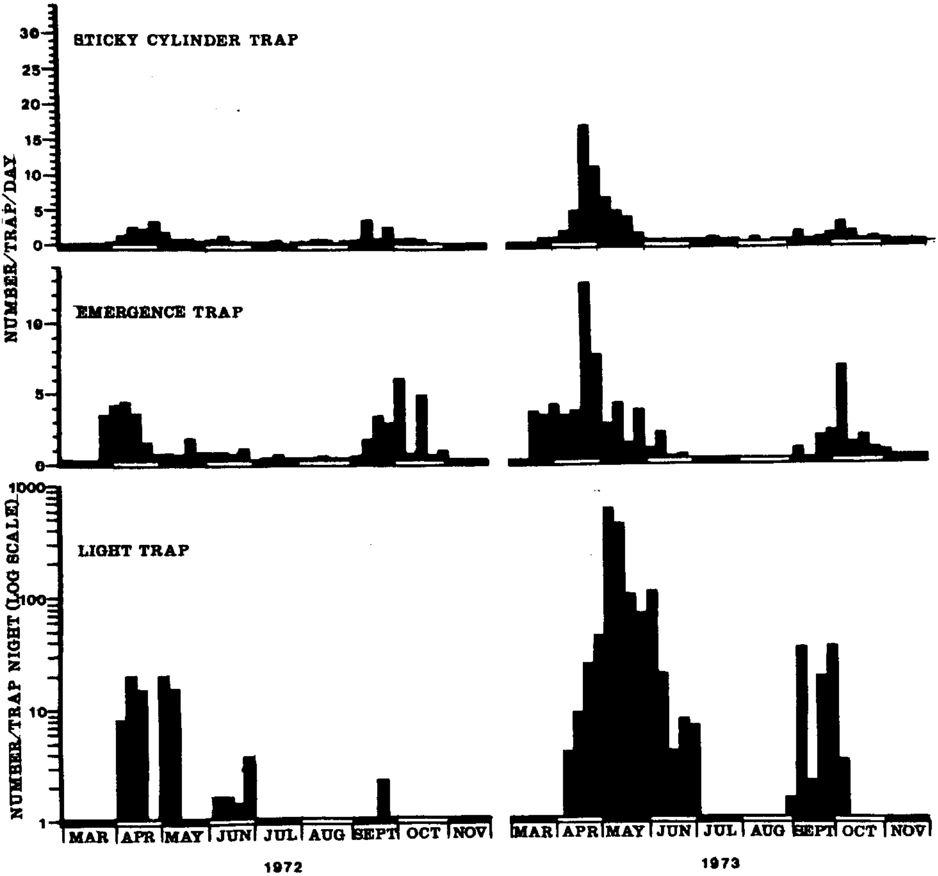

Fig. 1. Numbers of *Culicoides hollensis* collected in 1972 and 1973 by a light trap, emergence traps and sticky cylinder traps in and near a *Spartina* salt marsh, Carteret County, North Carolina.

more distinct in 1973 than in 1972. Most of the gnats were trapped during periods when the average weekly temperature was 17–21° C. Mostly females were collected in the light trap.

In the emergence traps, *C. hollensis* were collected from March through November, appearing about a week earlier and disappearing 3 weeks later in 1973 than 1972. A spring peak occurred in late March through early April in 1972, and late April in 1973. Few specimens were obtained from June through August. A fall peak occurred in late September through early October in both years. The fall collections in 1972 were greater than the spring ones; the reverse was true in 1973. No gnats were collected from December 1973 to early March 1974. Large numbers of both males and females were collected in the emergence traps.

On the sticky cylinder traps, *C. hollensis* were caught from late March through October. A spring peak occurred in late April both years. Few gnats were trapped from June through August. A fall peak was indicated in late September through early October. The spring and fall peaks were approximately equal in 1972, but the spring peak was distinctly larger in 1973.

Table 2. Total number and percent of each sex of *Culicoides hollensis* and *C. furens* collected by a light trap, emergence traps, and sticky cylinder traps for 1972 and 1973.

Traps	1972 Total	1972 % ♂	1972 % ♀	1973 Total	1973 % ♂	1973 % ♀
			C. hollensis			
Light	569	2.3	97.7	10,868	0.9	99.1
Emergence	5,801	55.9	44.1	15,891	54.8	45.3
Sticky cylinder	3,051	6.7	93.3	2,599	1.0	99.0
			C. furens			
Light	1,901	8.1	91.9	731,521	2.6	97.4
Emergence	3,157	40.2	59.8	17,912	44.0	56.0
Sticky cylinder	12,565	28.3	71.7	9,044	32.3	67.7

Mostly females were caught on the sticky cylinder traps.

The population of *C. hollensis* in the study area was much greater in 1973 than in 1972. This is illustrated by comparing the catches from the same 3 sticky cylinder traps operated in the same position in a zone of short *Spartina alterniflora* for the 2 years. A total of 909 specimens were caught on those traps in 1973, and 670 in 1972.

The light trap collections showed the same general seasonal trends as the emergence traps and sticky cylinder traps. However, the light trap did not reveal the beginning or ending of the seasonal incidence and the detailed fluctuations as well as the other methods. The data from the emergence traps and the sticky cylinder traps agreed fairly well with each other. The sticky cylinder traps were less effective than the emergence traps in detecting low numbers of gnats near the end of the season. The emergence traps collected nearly equal numbers of males and females while the light trap and sticky traps caught very few males.

C. furens. Fig. 2 shows the seasonal catches of *C. furens* by the 3 trapping methods, and the sex ratios are given in Table 2. Few gnats were collected in the light trap in 1972; large numbers were collected in 1973. In both years these gnats first appeared in the collections in late April, and were collected through mid-September, and early October in 1972 and 1973, respectively. Three apparent peaks occurred in 1972: late May to early June, late June and mid-September (the smallest). The peaks were less obvious in 1973, although there was a peak in late May and a smaller peak in early September. The gnats were most abundant in early summer (May-June) and late summer (late August-September). Most of the gnats were trapped during periods when the average weekly temperature was 21–26° C. Mostly females were caught in the light trap.

In the emergence traps, *C. furens* were collected from late April through early October. In 1972 peaks occurred in June (several broods?), late July, and late August, while in 1973 peaks occurred in May, early July, early August, and early September. The early and late summer collections were about equal. Approximately 40 percent of the specimens were males.

On the sticky cylinder traps specimens were collected from late April through early October. In 1972 definite peaks occurred in June and late August, and possibly a small peak in late July. In 1973 peaks occurred in early May, early July, early August, and early September. About 28 percent of the specimens were males..

A comparison of the 3 sticky cylinder traps operated in the short *S. alterniflora* for the 2 years showed a greater number of *C. furens* present in 1973 than 1972. A total of 5942 specimens were caught on these traps in 1973, compared to 4890 in 1972.

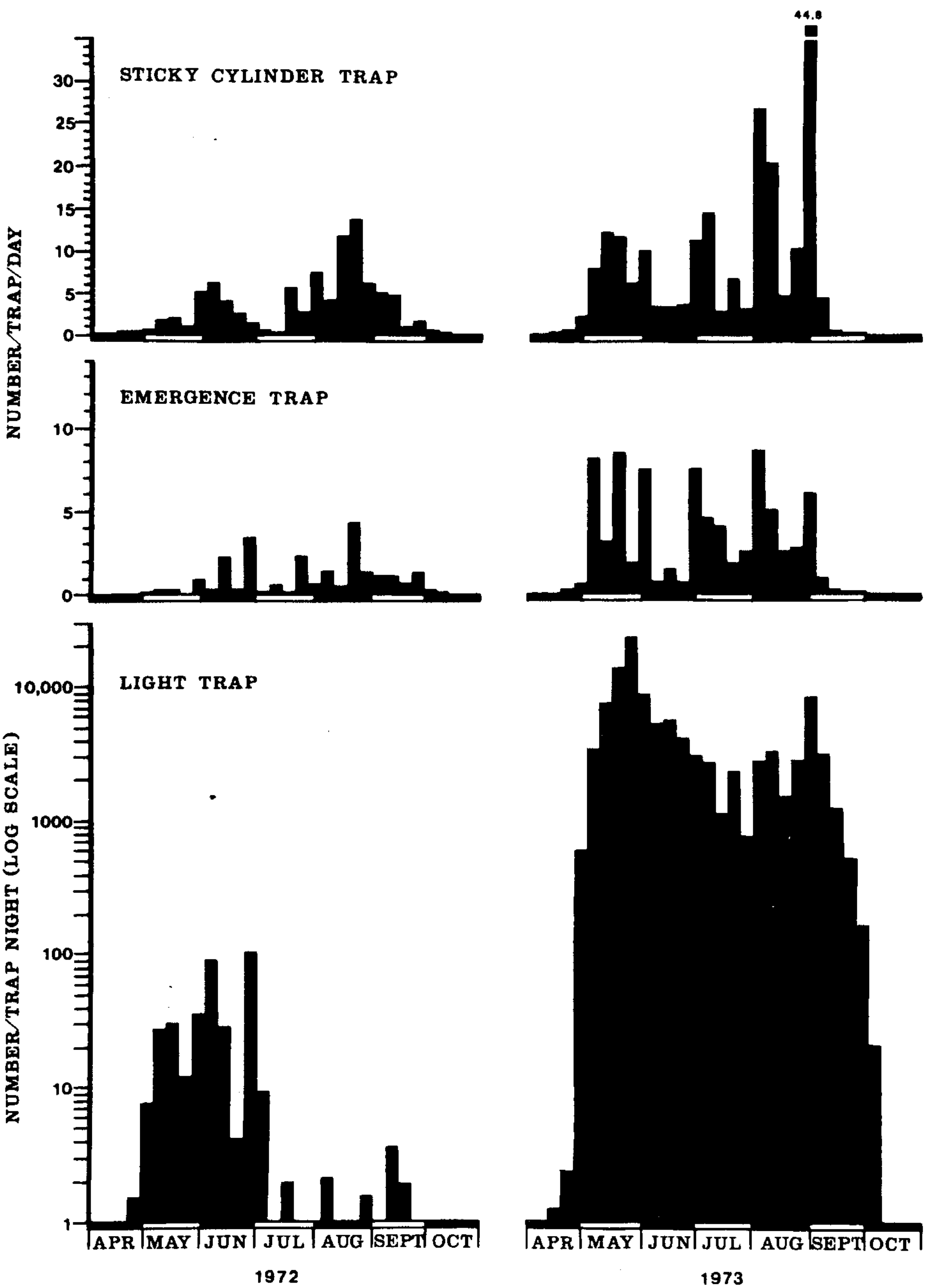

Fig. 2. Numbers of *Culicoides furens* collected in 1972 and 1973 by a light trap, emergence traps and sticky cylinder traps in and near a *Spartina* salt marsh, Carteret County, North Carolina.

The 3 trapping methods showed the same seasonal incidence of *C. furens*. However, the detailed seasonal fluctuations shown by emergence traps and sticky cylinder traps were not shown by the light trap data. The sticky cylinder data showed the August-September peak to be greater than the spring peak while the reverse was shown by the light trap. Fewer males than females were collected by all 3 methods, but emergence trap collections were close to a 1:1 ratio.

Correlation with environmental factors. Results of the multiple regression analysis were considered significant if the significance level of the partial sums of squares probability of F value was 0.05 or less and highly significant if 0.01 or less. Factors included were date, rain, temperature, percent time flooded and type of vegetation (except in analysis of light trap data).

The light trap collections of *C. hollensis* were not significantly correlated with any of the factors due to the low numbers caught in the light trap (see discussion). The light trap catches of *C. furens* were significantly correlated with temperature and date.

The correlations of emergence trap catches of *C. hollensis* with all 5 factors were highly significant. However, this was true for the flooding factor only when the data for the vegetation were dropped from the analysis and that illustrates the confounding effect in the regression analysis of these 2 factors which are interrelated. The correlations of emergence trap catches of *C. furens* with all factors except vegetation were highly significant. We consider the lack of significance for the correlation of trap catches with vegetation to be also an artifact of the confounding effect of the interrelated factors of vegetation and percent time flooded. We have shown by analysis of variance that emergence trap catches of *C. hollensis* and *C. furens* are significantly different in different plant zones (Kline and Axtell, 1976).

The correlations of sticky cylinder trap collections of *C. hollensis* with all the factors were highly significant. For *C. furens* there was a significant correlation with rain and highly significant correlations with temperature and vegetation while date was not significantly correlated. Again, we consider the confounding effect of the interrelated factors of vegetation and percent time flooded in the multiple regression analysis procedure to be responsible for the lack of significant correlation between catches and flooding.

DISCUSSION AND CONCLUSIONS

The species composition found in coastal North Carolina was similar to that found by other workers along the Atlantic and Gulf coasts of the United States (Beck 1958; Lewis 1959; Ah 1968; Khalaf 1969; Henry 1973). In all these studies *C. hollensis* and *C. furens* were the dominant salt marsh species.

Seasonal variation. Our results showed that *C. hollensis* was most abundant in the spring and fall, and *C. furens* was a summer species, abundant from May through early September. These results were in general agreement with those obtained by other workers on these species. In South Carolina Henry (1973) reported similar results for *C. furens*, but obtained only the spring peak for *C. hollensis*. This may have been due to her light traps being operated only sporadically from September through February. Khalaf (1967, 1969) reported that *C. hollensis* had a bimodal pattern in Louisiana. In contrast to our studies he stated that *C. hollensis* was present during the winter months. His results for *C. furens* showed 5–6 peaks from April through November, a longer period of incidence than in North Carolina. He also reported that *C. hollensis* was the most abundant species along the Gulf Coast when overall annual density was considered while in our study area *C. furens* was the most abundant species.

Both species are active all year in Florida (Beck 1958). Linley et al. (1970) showed *C. furens* to be most abundant from March through May at Vero Beach, Florida, and Linley (1968) reported that *C. furens* is the most important pest species of this area. High incidence of *C. furens* has also been reported in Panama (Blanton et al. 1955) and Puerto Rico (Fox and Garcia-Moll 1961). Linley and Davies (1971) considered this species to be one of the most important economic pests of the Caribbean area.

In Northern states the seasonal duration is more limited. Jamnback et al. (1958) reported a bimodal pattern for *C. hollensis* in New York, in early and late summer. *C. furens* was present throughout the summer in low numbers. Lewis (1959) found both species to be present in the summer months only in Connecticut. *C. hollensis* had a single peak in mid-July and *C. furens* had 2 major peaks, 1 in mid-July and the other in early August. Wall and Doane (1960) considered *C. hollensis* to be the most abundant salt marsh species in Massachusetts.

Comparison of trapping methods. In our study and in other sampling we found that the light trap had these advantages: (1) collected large numbers, (2) collected the greatest diversity of species, (3) collections were relatively easy to process, and (4) specimens, if removed daily, were in good condition to mount. Disadvantages were: (1) detailed seasonal fluctuations were obscured, (2) few males were collected, and (3) all species were not equally attracted. The light trap provides incomplete data on the seasonal abundance of *C. hollensis* due to the daily activity behavior of this species. We observed that *C. hollensis* was most active from about 2 hours after sunrise to an hour after sunset, and consequently the populations were underestimated by the light trap samples. Therefore, the lack of significant correlations of light trap catches with environmental factors is not surprising. Fewer *C. hollensis* in light traps than in biting collections and larval sampling was noted by Wall and Doane (1960) in Massachusetts, and Jamnback et al. (1958) in New York. While we found no significant correlation of light trap catches of *C. furens* with percentage time flooded (avg. for the entire marsh), Fox and Garcia-Moll (1961), in apparent contrast, found that their light trap catches of *C. furens* over a period of 5 years had an adverse relationship with tide data obtained from tide tables (which are predicted values not actual readings).

We found that emergence traps had these advantages: (1) large numbers of gnats were collected, (2) detailed seasonal fluctuations in numbers of gnats were detected, (3) accurate sex ratios were obtained, (4) detected larval habitats, (5) independent of any power source, and (6) catches were not affected by wind. Disadvantages were: (1) collecting and processing collections required much time, (2) a very limited area was sampled and (3) specimens were in poor condition.

The significance of emergence trap catches with date and temperature was most likely a reflection of temperature. Linley et al. (1970) interpreted the seasonal emergence patterns of *C. furens* as largely temperature dependent. According to our emergence trap data, *C. furens* was most abundant in areas with a low percentage time flooded. Similar findings for *C. furens* in Jamaica were reported by Davies (1967). A possible explanation was offered by Linley's (1966) field observations that mature *C. furens* larvae move to dried parts of the soil prior to pupation. While Davies (1967) also reported that the rate of emergence of *C. furens* adults in Jamaica was unaffected by rainfall, our data indicated otherwise. However, most of the rainfall in 1973 was concentrated in a few heavy downpours, which could have resulted in the washing away of pupae.

Sticky cylinder traps were useful in that they: (1) monitored seasonal fluctuations in the numbers of gnats fairly accurately, (2) gave an indirect indication of larval habitats, and (3) were independent of any power source. Disadvantages were: (1) a great amount of time required to process collections, (2) specimens were in poor condition, (3) relative abundance of *C. hollensis* was not accurately depicted, and

(4) a low percentage of males, especially *C. hollensis,* were caught.

We feel that the significant correlation of sticky trap catches of *C. hollensis* and *C. furens* with rainfall was probably due to reduction of flight during frequent rain storms. In 1972 during the time *C. furens* was most abundant (May to mid-September) the measured precipitation was 713 mm compared to 501 mm during the same period in 1973. Also, the precipitation in 1972 occurred in frequent (mostly evening) storms that produced greater than 25 mm precipitation and occurred at seasonal peak periods of *Culicoides* abundance. In 1973, 5 storms, of which only 1 occurred during time of peak abundance, resulted in 249 mm of precipitation; most of the remaining storms were daytime showers that resulted in less than 12 mm of precipitation. This suggests that the frequency, intensity, and time of occurrence (seasonal and diel) of rain affect *Culicoides* collections on sticky cylinder traps.

In another report (Kline and Axtell 1976) we showed that the greatest number of *C. hollensis* larvae were recovered from areas of the tall form of *S. alterniflora,* thus the significant correlation of catches by emergence traps and sticky sticky cylinder traps with vegetation was not surprising. Since marsh vegetation largely reflects degree of flooding, it was expected that percentage of time a marsh area was flooded should also be an important factor in determining the presence of *Culicoides.* The lack of significant correlation of emergence trap catches with flooding time in the original analysis was probably due to the confounding of this variable with vegetation (i.e., the 2 assumed independent variables are, in fact, highly correlated). This was confirmed when significance was achieved after dropping the vegetation factor from the analysis. Further investigation is needed to determine whether the most important factor affecting the distribution of *C. hollensis* is the physical presence of the vegetation, or if it is the difference in kind and quantity of nutrients required by the immature stages which are reflected by the vegetation type or percentage of time a marsh site is inundated.

Light trap catches of *C. hollensis* and *C. furens* were not significantly correlated with moon phase, but it was noted that greater numbers of both species were caught during new moon than full moon. The effect of moonlight on insect collections has been discussed by Provost (1959), Bidlingmayer (1967) and Bowden (1973). Provost (1959) found New Jersey light traps took greater catches of mosquitoes at new moon than full moon. The reduced catch near full moon was generally thought to be caused by moonlight competing with the light of the trap. Provost (1959) emphasized that the effect of the moon phase can be exposed and analyzed only on the basis of a very large mass of operational data gathered over many months and preferably years. Probably our analysis was based upon too little data.

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