BIOLOGY OF IMMATURE CULICOIDES VARIIPENNIS SSP. AUSTRALIS (COQ.) (DIPTERA:CERATOPOGONIDAE) AT SALTVILLE, VA

by

Jefferson Archer Vaughan

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APPROVED:

E. C. Turner, Jr., Chairman

J. L. Eaton

R. L. Fleischer

J. R. Voshell, Jr.

W. H. Yongue, Jr.

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(ABSTRACT)

The larval and pupal biology of a unique population of *Culicoides variipennis* inhabiting the brine ponds of Saltville, VA was studied. Developmental threshold temperatures (^{o}C) and thermal constants (^{o}days) for larvae and pupae were 9.6^{o}C and 387^{o}days (larval stage) and 9.6^{o}C and 30^{o}days (pupal stage) respectively. Accumulated heat units recorded in the field ranged from 366-376^{o}days between successive generations in the summer. Heat accumulations required for completion of immature development of *C. variipennis* were found to be much greater (831^{o}days) for the overwintering generation. During the summer, larval/pupal distribution within the littoral zone of a brine pond was confined to the surface cm of mud at or near the shoreline.

Insects overwintered farther offshore, mostly as 3rd instars. In early March, most larvae had molted to 4th instars and migrated above shoreline to pupate. Adult emergence occurred in April. Three summer generations were documented for 1983-1984 at Saltville. Life tables and survivorship curves were calculated for the overwintering generation and the first summer generations for 1983 and 1984. For the overwintering generation, there was a relatively constant mortality rate between successive age-classes (Type II survivorship curve). During the summer, there was relatively little mortality between successive larval age-classes but a dramatic increase in mortality was evident at the pupal stage (Type I survivorship curve). Late instar larvae were found to migrate from the shoreline onto the exposed mudflats to pupate, thus becoming vulnerable to predation by ants and carabid beetles. Excellent survival rates of the larvae during the summer was attributed to habitat stability, the paucity of predators and parasites and abundant microfloral content (i.e. food) of the pond water. Intra-specific competition for food resources appeared to be alleviated somewhat by partitioning of those resources on a diurnal cycle.

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I dedicate this dissertation to the memory of my father

.

He was the first to teach me about the wonders of the natural world.

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CHAPTER 1

Introduction

Blood-sucking midges, particularly the genus Culicoides, have received increased attention in recent years. Reviews of Culicoides biology and ecology may be found in Linley (1976), Kettle (1977) and Blanton and Wirth (1979). A systematic review of Culicoides spp. of Virginia is presented by Battle and Turner (1971).

1.1 Disease Importance.

One species in particular, C. variipennis (Coquillet), has received special scrutiny because it serves as the vector for both bluetongue virus (Luedke, et al. 1967) and a closely-related virus causing epizootic hemorrhagic disease (Foster, et al. 1977). Epizootic hemorrhagic disease causes comparatively mild symptoms in livestock but is often fatal to white-tailed deer, with fatality rates known to approach 90% (Hoff and Trainer 1979). Economically, bluetongue is the more serious of the two viral diseases. It causes lameness, abortions, birth defects and occasionally the death of livestock, as well as international trade restrictions on U.S. animals and semen (Jones, et al. 1981). Bluetongue virus is more virulent in domestic sheep and goats than in cattle, however, cattle often serve as

reservoir hosts for the disease. Bluetongue in the USA occurs primarily in the west and southwest. Although the incidence of bluetongue is lacking in the east, the occurrence of the vector, C. variipennis, is widespread in the east and often associated with livestock production. Foster and Jones (1979), using a western population, showed that bluetongue virus titer in C. variipennis peaks 10 to 14 days after an infective bloodmeal. Mullens and Rutz (1984) demonstrated that the survivorship of eastern populations of C. variipennis under field conditions in New York state (i.e. 19 days) is long enough to allow replication and transmission of the virus. Apparently physiological or environmental barriers exist within the eastern form of C. variipennis preventing viral replication. I should point out that there is some confusion concerning the taxonomy of this insect. Wirth and Jones (1957) described five subspecies of C. variipennis, primarily on the basis of four adult morphological characters. Hensleigh and Atchley (1977) recommended dropping the trinomial nomenclature since they showed that variations in adult morphology are strongly related to larval environment. Recent work suggests that when New York midge populations (normally having a viral infective rate of ca. 2%) are reared under the conditions at the USDA-ARS facility in Denver, CO, their susceptiblity

rate to bluetongue virus replication increases dramatically (Jones, unpubl. data). All of this implies that the environmental conditions of the larval habitat may be quite important, not only with respect to adult morphology, but also perhaps to the vector competency of C. variipennis.

1.2 The Saltville Subspecies.

All studies reported here were done using midge larvae inhabiting the brine ponds of Saltville, VA (Fig. 1). Wirth and Jones (1957), after examining adult specimens from Saltville concluded that the Saltville population belonged to C. variipennis subsp. australis, a subpecies indigenous to the lower Mississippi Basin north to the Ozark Plateau, not the more typical eastern subspecies, C. variipennis variipennis. Indeed, Downes (1978) proposed that the Saltville population may represent a distinct species, C. occidentalis. Culicoides variipennis australis are known to breed in ponds of high salinity, whereas the typical breeding sites for C. variipennis variipennis are the sunlit margins of farm ponds and lagoons containing substantial amount of animal excrement (Blanton and Wirth 1979, Hair et al. 1966). The major breeding sites at Saltville are brackish (salinity = 15-30ppt) as a result of ground water seepage from ancient marine deposits forming rock salt accumulations in the underlying limestone bedrock (Ray et

al. 1967). The ponds lie in a basin (ca. 32 hectares) surrounded by mountains and, as far as is known, there exist no other breeding sites of C. variipennis within 3km of Saltville. Therefore, it seems plausible that subspeciation (or even speciation) of the Saltville population could have occurred via geographical isolation. However, Zimmerman et al. (1982) found both subspecies, as described by Downes (1967), as well as intermediate forms within the same mating swarm in Saltville. Based upon anatomical measurements of adults reared from different areas of the state as well as from Saltville, Zimmerman (1981) concluded that C. variipennis should be considered as a single, albeit somewhat plastic, species with ecotypic variability attributable to differing environmental parameters in different breeding sites. During preliminary sampling, I found C. variipennis larvae inhabiting various Saltville sites with wide ranges of salinity (0.9ppt-36.0ppt) and pH (6.5-8.6). Clearly, the possibility of subspeciation at Saltville warrants closer investigation, as well as the effect that larval environment has on the vector competence of adults. Rather than become entangled in a debate on taxonomy, I chose to study certain aspects of larval ecology and biology of the Saltville midge population. The reasons for this were two-fold. First, the adult ecology for this

unique population has already been studied in depth (Zimmerman 1981). Second, the relative abundance of midge larvae at Saltville was much greater than in more "typical" habitats sampled in and around Blacksburg, VA. At Saltville, I was assured of obtaining large numbers of larvae everytime I took a sample. In addition, the Saltville breeding sites were virtual monocultures and only once or twice did I encounter Culicoides larvae of a different species when sampling the water's edge. Pending further studies on the taxonomy of this insect (e.g. isozymes), I will acknowlege the more conservative and widely-accepted trinomial designation, C. variipennis australis, however, for brevity's sake, will refer to the Saltville population as C. variipennis in this dissertation.

1.3 Objectives.

This research addresses the larval/pupal ecology and biology of a single unique population of C. variipennis. Specific objectives include:

1. Determination of developmental times, developmental threshold temperatures and thermal constants for the egg, larval and pupal stages under constant laboratory temperatures. This objective is addressed in Chapter 2 and serves as a base on which temperature data collected from the field is interpreted.

2. Investigation of the seasonal microdistribution of the immature stages within the littoral zone of a pond. This objective involved monthly sampling at different horizontal and vertical distances within the microhabitat throughout an entire year. It is discussed in Chapter 3.

3. Investigation of the diel periodicity exhibited by larvae in microhabitat distribution and studies into its probable causes. This is addressed in Chapter 4.

4. Determination of field survival and population dynamics of immature C. variipennis at Saltville, VA. This comprises the majority of Chapter 5.

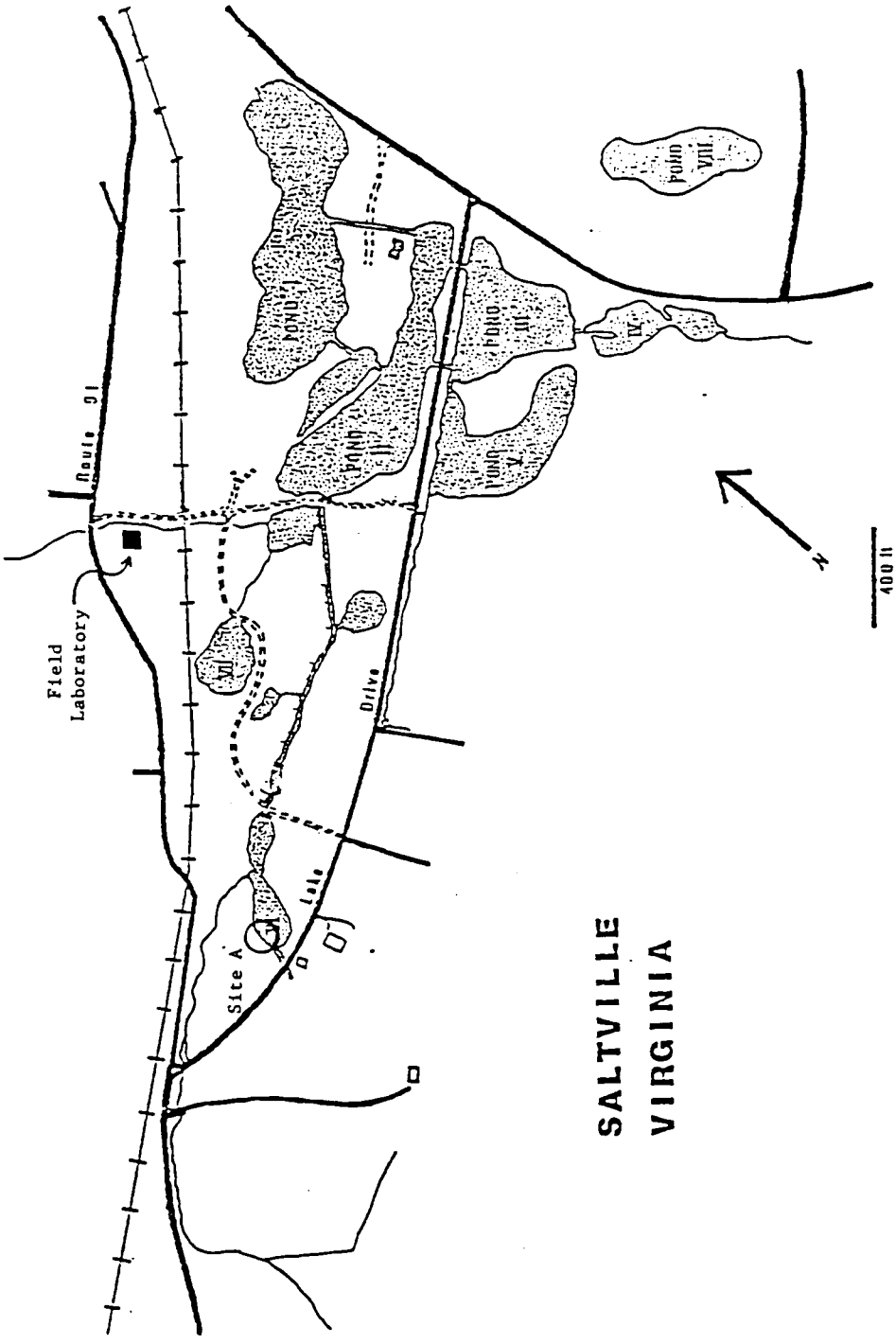

Figure 1. Brine ponds of Saltville, VA

CHAPTER 2

Development of immature Culicoides variipennis ssp. australis (Coq.) at Constant Laboratory Temperatures

2.1 INTRODUCTION

Because temperature plays such a dominant role in insect development, the seasonal dynamics of most insect populations are strongly influenced by temperature. Developmental rates obtained in the laboratory can often be used in obtaining approximations of thresholds and thermal constants useful in the interpretation of field data. Until recently, developmental studies with Culicoides spp. have focused upon the effect that different rearing temperatures have upon adult characteristics (Hensleigh and Atchley 1977) or upon the quality control factors involved for optimal colonization (Akey et al. 1978). Kitaoka (1982) tested the effect of temperature on larval duration and adult size for C. arakawae and C. maculatus. He reported that C. arakawae was more adaptable to higher temperatures than C. maculatus, but both species had close to the same "developmental zero" (threshold?) of 15-16°C. Mullens and Rutz (1983a) reared immature C. variipennis in natural substrate under constant temperature conditions. They reported developmental

threshold temperatures and thermal constants for each lifestage, with total immature development having respective values of 10.5°C and 284°days. Their estimates were for C. variipennis ssp. variipennis from New York State. Developmental parameters for insects often differ with populations of a species in different latitudes (Taylor 1981). It is for this reason that I elected to undertake developmental studies using the Saltville population of C. variipennis ssp. australis, not only for use in further field studies at Saltville, but also to compare them with developmental parameters of New York midge populations. With minor modifications, I patterned my procedures after that of Mullens and Rutz (1983a).

2.2 MATERIALS AND METHODS

Egg, larval and pupal stages of C. variipennis were reared at 20±1.9°C, 23±1.8°C, 27±1.4°C, 30±1.5°C and 35±1.7°C at a 14:10 (L:D) photoperiod.

2.2.1 Egg Stage.

Gravid adult females were live-collected at Saltville using UV light attraction and an aspirator. Oviposition vials containing damp filter paper were inserted into the sides of the cages containing the gnats. Cages were held at

ca. 27°C in darkness for ca. 4h after which oviposition vials were replaced. Filter papers containing the eggs were placed in petri plates in the rearing chambers. Dishes were checked at 4-7h intervals and hatched eggs were counted and removed. At the end of 9 days the test was terminated.

2.2.2 Pupal Stage.

Late instar larvae were collected from the field prior to spring emergence. Larvae were placed on damp filter paper in petri dishes at 27°C. Dishes were checked every 2 to 8h for pupation and new pupae were tranferred onto damp filter paper and placed into rearing chambers. Dishes were checked at 4 to 8h intervals for adult emergence. The test was terminated at the end of 7 days.

2.2.3 Larval Stage.

Newly-hatched larvae were reared to adults in rearing cartons (3.8l cylindrical ice cream containers with screen lids) into the bottom of which were fitted glass stender dishes containing ca. 200 ml of larval substrate. Larval substrate consisted of surface mud collected from the littoral zone of a Saltville pond known to support larval C. variipennis. Prior to use, the mud was heated to 70°C for 1h to eliminate macroorganisms, then cooled. Stender dishes containing sterilized mud were placed on a slight slope in

the bottom of the rearing cartons and freshly-collected Saltville pond water containing a rich assemblage of phytoplankton was added to cover most, but not all, of the substrate. Newly hatched larvae (12-24h old) were obtained from oviposition papers as described above. One hundred 1st instar larvae were added to each rearing carton and cartons were replicated 5X for each temperature. Cartons were lightly swirled to break up surface scum and checked for adult emergence on a daily basis. Tap water was added to the substrate as needed. The duration of the larval period was calculated by subtracting the pupal period for a given temperature from the elapsed larval-adult period for that temperature. At the apparent end of adult emergence, larval substrates were extracted for larvae (Boreham 1981) to confirm that all insects had completed development.

Emerging adults were collected by aspirator from the rearing cartons and placed into 70% EtOH. One wing was removed from each emerging adult and measured from the basal arculus to the tip with an ocular micrometer to compare their size at each temperature.

2.2.4 Statistical Analysis.

Developmental rate graphs for each lifestage were generated using linear regression, plotting developmental rates (1/days) against temperatures. Theoretical

development thresholds (t) for each lifestage were estimated by extrapolation of regression lines to the abscissa. In order to determine degree-day accumulations required for completion of development, thermal constants (k) were calculated using the equation $k=y(d-t)$, where y = developmental time in days, d = temperature in ^{o}C, and t = the theoretical developmental threshold (Andrewartha and Birch 1954). Thermal constants were calculated for each temperature and then averaged to obtain an overall thermal constant for each lifestage. Linear regression was also used to test for sex differences and to compare winglengths of emergent gnats. Duncan's Multiple Range Test was used to separate means.

2.3 RESULTS

The developmental times for each lifestage, as well as theoretical developmental threshold temperatures and thermal constants for larvae and pupae, are given in Table 1. Developmental times represent mean values for the egg and pupal stages. Modal values were judged to be more appropriate in describing the larval duration. The frequency distribution for larval duration was skewed to the right at most temperatures (Fig. 2a-d), tending to distort

the mean values as a measure of central tendency. The late-occurring "spike" in the 30°C rearing temperature (Fig.2c) was deemed to be an artifact and was disregarded (see discussion below). A developmental threshold temperature (and hence thermal constant) could not be justifiably calculated from the developmental rate curve for eggs (Fig. 3), which appeared rather erratic. Theoretical developmental threshold temperatures for larvae and pupae were the same (9.6°C). The data suggest that approximately 417°days are necessary for insects to complete development from hatch to adult emergence.

Stage survivorship at different temperatures is presented in Table 2. Eggs had lowered survival at the temperature extremes (20, 35°C). Most eggs at 20°C failed even to develop eyespots, whereas at 35°C many hatchlings apparently intiated eclosion but died before completely working free of the egg. Mean larval survivorship (10-18%) was less than that reported by Mullins and Rutz (1983a) and probably can be attributed to the greater larval densities used in this study and to lack of adequate light intensity needed to support the autotrophic microfloral community normally associated with C. variipennis habitats. Pupal survivorship was relatively good at all temperatures.

Regression analysis revealed that insects reared at lower temperatures were, for the most part, larger than those reared at higher temperatures (Table 3). A notable exception to this was emergent adults reared at 35°C. These insects (both male and female) were significantly larger than insects reared at 30°C. This may have been caused by an accelerated rate of bacterial growth within the rearing media at 35°C, contributing to better larval nutrition. Males were significantly smaller than females. No significant differences were observed between males and females for larval or pupal duration. Similar findings have been reported for this and other species of Culicoides (Akey et al. 1978, Davis et al. 1983, Hensleigh and Atchley 1977, Kitaoka 1982, Mullens and Rutz 1983a).

2.4 DISCUSSION

It is generally accepted that developmental rate curves for insects are sigmoid in nature (Davidson 1944) and there have been several different mathematical equations used to describe them (Lamb et al. 1984). Although not the most accurate, perhaps the most popular method utilizes a linear model. Linear models are much less cumbersome in obtaining estimates than are other models, but tend to overestimate

the true developmental threshold temperature of an insect particularly if only those temperatures exiting within the linear portion of the sigmoid curve are tested. In this study a relatively wide range of temperatures were chosen. The developmental rate curve for pupae seemed to "flatten out" at 35°C as it approached what appears to be the upper developmental threshold temperature (Fig. 3). The developmental rate curve for larvae appeared more linear at the temperatures chosen, suggesting a greater tolerance of the larvae to elevated temperatures. Because of the high degree of linearity with the larval developmental rate curve (r^2=0.97), my estimated developmental threshold temperature (9.6°C) may exceed somewhat the true developmental threshold temperature. However, since the slope of the rate curve is relatively flat (b=0.002) the margin of error is much reduced.

The erratic development of eggs is puzzling. In previous studies (Davis et al. 1983, Mullens and Rutz 1983), eggs were obtained by decapitation of gravid females so that the exact time of oviposition was known. A certain amount of resolution (ca. 6h) was sacrificied in my method by not knowing the precise time of oviposition, but I feel that this deficiency was overcome by increased sample size (n=4057), and the fact that decapitation may induce

premature oviposition of less-than-fully developed eggs (Davis et al. 1983, pers. obs.). Developmental rates among eggs from different females may not be synchronous at the same temperature, or perhaps the time elapsed between completion of embryonic development and successful hatch may vary among individuals. Observations revealed that temperature extremes have deleterious effects on larval eclosion and, hence, egg mortality (Table 2). Larval survivorship was rather low (10-18%) at all temperatures, indicating that unsuitable conditions existed in the rearing containers. Little is known concerning the dietary requirements of C. variipennis larvae, but it is presumed that the microfloral community within breeding habitats is a key constituent of larval nutrition (Parker et al. 1977) Microscopic examination of the rearing substrate revealed a wealth of bacterial flora as well as ciliated protozoans living in the mud. Examination of the natural habitat of this midge, however, reveals a much more autotrophic community of phytoflagellates and diatoms, suggesting that a saprozoic microfloral community with its associated byproducts is suboptimal for this insect's survival. At the apparent end of adult emergence, extraction of two replicates in the 30°C temperature group produced ca. 20 4th instar larvae, apparently in an arrested stage of

development. When placed into fresh media, they quickly and synchronously completed development as shown in a late-occurring peak of the frequency distribution (Fig. 2c). Other studies using field-collected eggs placed in a substrate devoid of significant microfloral growth, showed that many 1st instar larvae ingested the sterile mud and survived for 2wks or more, but remained at the 1st instar stage, developing very slowly if at all, regardless of temperature (20, 23, 27, 30^{o}C). Addition of green algae to some of the containers produced almost an immediate shift in the larval age-structure within those containers from predominantly 1st instars to predominantly 2nd instars. Thus it would appear that food quality affects developmental duration as well as survivorship in this insect. For this reason, I suspect that my figures on larval duration (and the thermal constant derived from them) may be somewhat larger than those actually occurring in the field where larval nutrition is probably better. Mullens and Rutz (1983a), who had good larval survivorship (60-75%), calculated a thermal constant for New York larvae as 212^{o}days. My calculated thermal constant was 387^{o}days. The theoretical developmental threshold temperature for Saltville larvae (9.6^{o}C) was similar to the threshold for New York midge larvae (10.7^{o}C) reported by Mullens and Rutz

(1983a). Using only those temperatures common to both studies (i.e. 20, 23, 27, 30°C), a comparison of larval and pupal developmental rate curves showed no significant difference in the developmental rate (i.e. slope) between New York and Saltville populations (F-test, $p<0.05$). I should point out, however, that the developmental rate for the New York larvae (0.004) was twice that of the Saltville larval rate (0.002) and that, considering only four points were used to generate each line (df=1,4), statistical significance, in this case, may not accurately reflect biological significance. It is known that more northern populations of an insect often display accelerated rates of development to compensate for a shorter growing season (Taylor 1981). Although differences in larval developmental rates between New York and Virginia C. variipennis populations appear slight from a statistical standpoint, such slight differences may represent adaptations of two populations to two slightly different climatic regions. Similar comparisons on C. variipennis populations from even more dissimilar regions could prove illuminating.

Table 1. Developmental times, developmental threshold temperatures (+), and thermal constants (k) for immature Culicoides variipennis.

		Developmental Time[1]						
Life Stage	N	20°	23°	27°	30°	35°	t(°C)	k(°days)
Egg (hrs)	4057	63.6±8.2	64.7±7.8	61.4±4.1	50.9±12.9	57.1±7.8	-	-
Larva (days)	2500	33	32	24	21	15	9.6	386.9±26.9
Pupa (hrs)	597	89.3±7.8	65.5±7.4	50.6±5.9	39.1±3.9	38.8±4.4	9.6	29.9±1.0

1. $\bar{x}$ ± s.d. for egg and pupal stages; mode for larval stage.

Table 2. Survivorship (%) of immature Culicoides variipennis under different temperatures.

		------ Temperature (°C) -------				
Life Stage	N	20	23	27	30	35
Egg	4057	23.0	63.4	58.8	68.0	17.7
Larva	2000	14.0	17.6	10.4	16.0	18.0
Pupa	597	80.0	87.7	94.3	94.4	88.9

Table 3. Winglengths (mm) of Culicoides variipennis reared at constant laboratory temperatures.

	All Temps	20°	23°	27°	30°	35°
Total (n=66)	1.43±.13	1.55±.09a[1]	1.48±.10b	1.42±.11c	1.27±.11d	1.43±.10c
Males[2] (n=34)	1.39±.12	1.51±.06a	1.42±.09b	1.38±.10b	1.26±.10c	1.39±.09b
Females (n=32)	1.47±.14	1.60±.10a	1.52±.09b	1.46±.10c	1.29±.13d	1.47±.08c

1. Means followed by same letter are not significantly different by Duncan's Multiple Range Test (p 0.05).

2. Differences in male and females significantly different (t-test, p 0.05).

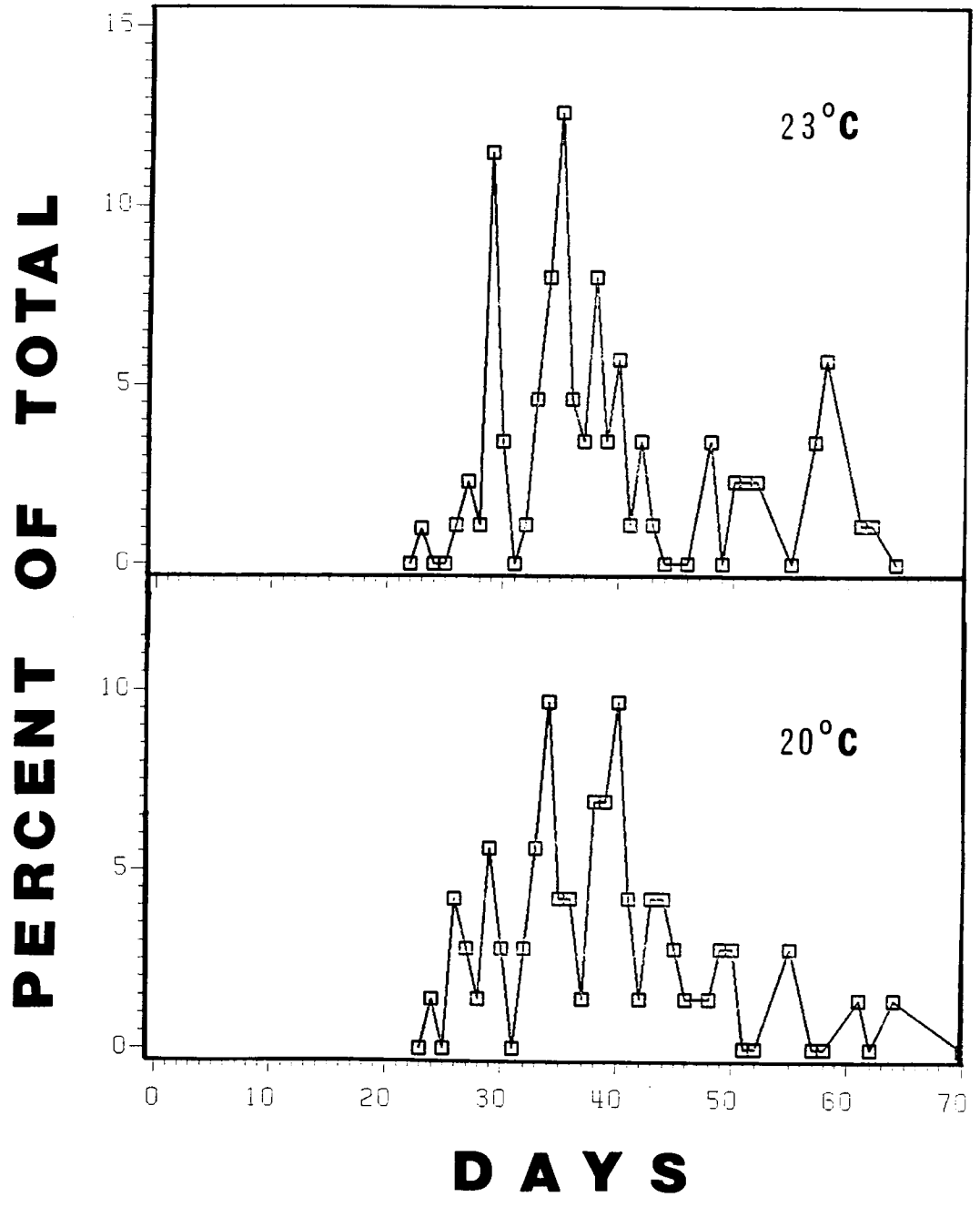

Figure 2a. Relative frequency distributions (% of total) of larval/pupal duration of Culicoides variipennis at constant temperatures of 20 and 23°C

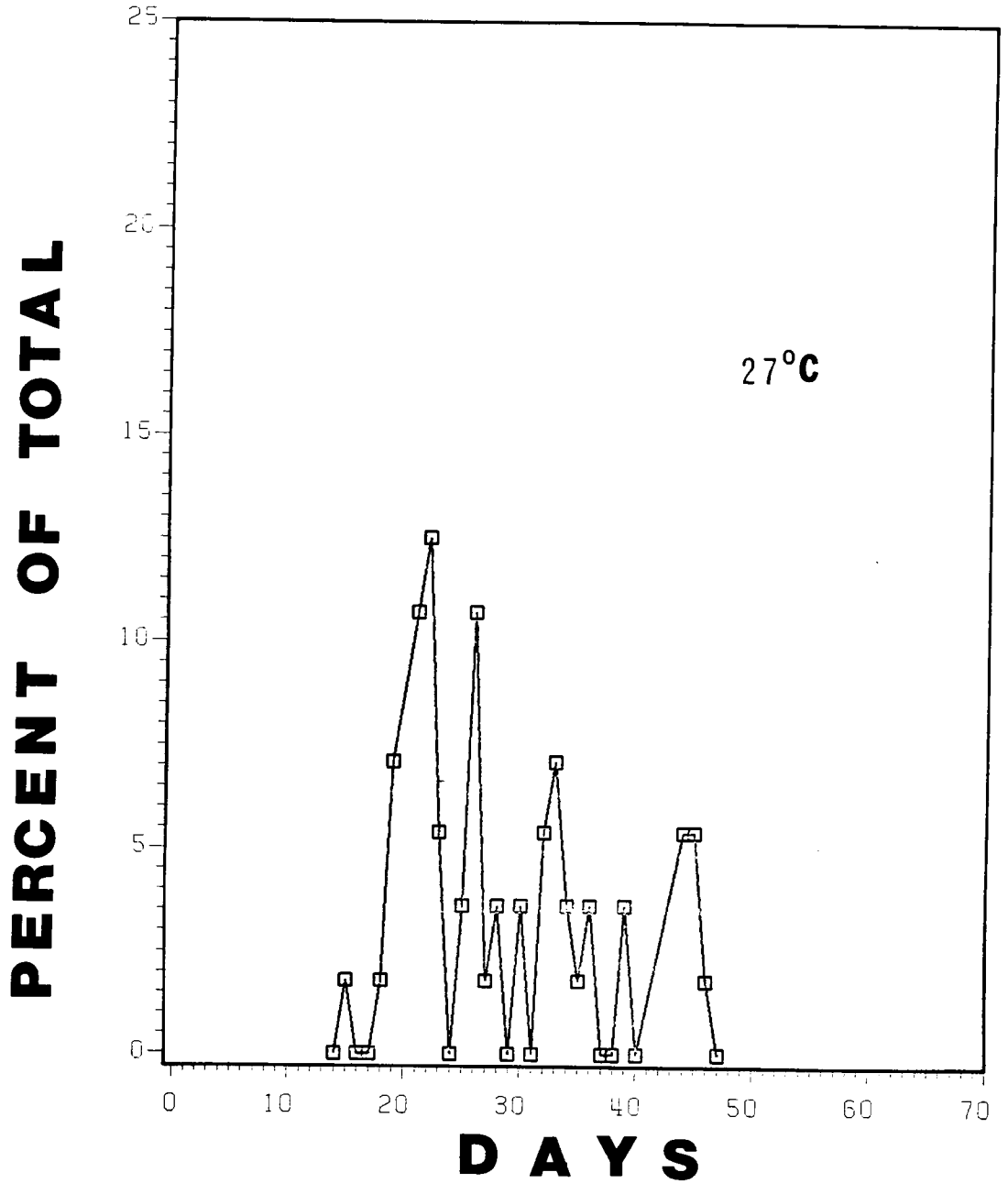

Figure 2b. Relative frequency distribution (% of total) of larval/pupal duration of Culicoides variipennis at constant temperature of 27°C

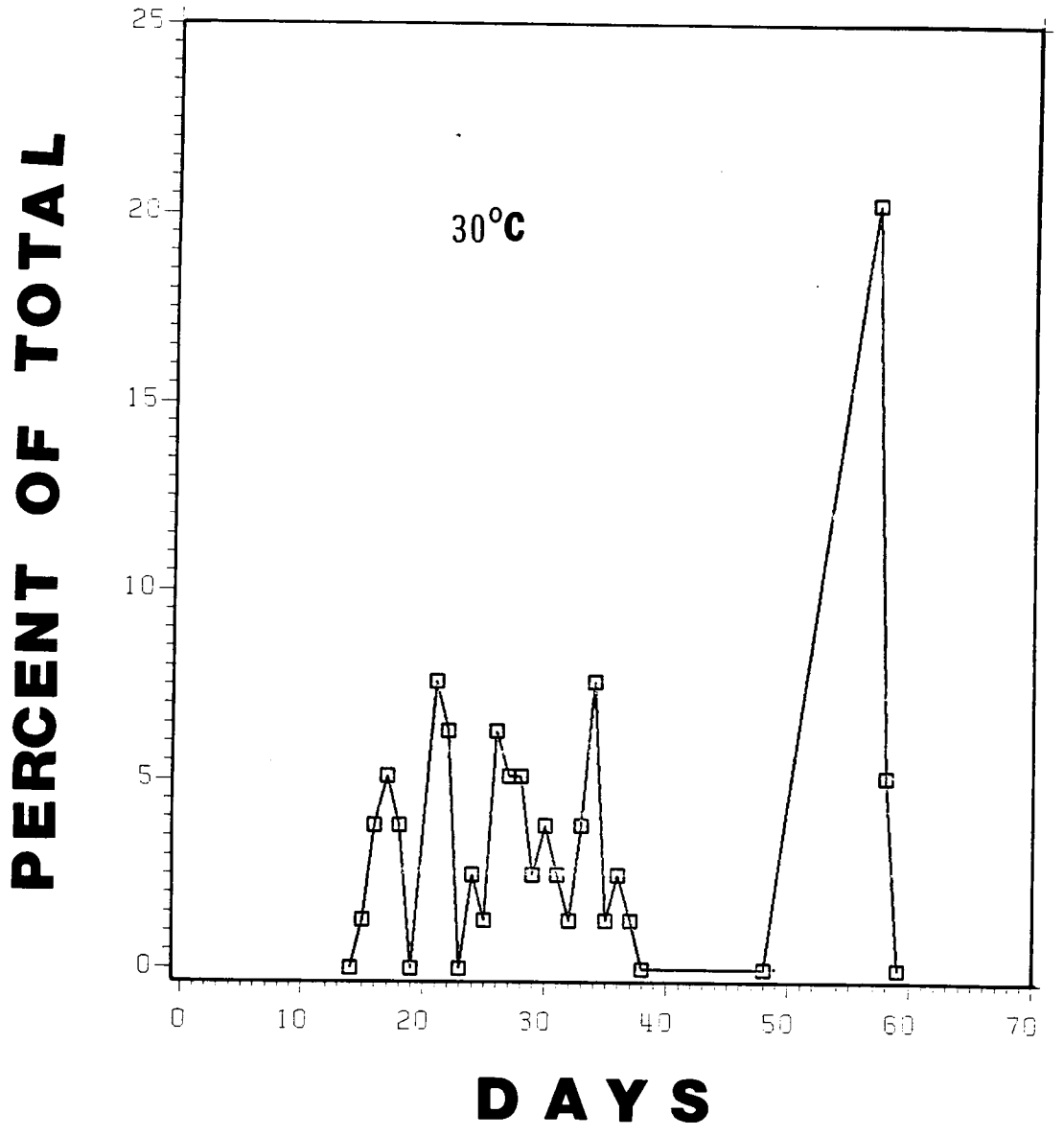

Figure 2c. Relative frequency distribution (% of total) of larval/pupal duration of Culicoides variipennis at constant temperature of 30°C

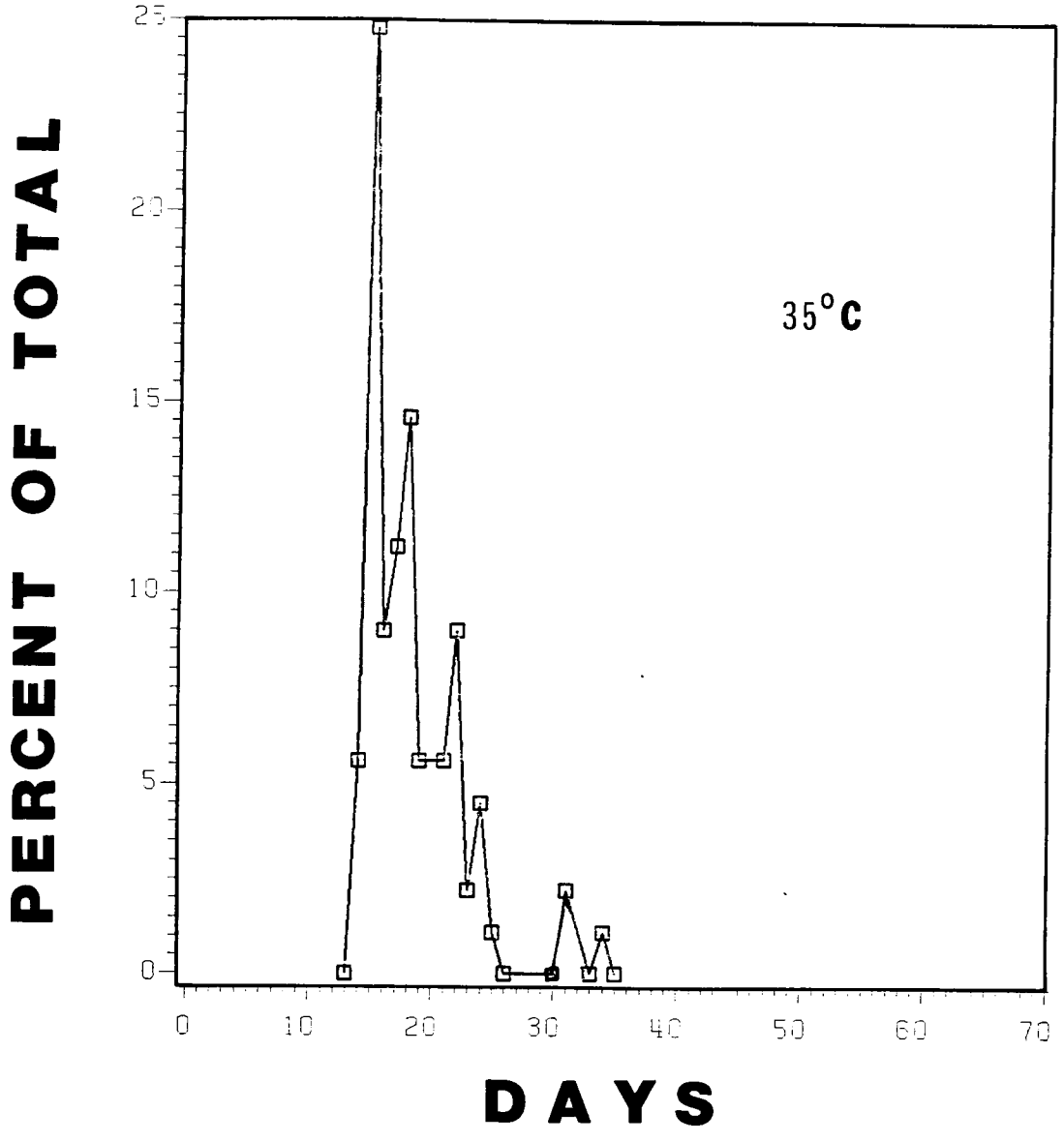

Figure 2d. Relative frequency distribution (% of total) of larval/pupal duration of Culicoides variipennis at constant temperature of 35°C

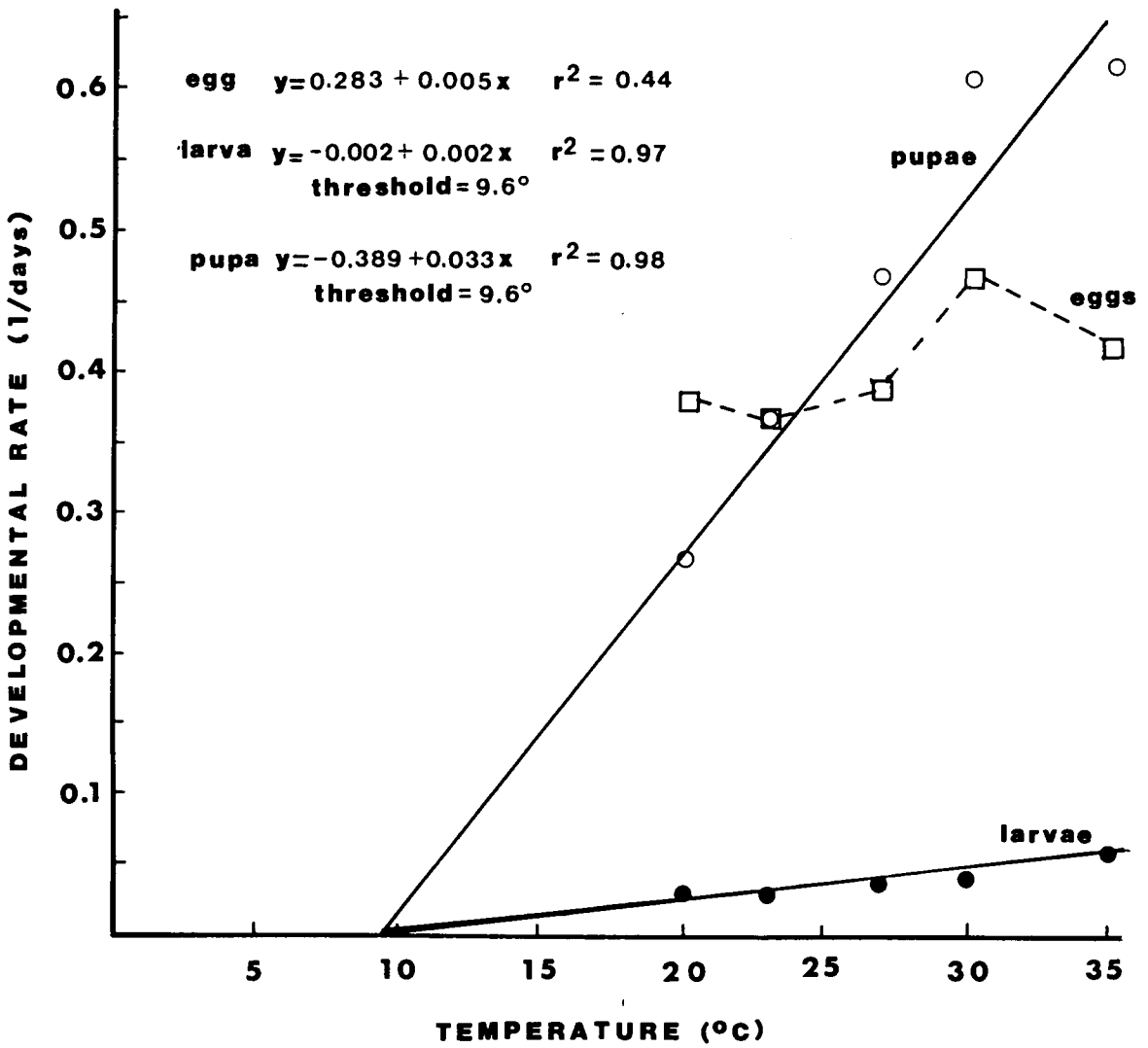

Figure 3. Developmental rate curves and threshold values for immature Culicoides variipennis

CHAPTER 3

Seasonal microdistribution of immature Culicoides variipennis ssp. australis (Coq.) in Saltville, VA

3.1 INTRODUCTION

The larvae of Culicoides variipennis (Coq.) are present in the littoral zone of farm ponds and lagoons throughout the year and is the overwintering stage of the insect in regions with rigorous winters. During summer months C. variipennis larvae inhabit the top cm of mud at or near shoreline (Barnard and Jones 1980). There have been no definitive studies on C. variipennis larval distribution during the winter, although Jones (1967) reported finding C. variipennis larvae in mud under 50cm of water during winter months. It has been suggested that overwintering larvae escape unfavorable winter temperatures by burrowing deep into the mud or moving out into mud under deep water. In Russia, Isaev (1977) observed that C. odibilis larvae move into mud beneath the water surface (i.e. horizontal movement) during the winter and return to the shoreline in the spring. Rieb and Guinier (1981) found similiar shifts in the larval distribution of C. clastrieri and C. odibilis. Stream-inhabiting Culicoides sp. were reported to inhabit stream margins during the autumn and spring but move to the

stream channel bottom during summer to avoid high temperatures (Dzhafarov 1964). Winter-collected C. variipennis larvae display a substantial delay in the onset of pupation upon warming when compared with summer larvae, suggesting that winter larvae undergo physiological diapause (Rowley 1967). Yet larvae are often observed actively swimming about and feeding during warm periods in the winter (Rowley 1967, Mullens 1982). This chapter investigates the seasonal microdistribution of immature C. variipennis with observations on winter bionomics.

3.2 MATERIALS AND METHODS

3.2.1 The Study Site.

All results pertain to a single brine pond in the Saltville basin. The pond is fed by drainage of the western edge of the saltmarsh and represents one of two major headwater ponds of the Saltville valley drainage pattern. The loose depositional silt along the pond's perimeter supports enormous numbers of Culicoides variipennis larvae. From April to October the warm sunlit waters of this pond are extremely rich in phytoplankton, mainly freshwater species despite salinities ranging from 10 to 30ppt. The dominant microfloral species are Chlamydomonas sp. and

several unidentified pennate (boat-shaped) diatom species. Several months prior to intiating the study, two catwalks were constructed extending from the shoreline ca. 6m outward into the water. This was done to minimize habitat disturbance and to avoid becoming mired in the deep ooze during sampling. The posts for the catwalk also served as convenient points of reference in which to gauge the water level of the pond from month to month. Continuous temperature readings throughout the year were taken with a recording thermometer with probes placed in the surface mud just below waterline.

3.3.2 Larval Extraction.

Prior to conducting ecological studies on C. variipennis larvae, a suitable method was required to separate insects from the mud. The method had to be; 1) efficient at extracting all stages from the mud, and 2) simple enough so that a large number of samples could be processed quickly and easily. Many larval extraction techniques have been described (Bidlingmayer 1957, Dove et al. 1932, Jamnback 1965, Jamnback and Wall 1958, Jamnback and Wirth 1963, Kettle and Lawson 1953, Kettle et al. 1956, Williams 1960) all of which are labor-intensive and time-consuming. These methods rely on either sieving, floatation or Berlese funnels. After experimentation, I chose the

modified salt-floatation procedure outlined by Boreham (1981). Briefly, this procedure is as follows. A plastic funnel that has been trimmed to fit is inverted and placed snugly over the mud sample forming a cone-shaped inner chamber (Fig. 4). A saturated salt solution (150g NaCl/l H_2O) is poured into this inner chamber through the mouth of the plastic cone. The inner chamber is partially filled and, holding a thumb over the opening, the unit is agitated briefly to create a salt-mud slurry. The inner chamber is then filled to the top of the cone with more saturated salt solution. Saline is added to the outer chamber, immersing the top of the cone so that insects are free to float/swim out of the inner cone and into the upper chamber. Units are left under darkness for a minimum of 2h. This was found to enhance extraction of the larvae. Extracted insects are harvested by placing a finger over the mouth of the funnel and decanting the upper chamber. Before employing the Boreham extraction method in ecological studies, I validated its effectiveness by tediously sieving and microscopically examining 100-150ml mud samples (n=3) following extraction. Validation revealed the Boreham extraction procedure was 96±2.1% efficient in removing larvae from substrate samples. There appeared little difference in the extraction efficiency between different larval instars. Thus the

Boreham extraction procedure was used in all ensuing studies as the means for separating larvae/pupae from mud samples.

3.2.3 The sampling design and sampler.

Samples were taken at monthly intervals (ca. midday) for an entire year. Horizontal distribution of the insects was obtained by sampling predetermined distances (ca. 25cm) along transects perpendicular to the shoreline. These horizontal sampling distances were always measured in relation to the shoreline, a natural limnological boundary, on the day of sampling. However, since the shoreline was subject to change, particularly with spring rains and snowmelt, monthly sampling was only undertaken after the pond had drained and settled somewhat following inundation. Intially, four horizontal distances were chosen; one above shoreline (AB), shoreline (SL), and two below shoreline (BL1,BL2). During the winter it became apparent that larvae were moving out of the sampling area so another sampling distance was added farther out from shore (BL3).

At each horizontal sampling distance, vertical distribution of the insects was simultaneously ascertained by taking the samples with a variable-depth sampler (Fig. 5) capable of partitioning the mud into one-cm thick increments to a total depth of 5 cm. The sampler, constructed of Plexiglass, was inserted into the mud with the top partition

closed and the remaining partitions withdrawn. Upon reaching a depth of 5cm the surface mud could be observed to make contact with the top partition and the remaining partitions were then slid into place, accurately partitioning the mud. The sampler was then gently eased out of the mud. By withdrawing the partitions one-by-one, the different mud depths could then be rinsed into appropriately-labelled plastic containers. This device was used to take all samples with exception of occasional above-shoreline samples where bordering grass roots made it impossible. In these instances, a steel coring cylinder of the same approximate volume (i.e. $200cm^3$) had to be employed. In all, 80-100 samples (4 or 5 horizontal distances X 4 vertical distances X 5 replicates) were taken each month for 12 months.

In addition, mud samples from the shoreline habitat were taken at quarterly intervals throughout the year. Samples were oven-dried and standard soil analyses were performed by the Virginia Tech Soil Testing Laboratory.

3.2.4 Processing.

Insects were extracted from the mud using the modified salt-floatation method described by Boreham (1981). Extracted insects were taken through a coarse straining, retaining pupae and larger instars. Retained insects were

rinsed into a petri plate with 95% EtOH. The flow-though was filtered through fine nylon mesh (41µ opening) with the aid of a Buchner funnel and vacuum pump. First and second instar larvae were effectively retained on the nylon filter. These were washed off with water into alcohol. Insects were enumerated, age-graded according to head capsule width, and stored in 95% EtOH. Upon completion of sampling, larval subsamples representative of each season (29 Nov, 26 Jan, 10 May, 31 July) were removed and head capsules measured using an ocular micrometer at 100-150X in order to determine if seasonal differences existed in mean larval size. Late-winter larvae were also examined for the presence or absence of possible cryoprotectant agents (i.e. polyols) using high-performance thin-layer chromatography (HPTLC). Live larvae were macerated with hand-held tissue grinders in distilled water. Macerated samples were centrifuged and the supernatant extracted with chloroform to remove lipids, fats, etc. Half microliter samples were spotted onto silca-gel HPTLC plates along with various polyol standards (5mg/ml). Seven different solvent systems and two different indicators were used for detection and validation.

3.3 RESULTS

3.3.1 Larval/pupal abundance.

Figure 6 shows the average weekly mud temperatures for 1983-84. Temperatures declined steadily from September to December. In late December through January, the habitat remained frozen, yet thawed abruptly in mid-February and continued to warm as summer approached. Fluctuations in average weekly temperatures (indicated by the vertical bars) were greatest during the summer and autumn months. Table 4 shows age-grade percentages of the total catch for each monthly sample. Pupae were absent throughout the colder months. The majority of larvae overwintered as 3rd instars and did not molt to 4th instars until well after the Winter Solstice. There appeared to be rather substantial mortality of larvae during the course of the winter and absolute density declined steadily from November to late June. During September insect distribution was largely confined to the surface mud, very similar to the pattern noted during summer months. In October and November larvae burrowed progressively farther down into the mud (Fig. 7). In late December/early January the entire pond was frozen, with ca. 15cm of ice above and the underlying mud freezing to a depth of 2-3cm. Ice cover at each sampling location had to be removed with a hatchet before employing the variable-depth

sampler. The majority of larvae at this time were recovered from a narrow liquid interface existing between the ice and frozen mud (Fig. 8). In addition, larvae appeared to have moved offshore and it was for this reason that another horizontal distance (BL-3) was added in successive sampling regimes. Indeed, in late January most larvae were located at BL-3 and unreplicated samples taken at the end of the catwalk revealed larvae existing as far as 6m beyond the shoreline. In early March, following a warming trend in February, most larvae had molted to 4th instars (Table 4) and had moved up above shoreline apparently in preparation for pupation. In early spring (1 April) practically all insects collected were either pupae or 4th instars preparing to pupate (i.e. prepupae) and were aggregated in the surface mud above the shoreline (Fig. 9). The first adult emergence of the season occurred between 1 April - 1 May, with the first summer generation of larvae appearing in early May. From 10 June through the remainder of the summer (28 August), most larvae were collected near shoreline in the top cm of mud (Fig. 10).

3.3.2 Anatomical and chemical measurements.

Frequency distributions of larval head capsule widths (Fig. 11) show that autumn and winter larvae (29 Nov, 26 Jan) are larger than larvae collected in the spring and

summer (10 May, 31 July). This is particularly evident with 4th instar larvae. Most likely autumn larvae and winter larvae represent the same generation (i.e. overwintering generation), whereas most spring larvae probably represent the first summer generation. Larvae were smallest in the summer. This was true even for 1st instar larvae. Seasonal changes in substrate composition (Table 5) demonstrate a lowered index in the quantity of digestable material (i.e. % organic matter) in the habitat during the summer. The increased dissolved O_2 of the water during the spring can probably be attributed to spring algal blooms which, interestingly enough, corresponded with increasing salinity and substrate acidity. Nitrate levels were lowered during the summer, perhaps as a result of its metabolic utilization by spring algal blooms.

Glycerol was the sole polyol detected in late winter larvae using HPTLC. Glucose, which may also serve as an additional component in insect cryoprotectant systems (Baust and Lee 1983), was also present. Other polyols known to exist in cold-adapted insects (sorbitol, mannitol, ribitol) were not present.

3.4 DISCUSSION

Larval/pupal distribution during the summer months was largely confined to the surface mud at or near shoreline as described by previous workers (Barnard and Jones 1980). Microscopic examination of mud depths revealed that the microfloral abundance of the surface mud during the summer months was comparable to that of the pond, with the notable exception of the above shoreline site which was considerably less. Deeper mud was increasingly barren of phytoflagellates and diatoms. During algal blooms in the spring and early summer, C. variipennis larvae were observed to have "green guts", suggesting that phytoplankton, when in abundance, is an important constitutent of larval diet. With the approach of winter and subsequent decline in phytoplankton abundance, larvae appeared to take on a "rusty" color. This coloration was readily leached from their bodies with 95% ethanol and was flourescent under UV light. The orange coloration was most intense in liquid extracts prepared from late-autumn larvae (29 Nov.) and late-winter (4 March) larvae. It is not known whether these compounds represent xanthines and/or other compounds picked up in the diet (i.e. autumnal leaf-fall) or if the color is due to some endogenous product produced by the insects in preparation for winter.

As temperature and daylength decreased, larvae began to burrow into the mud, preferring to remain near the shoreline. Burrowing behavior is most likely attributable to progressive changes in temperature and photoperiod. Measurements taken with a hand-held thermometer during autumn sampling revealed the insulative properties associated with the mud. Invariably, autumn mud temperatures 2-3cm below the surface were 1-2^{o}C warmer than surface mud, suggesting that larvae were actively following a preferred temperature gradient. However, when the pond and underlying mud froze, most larvae were recovered far from shore, aggregated at the interface between ice and mud. This narrow region remained liquid even though the mud beneath was frozen to a depth of 3cm. It is difficult to discern whether the observed shift in larval distribution at this time resulted from active mobility of the larvae (which appeared rather sluggish) or whether larvae were moved passively in the course of ice crystal formation starting at the pond's edge and moving inward. A substantial number of recovered larvae were completely ice-bound yet, upon thawing, were alive and active. The exact degree to which larval C. variipennis can withstand sub-freezing temperatures is not known. Rowley (1967) reported that 62% of winter-collected C. variipennis were able to survive

being frozen in a tray of ice and mud for 6wks. Likewise, Dzhafarov (1964) reported 50% survivorship of Culicoides sp. larvae collected from mud measured at -10°C. I found glycerol to be present in late-winter larvae collected in Saltville. Nevertheless, the Saltville population sustained a 65% decrease in absolute density from 29 Nov. to 2-4 Jan. (Table 4) when weekly mean temperatures dropped precipitously. Such massive mortality did not occur in the next sampling interim. The age-structure of the population did appear to shift towards older larvae. Considering that only 0.25 degree-days accumulated between 2-4 Jan. and 26-31 Jan. (i.e. negligible development), it seems likely that the larger instars were surviving the freeze better than the more fragile early instars. Interestingly, another episode of mortality occurred following an abrupt thaw in mid-February. It is evident that C. variipennis larvae are able to withstand being frozen in mud for a period of weeks, however it would appear that rapid freezing and/or thawing may be detrimental.

Distribution of insects during the spring and summer was focused in the surface cm of mud at or near shoreline. As vernal temperatures warmed, overwintering larvae migrated from below the shoreline to above the shoreline to pupate. The numbers of insects collected 1 April were low (0.29

larvae/ml) undoubtedly due to emigration of emergent adults. The first summer generation appeared on 5 May, but larval densities remained relatively low (0.26 larvae/ml). Reasons for this are speculative but probably result either from poor survivorship of the first summer generation hatchlings or from reduced success of the previous generation in mating, obtaining a bloodmeal and returning to the breeding site to lay eggs. The larval population did not increase until 28 June (8.56 larvae/ml) with the advent of what appears to be the second summer generation. Although larvae became more numerous in the summer they were also smaller. Factors contributing to smaller individuals during the summer probably include elevated temperatures, lowered organic content of ingested mud and the effects of crowding. It is of interest to note that even the first instar larvae during July were smaller than their counterparts collected during cooler months. Although the exact duration is not known, it seems unlikely that the first stadium for this insect exceeds more than a few days during the summer and that the size of first instars is more likely a product of embryonic nutrition rather than one of post-hatch nutrition and temperature. Further studies will be needed to determine if egg size and yolk deposition by parent females and/or yolk utilization by developing embryos change according to season.

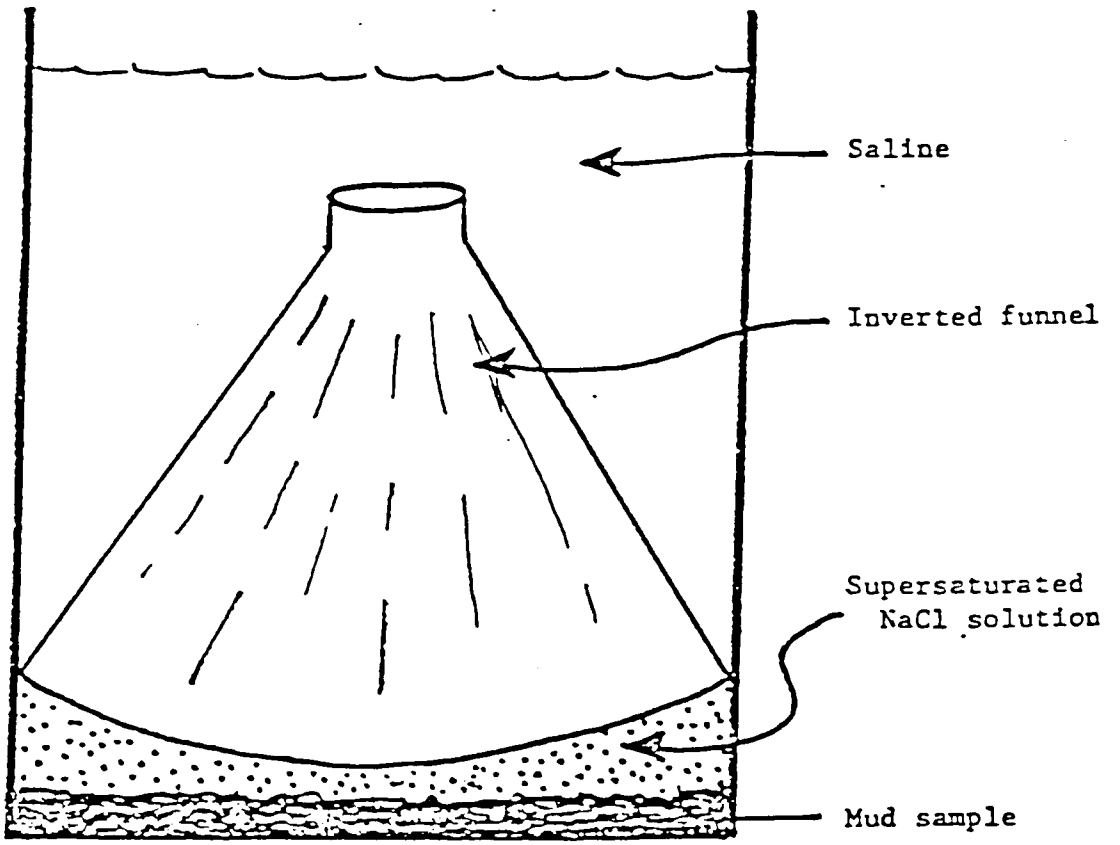

Figure 4. Larval/pupal extraction method (from Boreham 1981). Insects swim/float up through hole in funnel. After ca. 2h, saline solution containing insects is decanted.....

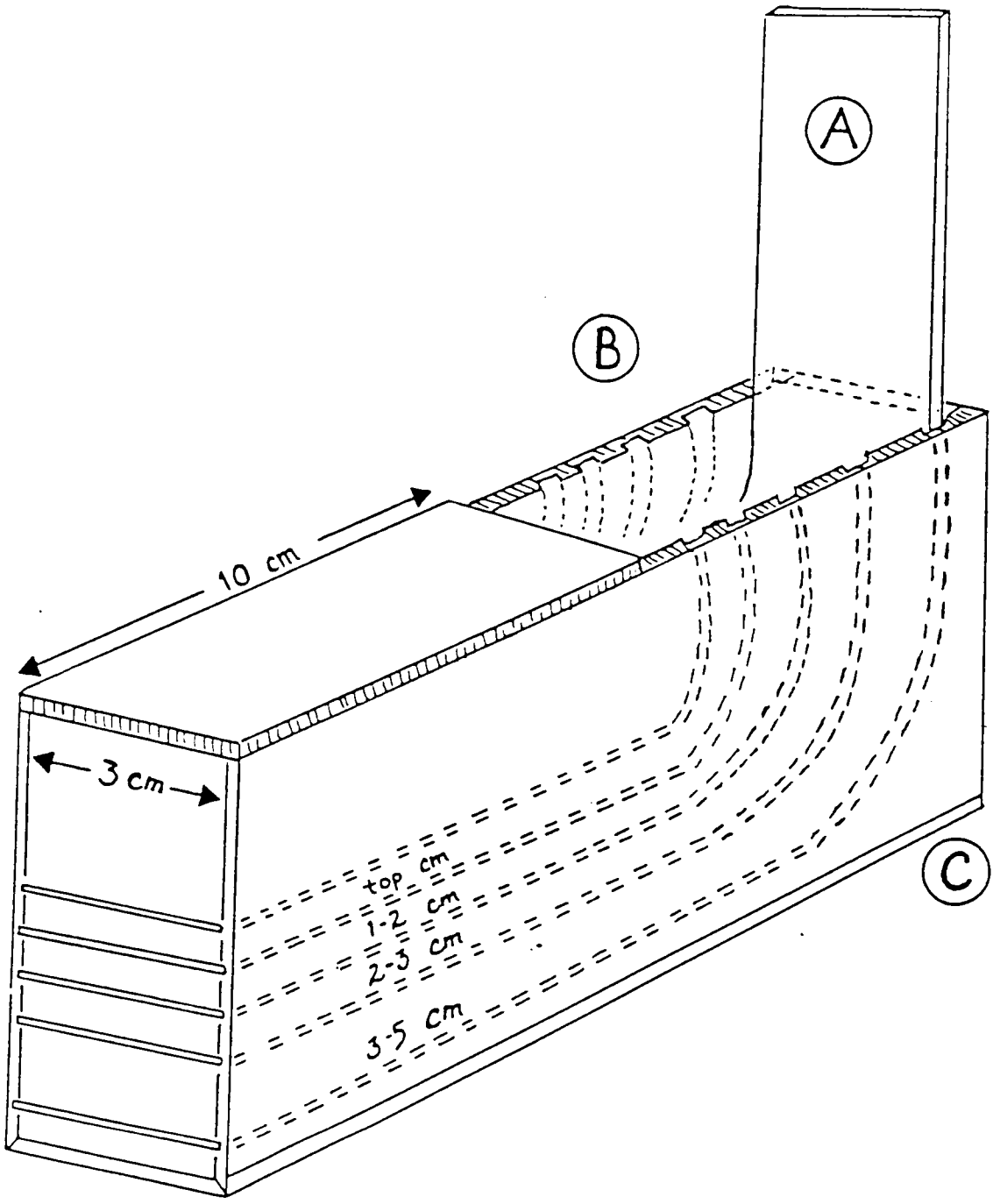

Figure 5. Variable-depth sampler. A. flexible plastic used to partition mud sample, B. slots, C. beveled edge for cutting into mud

Table 4. Total age-grade in percentages of Culicoides variipennis in monthly spatial distribution study. Saltville, VA 1983-1984.

	PERCENT RECOVERED OF EACH LARVAL INSTAR					
Date	I	II	III	IV	P	TOTAL # PER LITER
28 Sept	0.48	3.04	3.67	44.32	48.52	693
31 Oct	4.81	27.09	56.57	11.53	0.03	4370
29 Nov	10.30	14.82	45.17	29.71	0.00	3229
2-4 Jan	0.22	32.33	57.29	10.15	0.00	1127
26-31 Jan	0.08	18.88	51.85	29.27	0.00	1324
4 March	0.11	2.01	16.40	81.48	0.00	472
1 April	0.00	0.18	0.88	84.56	14.39	285
5 May	23.20	32.94	18.91	24.95	0.00	256
10 June	5.26	24.34	21.93	41.67	6.80	228
28 June	8.72	39.33	36.99	15.25	0.75	8556
31 July	3.29	23.77	36.13	35.50	2.88	6086
28 Aug	6.36	16.25	44.66	29.81	2.92	5261

Table 5. Chemical parameters of study site according to time of year. Saltville, VA 1983-1984

	------WATER------		----------SUBSTRATE----------							
				%	---------- ppm ----------					
SEASON	Salinity (ppt)	Diss. O_2 (ppm)	pH	O.M.	P	K	Ca	Mg	Salts	NO_3-N
AUTUMN (4 Oct)	10.8	10.7	7.6	6.0	2	39	1200	86	-	190
WINTER (27 Jan)	10.0	14.4	7.0	5.7	1	20	1200	57	11520	165
SPRING (18 April)	21.4	19.4	6.2	2.5	3	23	1200	83	38400	190
SUMMER (2 July)	17.8	-	6.5	1.2	3	28	1200	120	30720	95

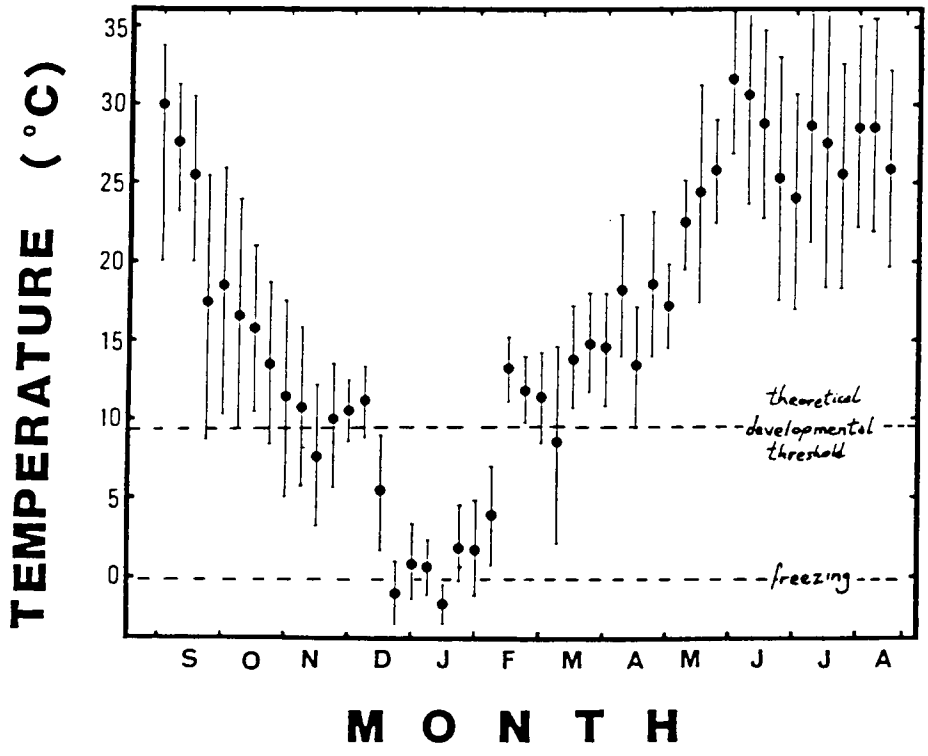

Figure 6. Average weekly mud temperatures at Saltville, VA 1983-1984. Vertical bars represent the average high and low temperatures for that week.

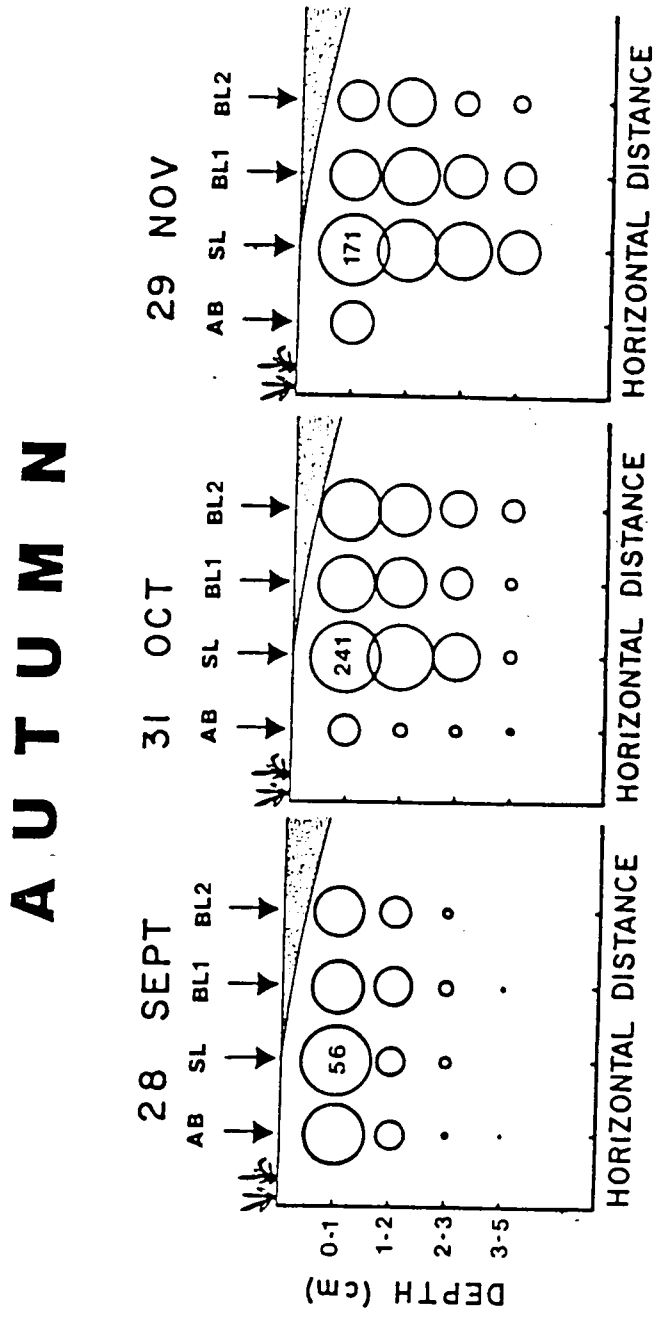

Figure 7. Spatial distribution of immature Culicoides variipennis. Area of circles indicates relative abundance with largest mean (n=5) for each sampling date given. AB = above shoreline, SL = shoreline BL = below shoreline. Saltville, VA Autumn, 1983

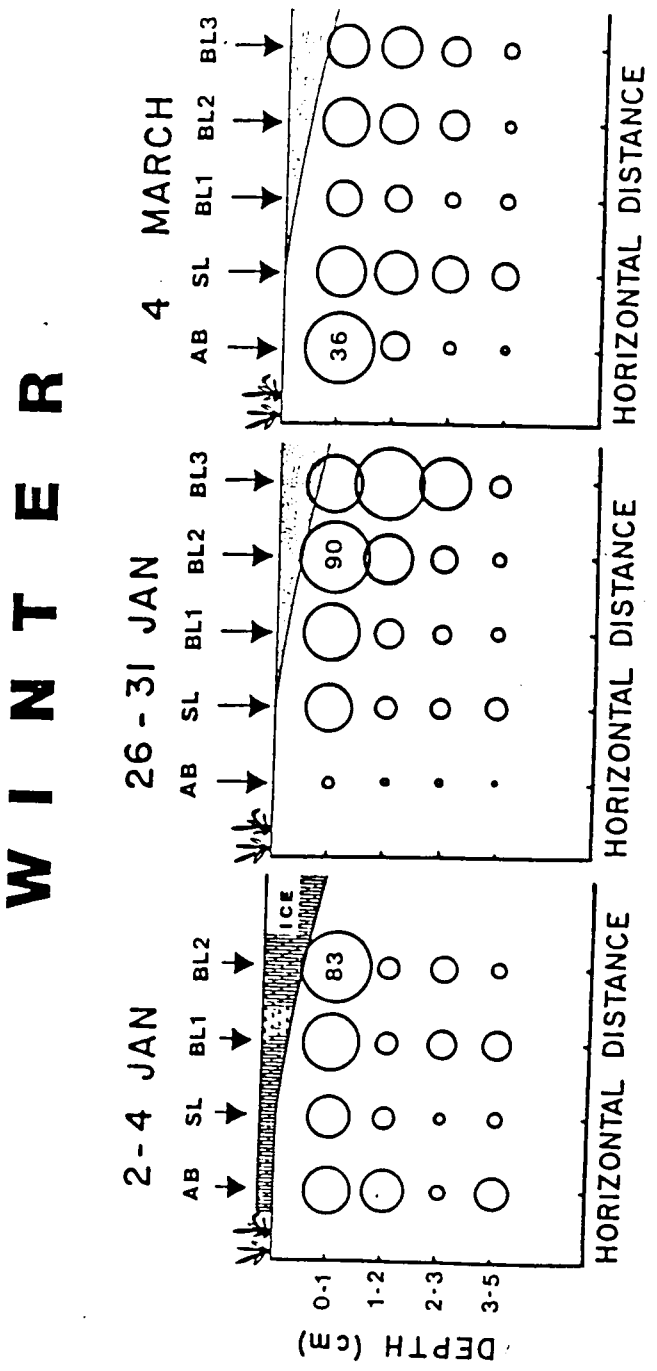

Figure 8. Spatial distribution of immature Culicoides variipennis. Area of circles indicates relative abundance with largest mean (n=5) for each sampling date given. AB = above shoreline, SL = shoreline BL = below shoreline. Saltville, VA Winter, 1984

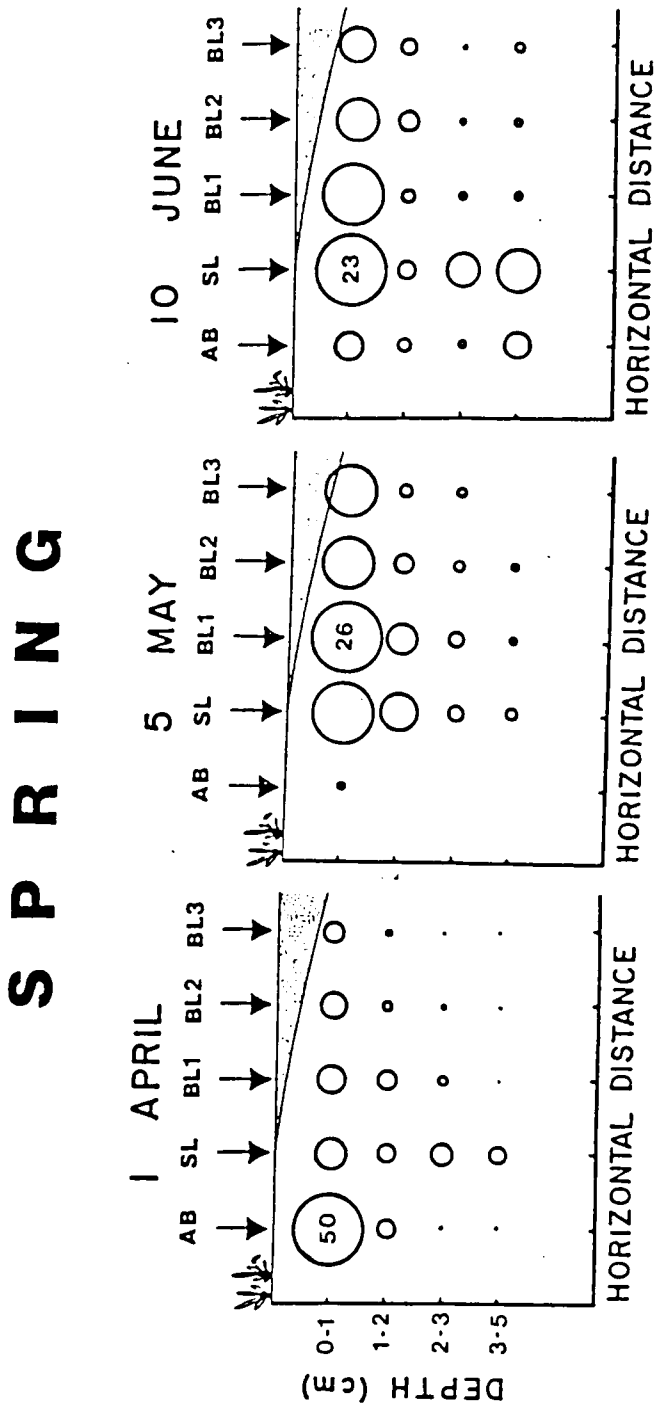

Figure 9. Spatial distribution of immature Culicoides variipennis. Area of circles indicates relative abundance with largest mean (n=5) for each sampling date given. AB = above shoreline, SL = shoreline BL = below shoreline. Saltville, VA Spring, 1984

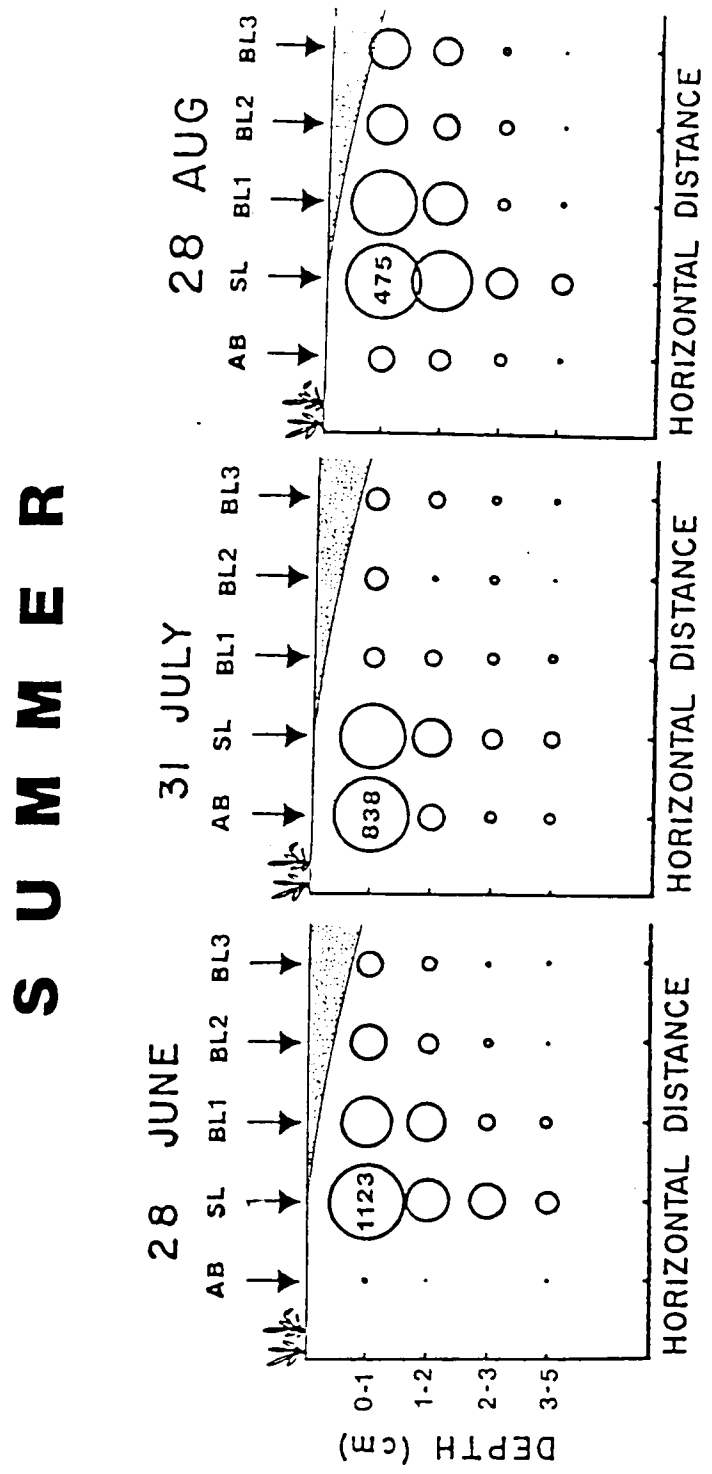

Figure 10. Spatial distribution of immature Culicoides variipennis. Area of circles indicates relative abundance with largest mean (n=5) for each sampling date given. AB = above shoreline, SL = shoreline, BL = below shoreline. Saltville, VA Summer 1984.

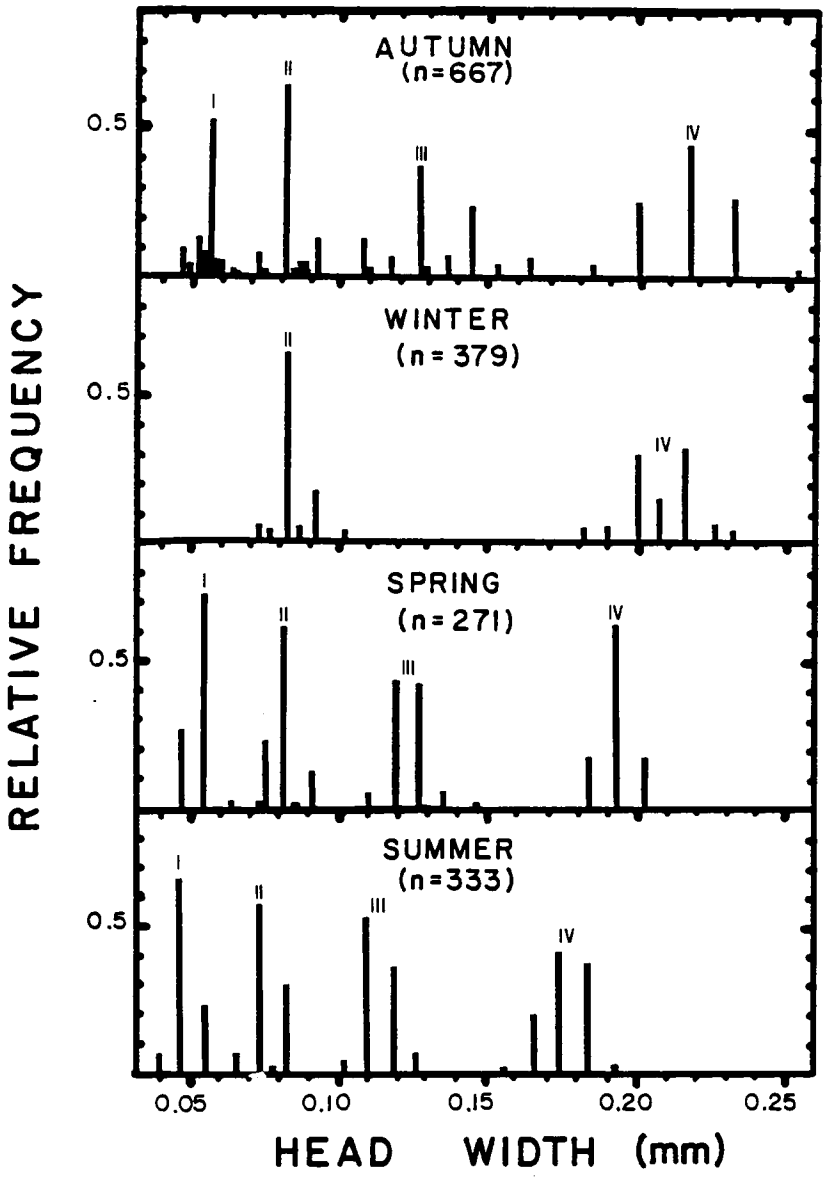

Figure 11. Relative frequency distributions of Culicoides variipennis head capsule widths. Saltville, VA 1983-1984

CHAPTER 4

Diurnal distribution of immature Culicoides variipennis ssp. australis (Coq.) at Saltville, VA

4.1 INTRODUCTION

Diurnal rhythms in insect activity are well-documented phenomena (Harker 1961). Many Culicoides spp. are generally regarded as crepuscular with respect to adult diurnal activity patterns (Blanton and Wirth 1979), preferring to rest during the day in secluded, shady places. Quantitative information regarding the diurnal activity patterns of immature Culicoides is scanty. Barnard and Jones (1980) reported that during the summer months in NE Colorado, most C. variipennis larvae were found to inhabit the surface cm of mud at or just below shoreline. Shifts in the relative abundance of larvae during the 24h period appeared horizontal with respect to the shoreline and little vertical movement of larvae into the substrate occurred. They reported larval mobility to be greatest during the daylight hours. Pupae were only found at or slightly above the shoreline. In contrast, little horizontal movement of larvae within the substrate was noted to occur with C. melleus, a species inhabiting estuarine sand (Linley and Adams 1972). Larval movement during the day was vertical.

Late instar C. melleus larvae tended to migrate downward into the sand as the day progressed from morning (1030) to afternoon (1530) and vice versa as the afternoon (1530) approached dusk (1900). This vertical movement within the sand was attributed to progressive changes in light and temperature. My primary goal was to investigate diurnal fluctuation in the spatial microdistribution of immature C. variipennis at Saltville, VA. Two studies were conducted; Study 1 was designed to discern whether the diurnal shifts were horizontal or vertical in nature, and Study 2 analyzed potential factors contributing to observed shifts.

4.2 MATERIALS AND METHODS

Results from both studies pertain to the same brine pond. Although the sampling design for each study varied, both studies employed the extraction technique described by Boreham (1981) and a variable-depth sampler; which was constructed of Plexiglass and designed such that the mud could be partitioned into one-cm thick increments to a total depth of 5cm. In both studies, sampling was conducted at 4h intervals 6 times during a 24h period. Samples were returned to an on-site field laboratory for insect extraction and enumeration.

4.2.1 Study 1.

This study was conducted 26-27 July 1983 under clear skies. The moon (3 days past full) was bright but, as typical in Appalachian valleys, fog formed in the pre-dawn hours and did not lift until several hours after sunrise. Changes in horizontal distribution were monitored by sampling three predetermined distances (ca. 10cm apart) along transects perpendicular to the shoreline. Distances were designated as above shoreline (AB), shoreline (SL), and below shoreline (BL). Each of these horizontal distances was further partitioned into four substrate depths (0-1cm, 1-2cm, 2-3cm, 3-5cm) with the variable-depth sampler. Samples were replicated four times making a total of 288 samples. Upon extraction, the water containing extracted insects (ca. 450ml) was decanted into labelled plastic cups for enumeration. Estimates of larval density were determined by swirling and vibrating the cup, then quickly withdrawing three separate 10ml aliquots with a pipette. The average number of age-graded larvae from the three aliquots were multiplied by a correction factor of 45 in order to arrive at an estimate of larvae in each 450ml container. Pupae were counted directly. Temperature probes placed in the surface mud at each horizontal distance monitored temperature fluctuations throughout the 24h period.

4.2.2 Study 2.

A different 24h sampling regime was conducted the following year, 17-18 August 1984. Only one vertical depth was examined, the top 2cm, however 6 horizontal distances ca. 20cm apart were sampled; two above shoreline (AB2 and AB1), shoreline (SL), and three below shoreline (BL1, BL2, BL3). As before, samples were taken at 4h intervals during the 24h period and each sample was replicated four times, making a total of 144 samples. In addition to larval sampling, I recorded data concerning physical and biological factors which may have affected diurnal shifts in larval microdistribution. These factors include temperature, light intensity, microhabitat food availability and larval feeding. Temperature of the surface mud was measured with a hand-held thermometer at each location (X4) for each time interval. Ambient light intensity at the site was measured for each time interval with a Li-Cor Photometer (Model LI-185). Before extracting mud samples a small amount of fluid from each sample was withdrawn with a bulb aspirator and aliquots from like replicates were pooled. This was done to obtain an estimate of microfloral abundance (i.e. food availability) in the microhabitat. While the mud samples were extracting, a few drops from each garnered aliquot was placed on a glass microscope slide, covered with

a cover slip and examined. Relative abundance was recorded as the number of microorganisms observed per field for 15 sec. Four fields were observed per slide and microorganisms were categorized as protozoans (including phytoflagellates), diatoms, filamenteous algae, rotifers and others (e.g. spirochetes, bacteria and blue-green algae). Upon completion of larval extraction, insects were harvested and taken through a coarse straining, retaining 3th and 4th instars. The flow-through was discarded since I was only interested in examining later instars. To test for diel periodicity in feeding, larvae were immediately narcotized in carbonated water to avoid regurgitation and defecation by the larvae as is often the case with a violent death in ethanol. Larvae were later transferred to 70% ethanol. Estimates of total numbers were determined by pouring preserved larvae into large (14cm diam.) petri plates which had been partitioned into 16 radiating sectors of equal size with small dowels adhered to the bottom. The insects in three randomly-chosen sectors were enumerated and age-graded. The average was multiplied by 16 to obtain an estimate of the number of larvae in the sample. Estimates were transformed (log n+1) and analysis of variance performed to test for the effect of time, location and replication on larval density. Means were separated using Duncan's Multiple Range Test (α 0.05).

After enumeration, a subsample of larvae (12-125) from each location and time was cleared in 10% KOH and presence or absence of a food bolus in individual larvae recorded. The maximum time elapsed between sampling and larval narcotization was 4h. Since it is not known how long it takes for food to pass through the alimentary tract of Culicoides larvae, a rough estimate of larval gut clearing time was obtained in the laboratory. Field-collected late instar larvae were kept in natural substrate in a refrigerator for ca. 1wk in order to reduce microfloral abundance without harming the insects. Upon warming, a "pulse" of diatom/algae culture was added to the substrate. Larvae were allowed to feed for 24h at 27°C, then extracted (Boreham 1981). Larvae were kept under starvation in tap water (25°C) and at ca. half-hour intervals were removed, narcotized, cleared in KOH and examined for food.

4.3 RESULTS

4.3.1 Study 1.

The mean numbers of age-graded larvae and pupae for each time interval and location have been graphed as proportionalities of one another (i.e. area of dots) using the largest mean observation for each age category as the

common denominator. The numerical value for the largest mean observation in each age category (i.e. largest dot) is given at the right-hand side of each figure. Most insects collected were third instar larvae. As indicated by previous workers, pupae tended to localize in discrete areas above the shoreline in the top cm of mud (Fig. 12). There was little, if any, movement from this region during the 24h period, even though temperatures in this zone ranged from 13.3°C to 34.4°C. Likewise, first instar larvae were localized above shoreline in the top cm of mud and little migration from this region occurred during the 24h period (Fig. 13). Most likely this can be attributed to the minute size of first instar larvae (hence limited mobility) and the apparent placement of eggs by parent females within this zone. There did appear to be a slight tendency for second instar larvae to move towards the shoreline during the morning daylight hours (0730 - 1130). As expected, the larger third and fourth instars accounted for the majority of diel larval migration within the habitat (Fig. 14). Most movement was horizontal, being confined to the top cm of mud. From the pre-dawn hours (0330) and through the daylight hours (0730 - 1530), late instar larval density progressively shifted from above shoreline to shoreline. As the hottest part of the day approached (1530), fourth instar

larvae moved further from the shore and, because of the low numbers recovered at this time, may have actually ventured beyond the sampling area. Near sunset (1930), late instar larvae redistributed themselves and the greatest incidence of vertical movement into the substrate was noted.

4.3.2 Study 2.

The mean numbers of late instar larvae for each time and location are presented in Table 6, with the overall trends (as proportions) depicted in Figure 15. Throughout the 24h period a large percentage of larvae were concentrated at shoreline. Apparently, there exist some factors in this region which are preferred by C. variipennis larvae. Nevertheless subtle shifts in larval distribution did occur. Larvae appeared to move offshore during the morning and afternoon daylight hours (1130-1530), moving towards shore and above in the late afternoon (1930) and becoming highly concentrated at shoreline during darkness (2330-0330). After sunrise (0730), larvae again began to migrate below shoreline. Thus, findings in 1984 (Study 2) agree with the preliminary study of 1983 (Study 1); that is, a portion of the late instar larval population move out below shoreline during the day but come up to the shore at night. The diel fluctuations in substrate temperature for each horizontal distance and time are given in Figure 16.

For most locations the diel fluctuations in substrate temperatures were similar (ca. 14°C), however diel temperature fluctuation at the shoreline was more moderate (ca. 11°C). Undoubtedly this was due to evaporative cooling during the day and insulative warming by the heated water at night. Interestingly, substrate temperatures were consistently warmer by several degrees in locations under water (i.e. BL1, BL2, BL3) than above shoreline and shoreline locations. This was true even during the daylight hours. Light intensity and ambient temperature are given in Table 7. As expected, air temperature was warmest in the afternoon (1530). Light intensity dropped somewhat during this time because of increasing cloudiness. Clouds continued to build throughout the 24h period and during the last sampling interval (0730) a light drizzle commenced.

Dominant microfloral species within the microhabitat included Chlamydomonas sp. (Protozoa: Phytomastigophora) and several unidentified pennate diatom species. Findings from the microfloral abundance data were inconclusive. There appeared no discernable trends and, at the level of resolution at which this parameter was examined, temporal and spatial differences in food availability (i.e. microfloral abundance) within the microhabitat were indistinguishable, with the exception that Chlamydomonas

abundance was much reduced above the shoreline throughout the 24h period. Diatoms were present throughout. At shoreline where larvae were consistently more abundant, gut analysis data (Fig. 17) revealed higher percentages of gut-filling occurred during the night (2330, 0330) and early morning (0730). The same trend was evident 20cm above shoreline (AB1). In contrast, during the mid-morning (1130) and afternoon (1530) gut-filling percentages for below shoreline sites (BL1, BL2, BL3) surpassed those at shoreline which were declining concurrently. With exception of the AB2 site where comparatively few larvae existed, it appeared that most feeding occurred at night (2330).

Results from the larval gut clearance trials (Table 8) showed that a minimum of 4-5h elapsed before substantial voiding of gut contents occurred in late instar C. variipennis. Over half of the insects still had food in their guts at the end of 7h. Insects in these laboratory trials were subjected to the same procedures of extraction and processing as those in Study 2. Since the maximum time elapsed between larval capture and narcotization was 4h, I feel that gut analyses for Study 2 are valid. However, extreme caution must be exercised in equating larval gut clearance times under these conditions with actual gut clearance times in the field. For aquatic amphipods,

turnover time of gut contents in the laboratory tends to be longer than gut turnover time in the field (Marchant and Hynes 1981). The gut clearance time reported here for C. variipennis larvae could have been strongly influenced by the supersaturated salt solution and agitation used to extract the larvae from the mud.

4.4 DISCUSSION

In Study 1 it was determined that pupal and early instar larval C. variipennis inhabit discrete zones within the breeding site and do not (or can not) actively leave this zone, despite wide temperature fluctuations occurring throughout the day. Older and larger larvae, however, did display patterns of diel migration. This diel migration was restricted largely to the surface cm of mud and therefore should be considered horizontal migration. Undoubtedly the food and oxygen requirements of the insect are more favorable in the loosely-packed light brown surface mud than in the thick black anaerobic mud beneath. As ambient temperature and light increased during the day, a certain portion of the late instar larval population moved towards the water. Conversely, as ambient temperature and light decreased, larvae migrated up to the shoreline and above.

This general trend was found to be evident at Saltville both in 1983 where sampling distances were 10cm apart and in 1984 with sampling distances 20cm apart, as well as in larval sampling conducted in NE Colorado by Barnard and Jones 1980. Thus corroborative evidence indicates that diel shifts in larval microdistribution are a real phenomenon for this insect. The difficulty arises in attributing causal factors to microdistribution patterns.

Surely one reason 4th instar larvae migrate above shoreline is to pupate. Metamorphosing Culicoides larvae must leave the water in order to inflate pupal thoracic air sacs for bouyancy and pupate successfully (Weerekoon 1953, Linley 1966). Weerekoon (1953) reported that late instar Palpomyia sp. (a lake-inhabiting ceratopogonid) which are ready to pupate leave the benthos at night in order to swim along the water surface to the lake's edge and pupate. Likewise, my data suggest that the search for pupational sites above the shoreline by 4th instar C. variipennis occurs primarily at night. However, prepupational behavior by 4th instar larvae does not, by itself, provide a complete explanation for diurnal shifts in larval distribution. Similar shifts were noted to occur with 3rd instar larvae and 4th instars not near metamorphosis (i.e. feeding). Although it has been shown that Culicoides larvae will

select a preferred temperature range (18-25°C) along a temperature gradient in the laboratory (Linley and Adams 1972), observed shifts in larval diel distribution cannot be adequately explained by temperature either. In Study 2, substrate temperatures, although fluctuating during the 24h period, were consistently warmer farther out from shore, becoming quite hot in the mid-afternoon (32-35°C). Yet it was during this time that a substantial portion of the late instar larval population (ca. 50%) was located below shoreline (Fig. 15). At night, the majority of larvae (ca. 70%) were densely aggregated at the cooler shoreline (17-18°C), avoiding the warmer mud (19-22°C) offshore. Certainly much of the larval population remained at shoreline throughout the 24h period where temperature fluctuations were most moderate, however apparently there exist overriding factors causing some late instar larvae to reject what would appear to be preferred temperature ranges.

Although the exact dietary requirements are not known, larval C. variipennis are considered to be "collector-gatherers", ingesting mud and obtaining nutrients from the substrate and microflora contained within. The degree (if any) to which larvae selectively feed on microflora by sieving mud through their epipharyngeal combs is not known, but observations indicate that a substantial amount of mud

is to be found within the alimentary canal of these insects. Microscopic examination of surface mud revealed an abundance of phytoflagellates and diatoms but could not detect temporal or spatial differences in microflora abundance. There were some noticeable trends, however, in larval gut analysis (Fig. 17). As larvae clustered about the shoreline during the night, a great deal of feeding occurred, as evidenced by the large percentage of the population having filled guts at that time. At midnight, larvae were observed to be actively wriggling about upon the surface of the mud, perhaps seeking pupational sites or foraging for food. During mid-afternoon, most larvae at shoreline were barely visible, being buried in the mud with only the head and thorax protruding. It was also at this time (1530), that shoreline feeding was at its lowest level, despite large numbers of larvae present. Thus it would appear that most of the larvae electing to remain at shoreline throughout the 24h period probably do most of their feeding at night.

It remains unclear why a substantial portion of the late instar larval population moved offshore during daylight hours. It seems unlikely that migrating larvae were attracted by temperature, which became increasingly warmer (up to 35^{o}C) offshore during the day. A more plausible possibility may be the combined effects of crowding and

competition for food resources. Shoreline density at night exceeded 20 larvae/ml, not including younger instars since they were discarded in processing. Perhaps such densities cannot be tolerated throughout the 24h period, resulting in a portion of the larger, more mobile instars dispersing during the day and only returning to shoreline at night to feed. If such is the case, then partitioning of space and food resources would appear to follow diurnal periodicities.

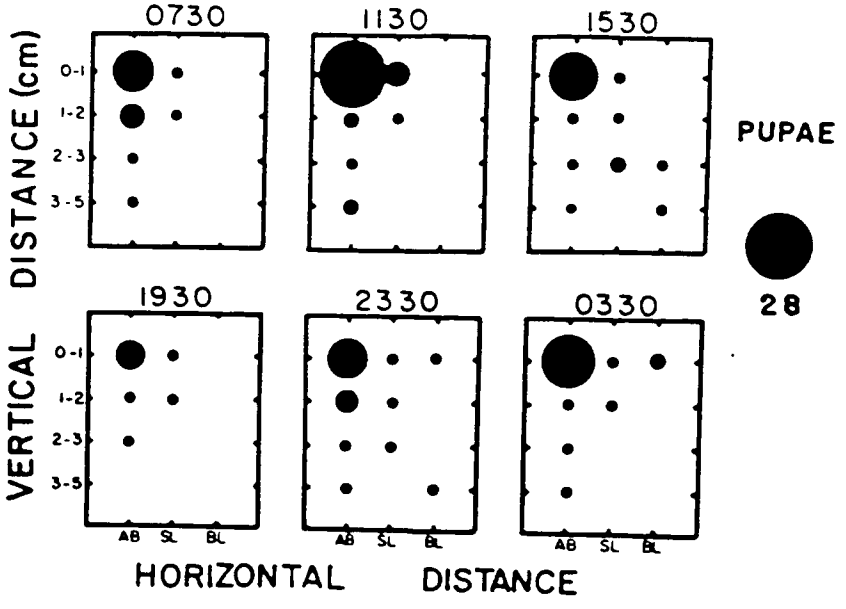

Figure 12. Diel distribution of pupal Culicoides variipennis. AB = above shoreline, SL = shoreline, BL = below shoreline (n = 4) Study 1. Saltville, VA 26-27 July 1983.

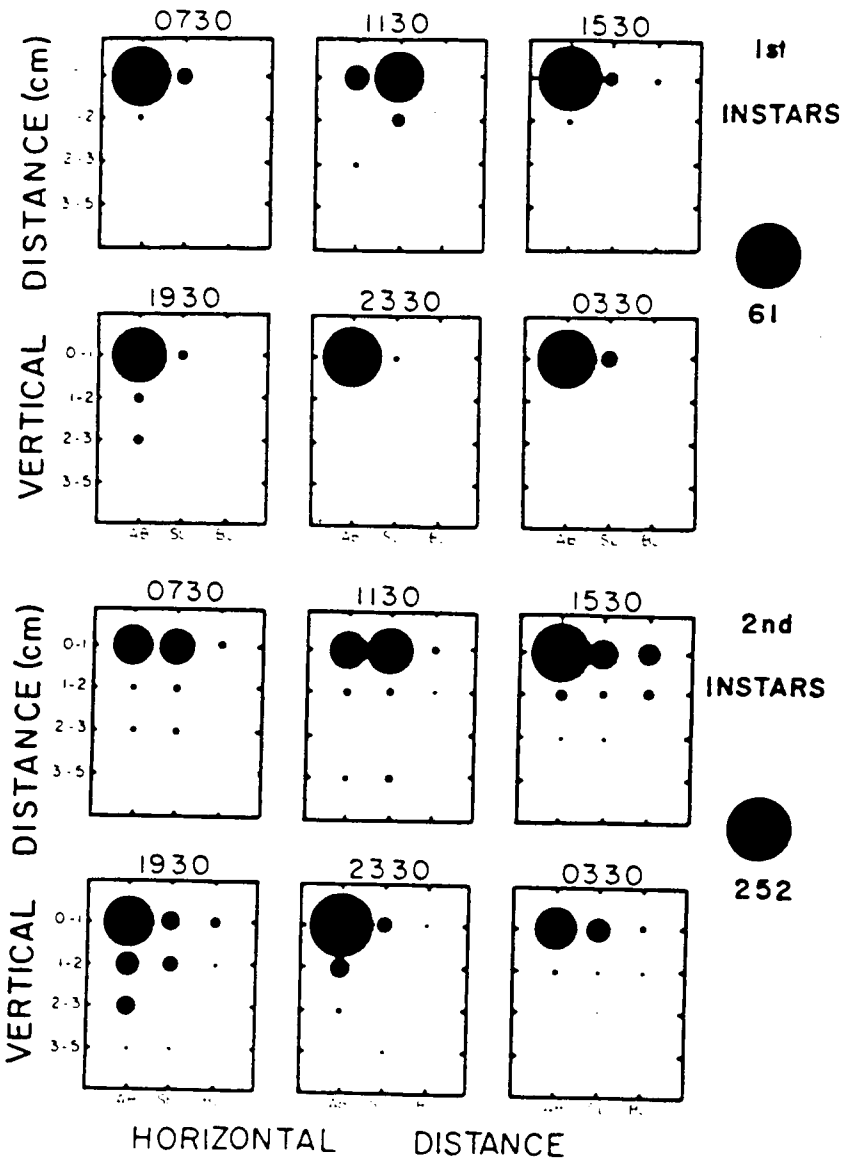

Figure 13. Diel distribution of early instar Culicoides variipennis. AB = above shoreline, SL = shore line, BL = below shoreline. (n = 4). Study 1 Saltville, VA. 26-27 July 1983.

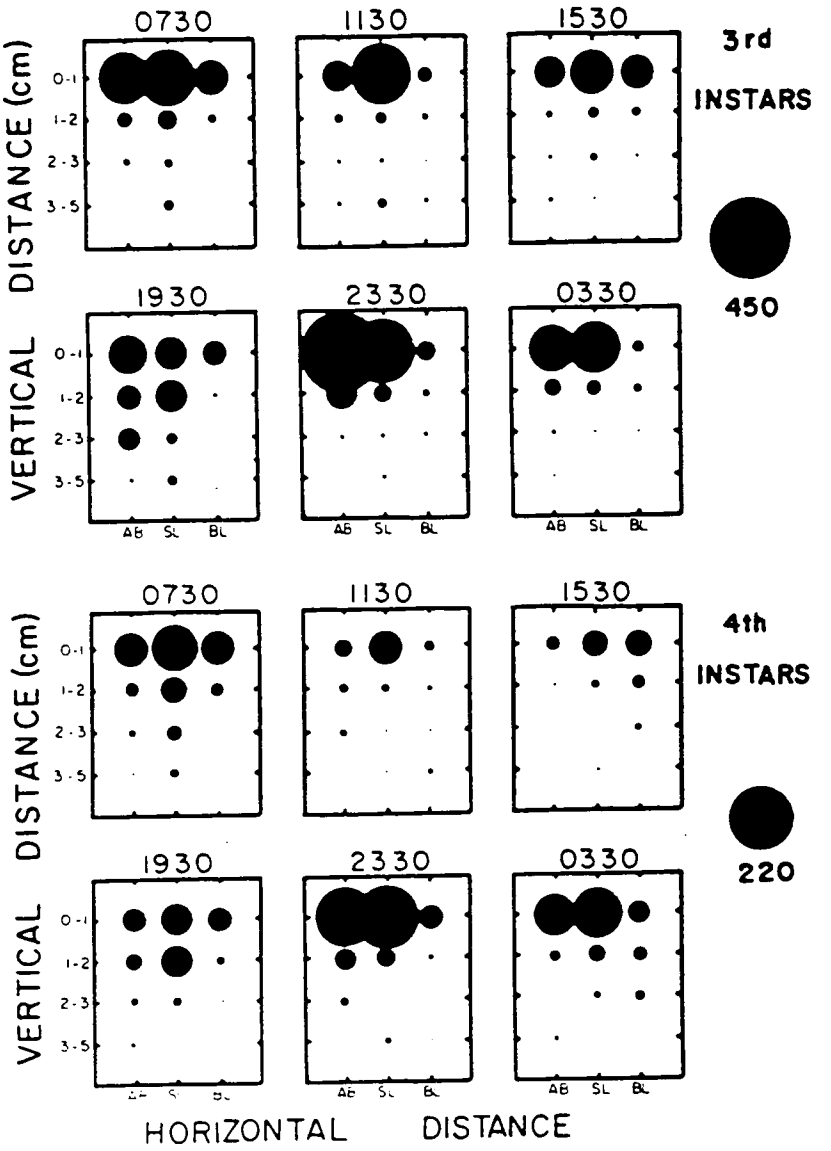

Figure 14. Diel distribution of late instar Culicoides variipennis. AB = above shorlline, SL = shoreline, BL = below shoreline. (n=4) Study 1, Saltville, VA 26-27 July 1983.

Table 6. Diel distribution of late instar Culicoides variipennis. Each mean value (n=4) is followed by 2 letters; the first letter compares the means within a row, the second letter compares the means within a column. Means followed by the same letter are not significantly different at the 0.05 level. AB = above shoreline, SL = shoreline, BL = below shoreline. Study 2. Saltville, VA 17-18 August 1984.

TIME OF	HORIZONTAL DISTANCE					
DAY	AB-2	AB-1	SL	BL-1	BL-2	BL-3
1130	9 c/ab	7 c/a	888 a/ab	800 a/a	115 b/bc	209 ab/a
1530	2 b/b	11 b/a	1390 a/a	769 a/a	273 a/a	282 a/a
1930	10 c/ab	98 bc/a	1248 a/ab	403 ab/a	180 ab/ab	98 bc/ab
2330	8 c/ab	137 b/a	1256 a/ab	149 b/b	56 bc/c	55 bc/b
0330	5 c/ab	75 b/a	1684 a/a	155 b/b	219 b/ab	195 b/a
0730	15 c/a	139 b/a	612 a/b	609 a/a	124 b/abc	128 b/ab

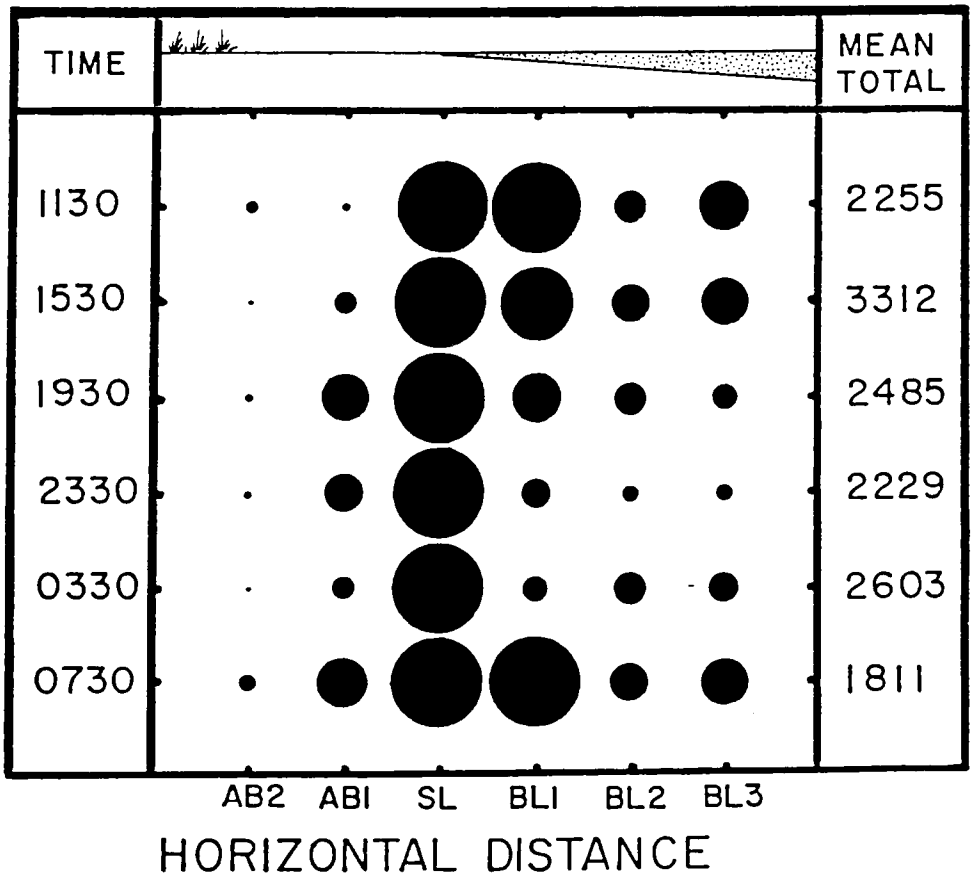

Figure 15. Diel distribution of late instar Culicoides variipennis. AB = above shoreline, SL = shoreline, BL = below shoreline (n=4). Study 2. Saltville, VA 17-18 August 1984.....

Table 7. Ambient temperature (^{o}C) and light instensity (lux) throughout 24h period. Study 2, Saltville, VA 17-18 August 1984.

	TIME OF DAY					
	1130	1530	1930	2330	0330	0730
Ambient Temp (^{o}C)	29.4	33.9	24.4	16.7	16.7	15.0
Ambient Light (lux)	80,000	55,000	5100	4	4	5000

Table 8 Gut Clearance Times

time (hrs) post-starvation	N	%empty guts	%filled guts	Gut Region: foregut only	foregut + midgut	midgut only	midgut + hindgut	hindgut only	entire gut
0.00	85	1.2	98.8	0.0	6.1	25.7	27.2	0.0	40.9
0.25	28	17.8	82.1	0.0	3.6	35.7	35.7	0.0	7.2
0.30	16	0.0	100.0	0.0	0.0	31.2	68.7	0.0	6.2
0.75	29	10.3	89.6	0.0	0.0	44.8	41.4	0.0	3.4
1.0	49	2.0	98.0	0.0	0.0	44.9	53.1	0.0	0.0
1.5	76	7.9	92.1	0.0	0.0	50.0	38.2	2.6	1.3
2.0	20	15.0	85.0	0.0	0.0	65.0	20.0	0.0	0.0
2.5	36	8.3	91.7	0.0	0.0	55.6	27.8	8.3	0.0
3.0	64	4.7	95.3	0.0	0.0	50.0	45.3	0.0	0.0
3.5	21	14.3	85.7	0.0	0.0	52.4	33.3	0.0	0.0
4.0	13	30.8	69.3	0.0	0.0	46.1	23.1	0.0	0.0
4.5	34	8.8	91.2	0.0	0.0	50.0	35.3	5.9	0.0
5.0	37	37.8	62.2	0.0	0.0	32.4	13.5	16.2	0.0
6.0	18	44.4	55.5	0.0	0.0	27.8	27.8	0.0	0.0
7.0	65	40.6	59.4	0.0	0.0	45.3	12.5	1.5	0.0

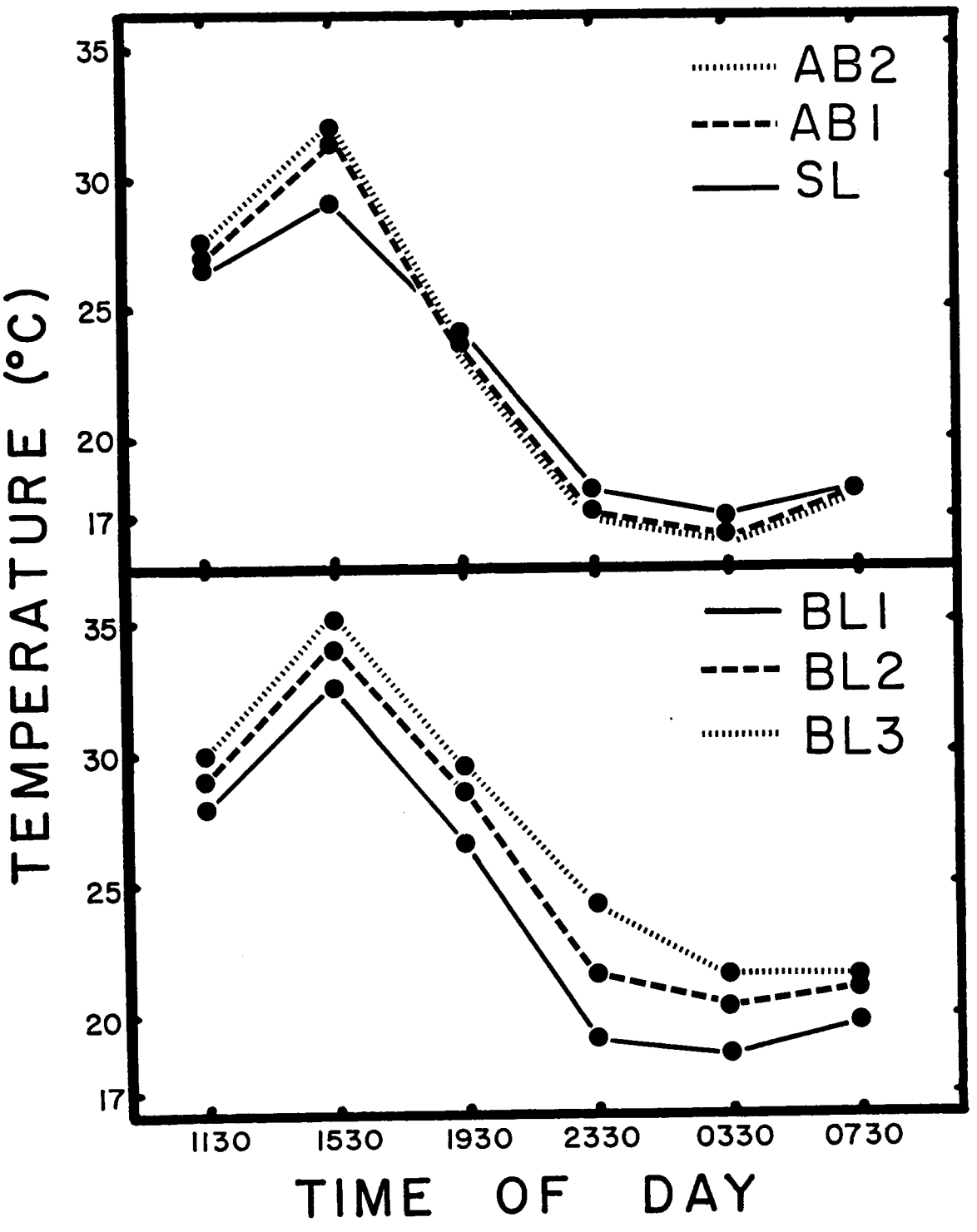

Figure 16. Surface mud temperatures throughout the 24h period. AB = above shoreline, SL = shoreline, BL = below shoreline (n=4). Saltville, VA 17-18 August 1984.

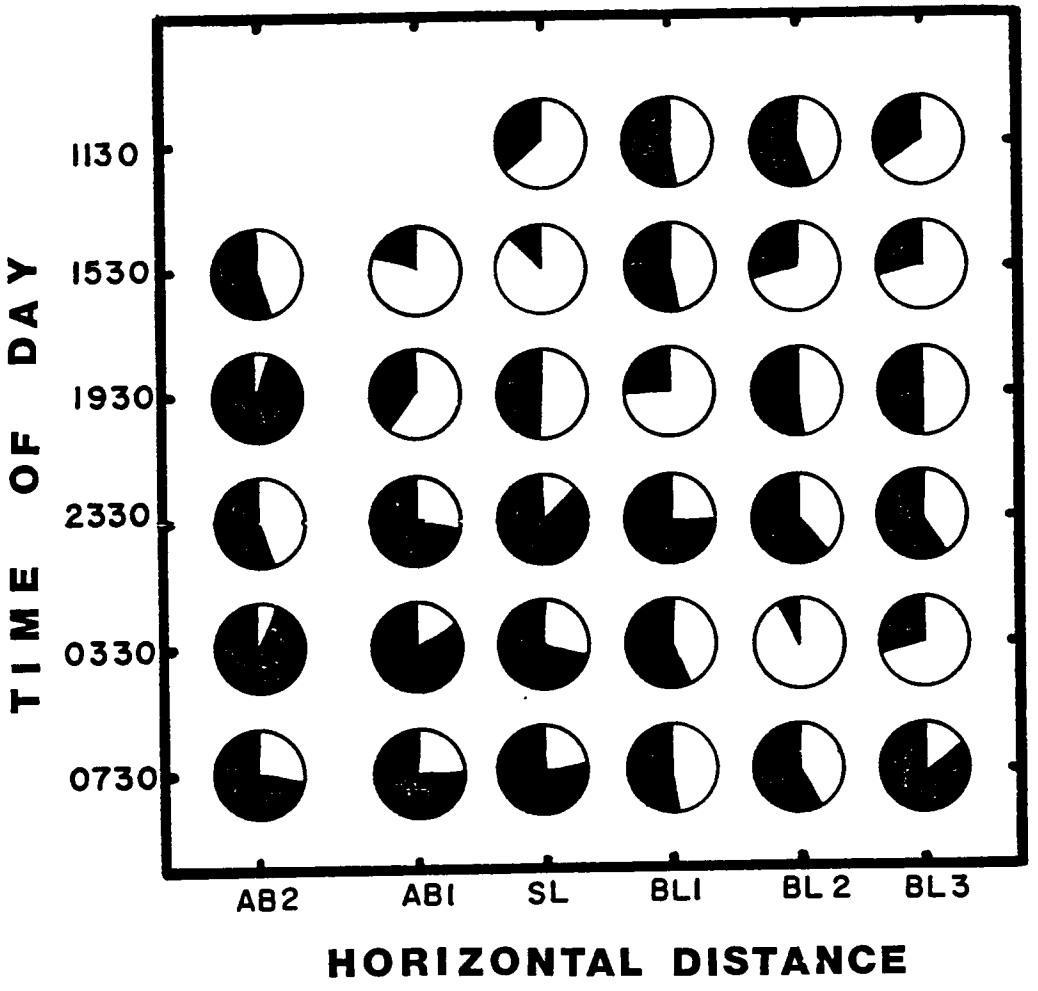

Figure 17. Percentages of late instar Culicoides variipennis with food bolus present (darkened area) in gut according to time and location throughout 24h period. AB = above shoreline, SL = shoreline, BL = below shoreline. Study 2. Saltville, VA 17-18 August 1984

CHAPTER 5

Life History and Survival of Immature *Culicoides variipennis* ssp. *australis* at Saltville, VA

5.1 INTRODUCTION

For a thorough understanding of the epidemiology of insect-transmitted diseases, information regarding the life cycle and survival of the vector is essential. The biting midge, *Culicoides variipennis* (Coq.) is the only known North American vector of bluetongue virus in cattle and sheep (Luedke *et al*. 1967). Adult gonotrophic age and survivorship has been studied in several species of *Culicoides*, including *C. variipennis* (Mullens and Rutz 1984, Zimmerman and Turner 1984). Although survivorship studies have been conducted on the immature stages of several species of mosquitos (Enfield and Pritchard 1977, Madder *et al*. 1983, Mogi *et al*. 1984), little work has been done on the stage-survivorship of immature *Culicoides*. The major obstacle to studies of this kind has been the difficulty in extracting all immature lifestages from the larval substrate with equal success. Methods in the past have been either too time-consuming to be feasible or too harsh, killing earlier instars before extraction could be completed. The

salt-floatation method of Boreham (1981) has proven to be efficient in extracting all larval instars of the salt-tolerant Saltville population of C. variipennis from the mud substrate in which they live. Using this method, I followed the age structure through time of an immature population of C. variipennis inhabiting a brine pond in Saltville, VA. The specific objectives of this study were:

1. To determine from larval population dynamics the voltinism patterns and relate these to thermal accumulations in degree-day units.

2. To determine from field data the developmental times and from these construct time-specific life tables for the immature stages of this species.

5.2 MATERIALS AND METHODS

5.2.1 Sampling

The breeding site used for this study has been described in Chapter 3. Samples of surface mud were taken on ca. weekly intervals during April-Sept 1983 and April-June 1984. Samples during the winter were taken at ca. monthly intervals. Continuous temperature readings were taken in the surface cm of mud just below the waterline from

August 1983 - August 1984. Because larval/pupal distribution within the habitat may differ according to lifestage, each replicated sample (X10) consisted of pooled amounts of mud taken from slightly above shoreline, shoreline and slightly below shoreline. Sample volume was determined by placing mud samples in graduated containers. Insects were extracted from the mud (Boreham 1981) and taken through a coarse straining to retain pupae and late instars. Early instars were recovered by filtering the flow-through onto fine nylon mesh (41μ) using a Buchner funnel and vacuum pump. Recovery of eggs was negligible, perhaps because sucker-like structures on the chorion of the eggs caused them to adhere to the substrate. Such structures have been reported in several species of Culicoides (Hill 1947). Extracted insects were rinsed with water into 90% EtOH. Estimates of total numbers were determined by pouring preserved larvae into large (14cm diam.) petri plates which were partitioned in 16 radiating sectors of equal size with small dowels adhered to the bottom. Plates were swirled and vibrated and the insects in three randomly-chosen sectors were enumerated and age-graded according to head capsule width. The average was multiplied by 16 to obtain an estimate of the number of larvae in the sample. To test the accuracy of this method, 78 such estimates were plotted

against their corresponding actual numbers. The slope of the regression line was 1.15, close to that of a perfect estimate (b=1.0). Thus the method of estimation appeared valid as well as time-efficient. To obtain absolute population estimates (larvae/10ml), each total was corrected for volume.

5.2.2 Calculation of Survivorship

Weekly population densities for each lifestage were converted to percentages and plotted against time (Fig. 18) in order to visualize overall population dynamics occurring thoughout the season. Using this, and by examining the rise and fall of numbers over time for each lifestage from the raw data, relatively discrete cohorts could be assigned for certain parts of the year. Unfortunately, my sampling intervals during the summer were too long (ca. 7 days) and larval development too rapid for meaningful cohorts to be constructed for the months of July-August. However, for those portions of the year where larval development was of sufficient length (i.e. spring and autumn), cohorts were constructed and survivorship rates could be calculated. For each cohort, the total number of individuals passing through each lifestage was determined. Southwood (1978) discusses several methods by which this can be achieved. I chose the graphical summation method for its simplicity and because I

felt its assumptions were valid for my data. Briefly, this method entails plotting successive estimates against days, allowing one square per individual per day. The total number of "individual-days" (i.e. the total number of squares under the curve) was then divided by the mean developmental time ("days") for that lifestage to arrive at an estimate of total "individuals" passing through that particular lifestage. This was done for each lifestage. Estimates of larval/pupal developmental times under field conditions were calculated by plotting the cumulative percentages of each lifestage against time on a log X probit scale (after Enfield and Pritchard 1977). The 50% point (similar to an LD-50) was taken to be the mean development time (t-50). Absolute estimates of each lifestage in each cohort resulting from these procedures were converted to logarithms and stage-specific mortality indices (k) could be calculated by subtraction of successive lifestages (Southwood et al. 1972). A common K, representing the total mortality acting upon a cohort, was then computed by adding up the individual k's for each life stage. In this way, total mortality between different cohorts could be compared. Graphical representations of stage-specific mortality (i.e. survivorship curves) for each cohort were constructed by plotting the log-transformed population estimates against

corresponding lifestages. A life-table (modified after Deevey 1947) for each cohort was calculated. The columns are as follows:

x = estimated developmental time (days) for the age class

lx = number surviving at the begining of the age class (starting with 1000)

dx = number dying during successive age intervals

ex = life expectancy

1000qx = rate per thousand alive at the start of age x

k = index of mortality occurring between successive lifestages

K = index of total mortality acting upon a cohort

5.3 RESULTS AND DISCUSSION

Spring emergence of adult midges from the overwintering larval generation appeared to take place in mid- to late-April for both 1983 and 1984. This is clearly evident in 1984 (see Figure 18). In 1983, I began my sampling too late in the season (21 April) to catch the advent of spring emergence. However, from the relatively large numbers of 1st and 2nd instars present, which are not known to constitute a major age class of the overwintering generation (see Chap. 3), it is my opinion that spring emergence

probably began one or two weeks previously, and was still continuing on 27 April 1983. I recorded a total of 4 generations (an overwintering generation and 3 summer generations) for 1983. The first summer generation probably completed development and emerged as adults in mid-June (ca. 60 days). The second generation emerged at the end of July/beginning of August (ca. 40 days). The third summer generation of 1983 emerged in late Sept./early Oct. (ca. 45 days). In 1984, I sampled the larval population up until August, during which I recorded two summer generations; the first emerged in mid-June (ca. 50 days), the second most likely emerged in early August (50-55 days). Continuous temperature data of the substrate were available for the overwintering generation 1983-1984 and two summer generations 1984. The degree-day accumulations for each of these generations are presented in Table 9.

Total degree-day accumulations for completion of immature development were similar for both summer generations (366 and 376^{o}days respectively). Degree-day accumulations required for the overwintering generation to complete development were much greater (831^{o}days). Most likely this difference can be attributed to larval diapause resulting from shortened daylength occurring during the winter. For many species in temperate climates, photoperiod

is the major factor in the intiation and termination of insect diapause, often overriding the effect of temperature (Tauber and Tauber 1976). Such appears to be the case for larval C. variipennis. Although larvae possess well-defined stemmata (larval eyes), the degree to which photoreception by these insects occurs beneath ice, water and mud is uncertain. Degree-day accumulations required for development of summer larvae/pupae (ca. 370°days) in the field are in fair agreement with the 417°days that I calculated for immature development (larva + pupa) in the laboratory (see Chap. 2) Thus I feel that degree-day values between 350-400 may be useful in predicting the emergence of C. variipennis at Saltville, VA. Caution should be exercised in applying my results from Saltville to prediction models intended for use in other regions. Mullens and Rutz (1983b) reported 502-655°days accmulating between peak emergences of 1st and 2nd summer generation adults for New York populations of C. variipennis. However, in the laboratory they reported a heat accumulation value of 285°days for total immature development. They attributed the discrepancy to possible effects of poor nutrition in the field as compared with that of laboratory trials or that field populations may have migrated to cooler areas within the microhabitat during the day. Therefore, I feel that

more research is needed from different areas of the country (especially in areas where bluetongue is prevalent) before a holistic model can be developed for C. variipennis emergence in the prediction of bluetongue outbreaks.

The cumulative percentages for each cohort lifestage throughout the duration of the cohort are given in Tables 10-12. The estimated mean developmental times (t50) for the first instar population is of longest duration for the first 1983 summer generation (20.5 days Table 10) and of shortest duration with the overwintering generation (4.93 days Table 11). Extended duration of the early instar population in the spring may have resulted from lower temperatures. In addition, the prolonged emergence and oviposition period exhibited by the overwintered parent generation contributed to this phenomenon. Perhaps warmer temperatures and a more synchronized adult emergence and oviposition contributed to somewhat quickened t50's for early instar populations during the autumn. However as daylength and temperatures decreased, t50 increased for the later instars, indicating that the autumn larval population was undergoing diapause. I did not feel justified in computing the t50 value for first instars in the May 1984 generation because there existed reasonable uncertainity as to the starting and ending dates. In general, t50 values calculated for

larval/pupal duration (t50 pupa) agreed with observed voltinism patterns (Table 9).

Survivorship curves for spring 1983, winter 1983-84 and spring 1984 are presented in Figures 19-21. Survivorship between larval instars for the May 1983 cohort seemed very good up until the pupal stage was reached, when it declined rapidly (Fig. 19). Thus, survivorship in the spring generation of 1983 was indicative of a Type I survivorship curve (Slobodkin 1962). In Type I survivorship, mortality factors act more acutely upon the older age-classes (in this case, the pupae). Survivorship for the overwintering generation (Fig. 20) more-closely approached a Type II survivorship curve, where the mortality rate is constant between one age to the next. The survivorship curve for the spring generation 1984 was not as clear-cut (Fig. 21). Population densities decreased from the 2nd to 3rd instar stage, yet increased from the 3rd to 4th instar stage. Of course this is biologically impossible. Obviously the discrepancy is a result of sampling error. Whether 3rd instars were under-sampled or 4th instars over-sampled is open to debate, but the previous year's data tend to favor the former, suggesting that a Type I survivorship curve is probably a more accurate description of life-stage mortality during the first summer generation of C. variipennis immatures at Saltville.

Further analysis of the stage-specific mortality of immature C. variipennis is given as a life-table (Tables 13-15). Indices of mortality between successive lifestages (k) were largest for the 4th instar-pupal interim in all cases. Total mortality (K) was greatest for the overwintering generation (Table 14). A common K could not be calculated for the May 1984 generation because of the sampling error discussed above. Decreasing life expectancy from one stage or age interval to the next is indicative of a Type I survivorship curve, whereas life expectancy is constant for a perfect Type II survivorship curve (Southwood 1978). There is a decreasing trend in life expectancy (ex) within all of these life tables, but the decline is much less dramatic in the overwintering populations than with summer generations. Thus, mortality factors operating upon the larval population appeared to differ according to season.

The excellent survival of C. variipennis larvae during the spring and early summer can probably be attributed to the abundance of food (phytoplankton), relative stability of the habitat and the paucity of predators or parasites. Phytoplankton abundance, though not measured quantitatively, was very high during April and May as evidenced by the greenish color of the water during blooms of Chlamydomonas

sp. Diatoms were present throughout the summer. It appears that during mid-summer when larval densities are high, there may even exist mechanisms by which food resources are partitioned on a diurnal cycle (see Chap. 4). The habitat did, on occasion, become flooded during the spring and early summer. In sampling before, during and after heavy rains I found that pupae, being bouyant, were washed far above the original shoreline and up onto the grassy slopes surrounding the mudflats. Larvae, on the other hand, remained at the original shoreline following inundation, despite a rise in pond level of ca. 1m. But three days later as the pond began to drain, larvae advanced to meet the receding shoreline. Thus a larval strategy of simply "waiting out the storm" may circumvent mortality due to flooding. Although the pond levels at Saltville drop during times of drought, they rarely, if ever, completely dry up (C.B. Totten, pers. comm.). Habitat dessication does not appear to be a factor in larval mortality at Saltville, although it is a definite factor in more typical C. variipennis habitats (i.e., farmponds, lagoons, and pasture springs). During sampling around Blacksburg, VA., I have extracted C. variipennis larvae living in the caked dirt of a dessicated habitat. Certainly the extent to which C variipennis larvae can withstand dehydration warrants closer examination.

It is difficult to attribute larval/pupal mortality directly to biotic factors within the habitat. Ceratopogonid larvae are known to be parasitized by several species of mermithid nematodes (Wirth 1977). Mullens (1982) reported nematode parasitism rates ranging from 7-10% in New York populations of C. variipennis. Although mermithid nematodes were also found to parasitize C variipennis larvae in Saltville, the frequency was so low as to be negligible. Salinity of Saltville breeding habitats may aid in reducing mermithid parasitism in these larvae.

Few potential invertebrate predators were observed to inhabit the area directly below the waterline. The mudflats directly above the water's edge, however, were traversed during the day by substantial numbers of small unidentified carabids and ants. This region in the microhabitat is the same region where mature larvae migrate in order to pupate. Considering the heavy mortality of C. variipennis pupae, it seems probable that insect predators such as carabids and ants were preying upon the pupae, contributing a large part to the increased rate of mortality of C. variipennis pupae.

One to several pairs of mallard ducks (Anas platyrhynchos L.) were frequently observed feeding in the littoral zone of the study site early in the morning. Although these birds are known to cause mortality of

mosquito larvae (Iverson 1971), their role in the mortality of C. variipennis larvae in Saltville could not be verified. However, observed feeding behavior consisted of taking mud into the bill, looking upwards and rapidly opening and closing the bill as if sieving or grinding the mud. Because of the high larval densities occurring within the duck's feeding range, it is difficult to imagine the birds not ingesting C. variipennis larvae as well as other aquatic insects (Corixids and Ephydrid larvae). If such is the case, then larval mortality caused by duck predation may be, to a large extent, random with respect to larval age class.

Table 9. Generation times and degree-day accumulations for three generations of immature of Culicoides variipennis at Saltville, VA 1983-84.

LARVAL GENERATION	DATE BEGIN	DATE END	DAYS	DEGREE-DAY ACCUMULATIONS
Overwinter 1983-84	4 Oct	27 Apr	206	831.1
1st Summer 1984	25 Apr	14 June	50	376.0
2nd Summer 1984	14 June	3-7 Aug	50-55[1]	366.3±21.2

[1]Exact date not known. The most likely range in which adult emergence occurred was projected and the degree-day accumulations were averaged over projected range.

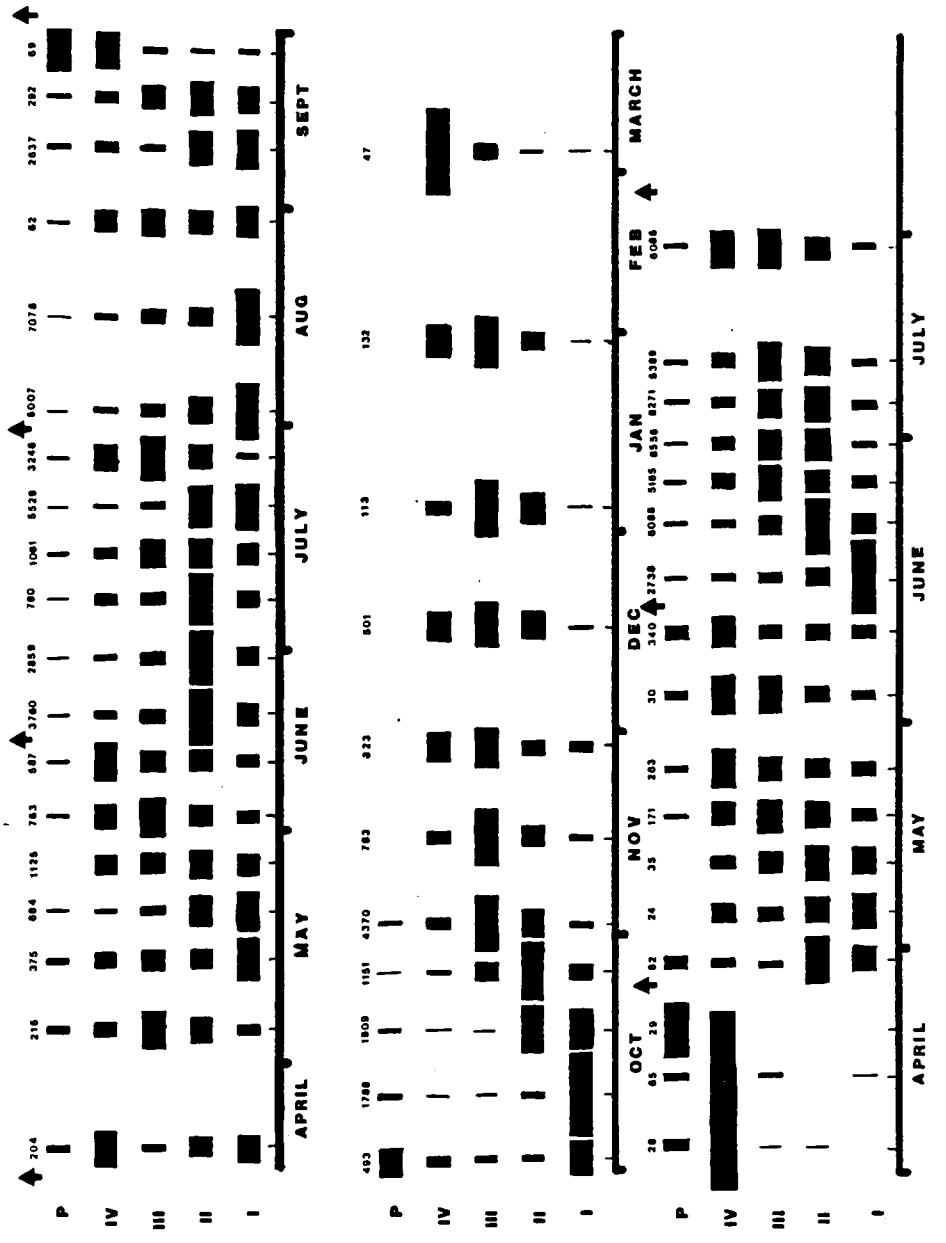

Figure 18. Life history and voltinism of Culicoides variipennis within littoral zone of brine pond. Saltville, VA 1983-1984

Table 10. Cumulative percentages and estimated development times (t50) of immature Culicoides variipennis under field conditions. Saltville, VA May-June 1983.

	Cumulative % of population completing instar				
DAY	I	II	III	IV	P
1	5.3	3.6	-	-	-
14	7.8	8.2	9.7	-	-
22	25.8	14.2	17.2	-	-
29	65.3	43.7	26.4	3.8	-
36	90.7	76.6	52.8	20.5	-
42	100.0	90.1	86.0	34.2	3.0
55	-	100.0	100.0	52.1	4.3
62	-	-	-	66.4	19.7
68	-	-	-	94.3	97.9
76	-	-	-	100.0	100.0
t50	20.5	25.8	31.1	48.8	62.7

Table 11. Cumulative percentages and estimated development times (t50) of immature Culicoides variipennis under field conditions. Saltville, VA 4 Oct - 27 April 1983-84 (overwintering gen.).

	Cumulative % of population completing instar				
DAY	I	II	III	IV	P
1	6.9	-	-	-	-
7	60.9	3.2	-	-	-
14	85.5	30.6	5.1	-	-
22	90.3	53.2	20.3	3.5	-
28	97.4	88.6	36.6	11.2	-
43	99.0	93.5	65.1	32.8	-
57	100.0	94.9	74.7	48.1	-
72	-	98.3	90.5	70.0	-
90	-	99.4	94.8	71.4	-
119	-	100.0	99.4	77.8	-
152	-	-	100.0	83.9	-
180	-	-	-	87.7	10.5
192	-	-	-	96.6	28.9
197	-	-	-	98.4	73.9
206	-	-	-	100.0	100.0
t50	4.9	18.7	35.3	62.1	192.7

Table 12. Cumulative percentage and estimated development times (t50) of immature Culicoides variipennis under field conditions. Saltville, VA May 1984.

	Cumulative % of population reaching instar				
DAY	I	II	III	IV	P
9	-	27.5	-	-	-
16	-	31.9	-	-	-
21	-	40.4	5.0	-	-
28	-	65.8	46.3	10.6	2.9
34	-	100.0	92.0	36.8	7.0
44	-	-	100.0	39.7	8.8
52	-	-	-	65.6	26.9
56	-	-	-	100.0	48.5
62	-	-	-	-	100.0
t50	-	18.3	27.8	41.1	53.5

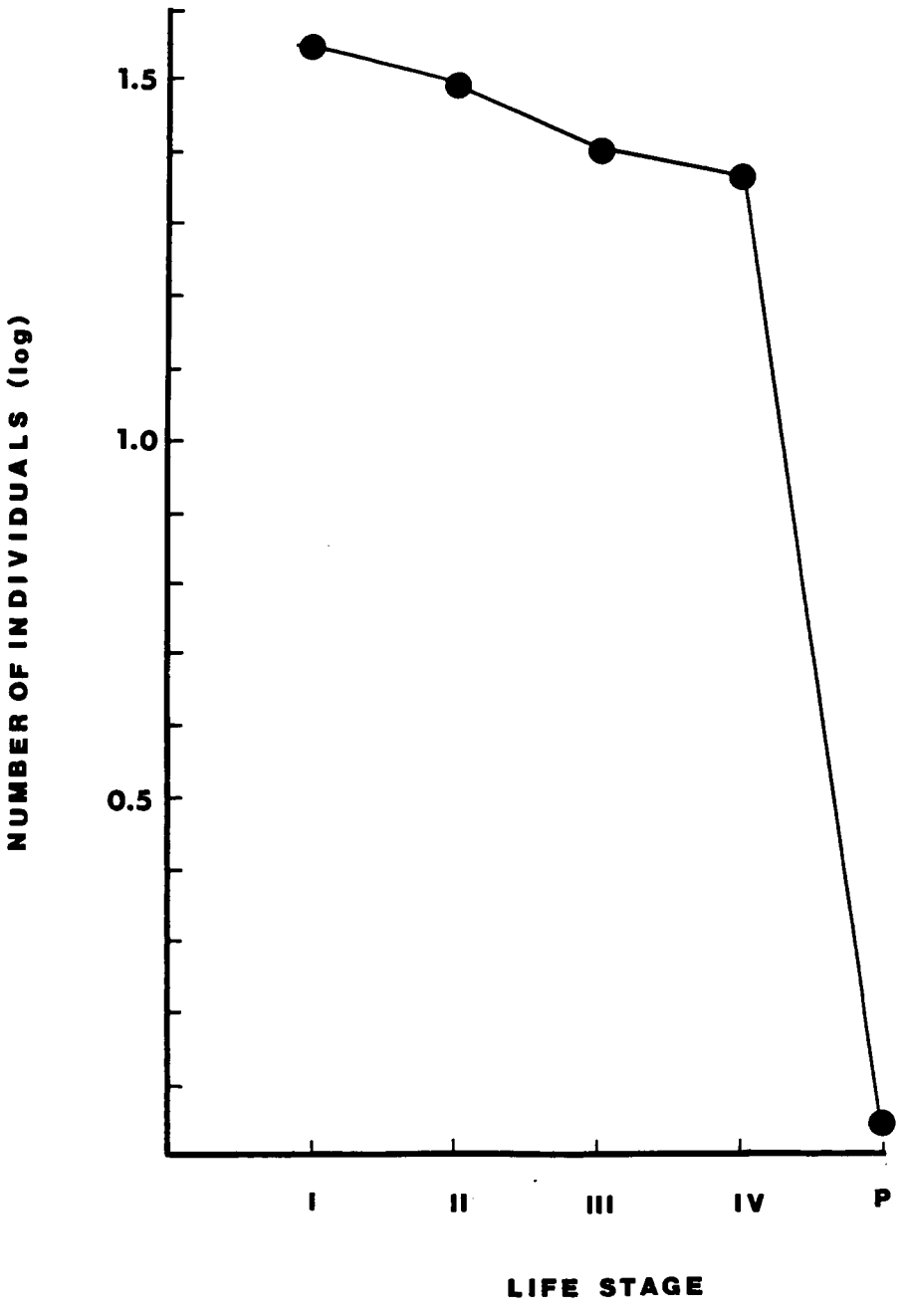

Figure 19. Stage-specific survivorship curve for immature Culicoides variipennis. Saltville, VA May - June 1983

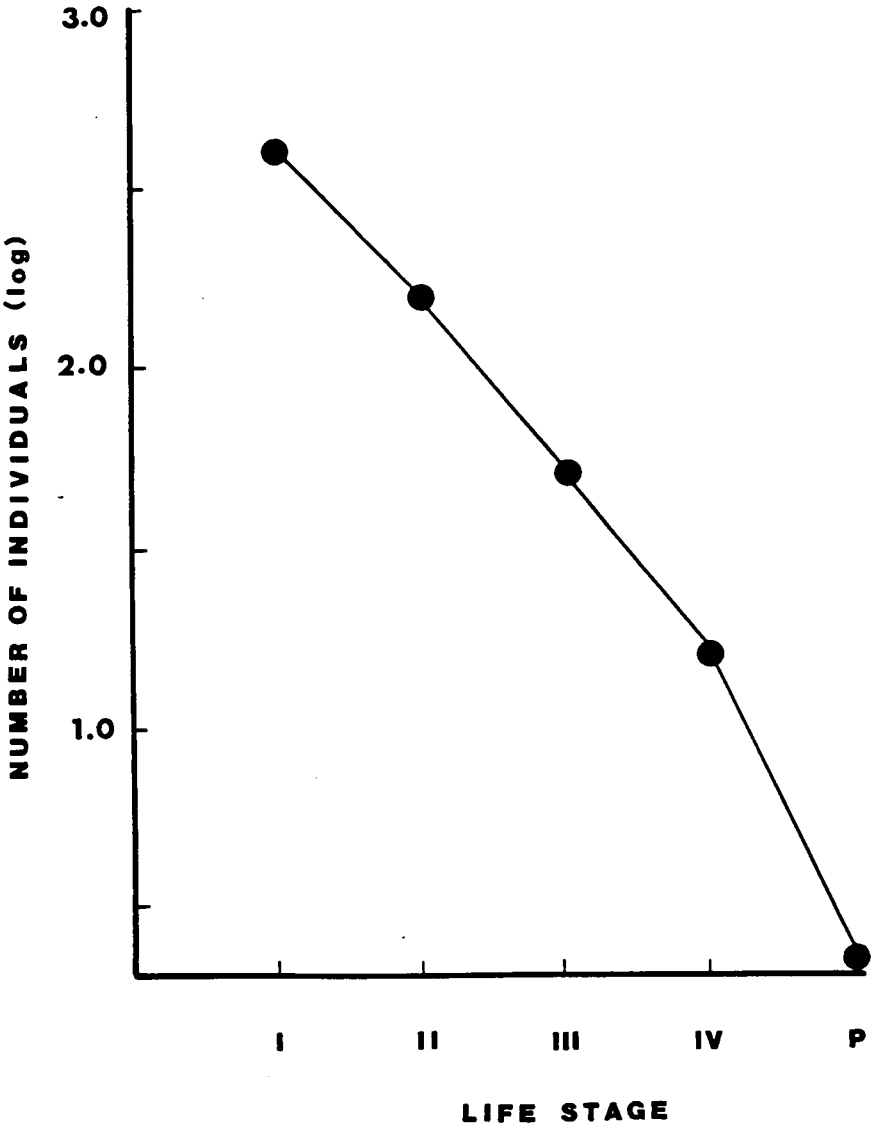

Figure 20. Stage-specific survivorship curve for immature Culicoides variipennis. Saltville, VA Oct - April 1983-1984 Overwintering generation

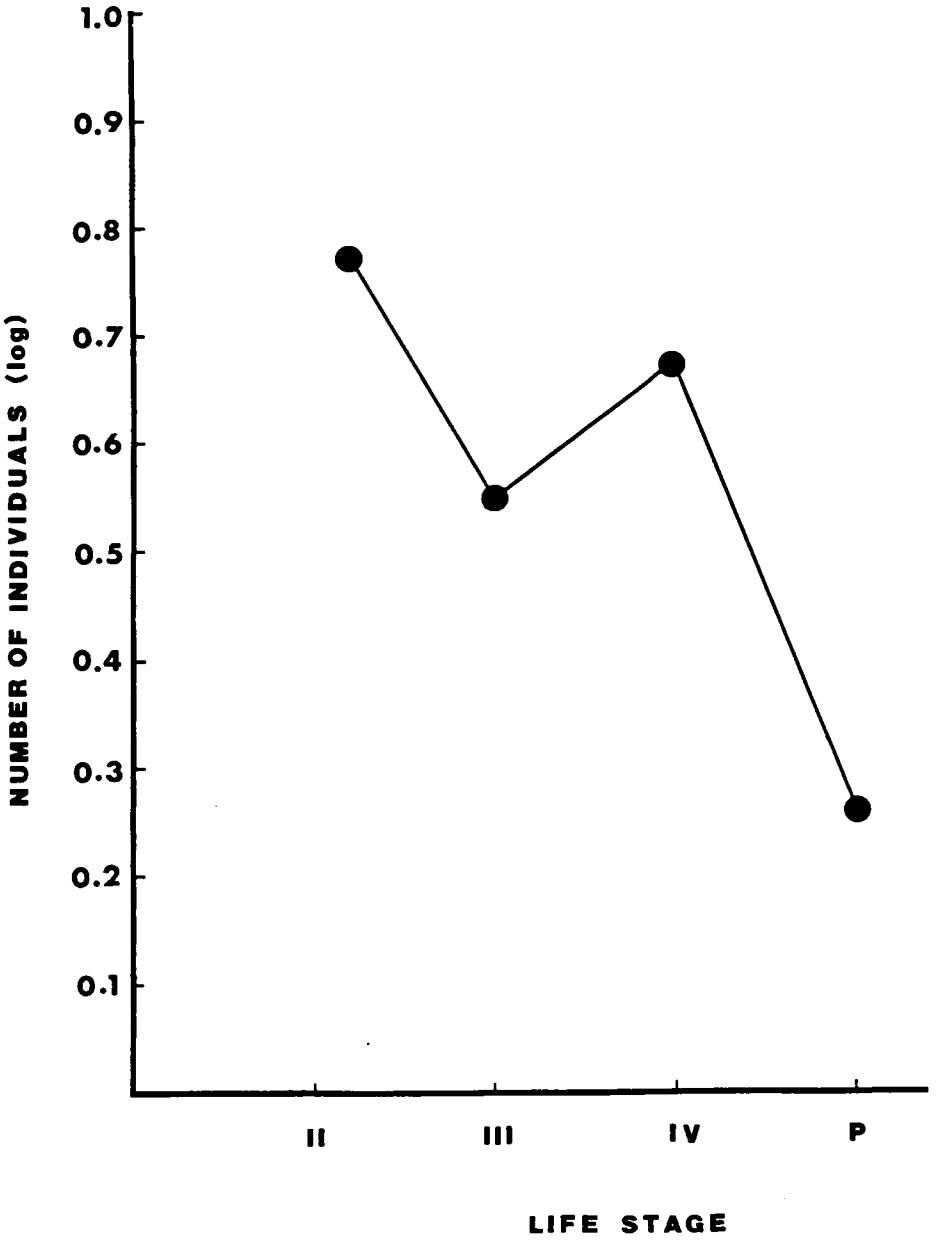

Figure 21. Stage-specific survivorship curve for immature Culicoides variipennis. Saltville, VA May - June 1984.

Table 13. Life table and k-factors for 1983 first summer generation of immature Culicoides variipennis. Saltville, VA 21 April - 6 July 1983.

21 APRIL - 6 JULY 1983

LIFE STAGE	x	lx	dx	ex	1000qx	k
I	20.5	1000	68	2.86	68	
						0.053
II	25.8	932	215	2.03	231	
						0.091
III	31.1	717	38	1.49	53	
						0.023
IV	48.8	679	647	0.55	953	
						1.323
P	62.7	32	32	0.50	1000	
						K = 1.490

Table 14. Life table and k-factors for 1983-84 overwintering generation of immature Culicoides variipennis. Saltville, VA 4 Oct 1983 - 27 April 1984.

4 OCT 1983 - 27 APRIL 1984

LIFE STAGE	x	lx	dx	ex	1000qx	k
I	5	1000	599	1.09	598.9	
						0.397
II	19	401	256	0.97	637.7	
						0.441
III	35	145	104	0.79	717.8	
						0.550
IV	62	41	40	0.51	990.2	
						1.970
P	193	0.4	0.4	0.50	1000.0	
						K = 3.357

Table 15. Life table and k-factors 1983 first summer generation of immature Culicoides variipennis. Saltville, VA 25 April - 14 June 1984.

25 APRIL - 14 JUNE 1984

LIFE STAGE	x	lx	dx	ex	1000qx	k
II	9	1000	422	2.17	422	
						0.238
III	29	578	-214	2.40	-370	
						-0.863
IV	48	792	487	0.88	615	
						0.415
P	54	305	305	0.55	1000	

CHAPTER 6

Summary

The research reported within this thesis focuses upon the biology and ecology of the immature stages of a single unique population of Culicoides variipennis at Saltville, VA. Preliminary sampling in 1982 was conducted at Saltville in order to locate the major breeding sites of this insect within the valley. An efficient method of larval extraction was validated for further studies and an on-site laboratory was established. In 1983-84, field studies were conducted at a major breeding site at Saltville, VA. Laboratory studies using the Saltville population were conducted at Virginia Tech, Blacksburg, VA. The specific research objectives summarized here include:

1. Determination of developmental times, developmental threshold temperatures and thermal constants for the immature stages of C. variipennis under constant laboratory temperatures.

2. Seasonal microdistribution of larvae and pupae within the littoral microhabitat.

3. Diurnal microdistribution of larvae and pupae within the littoral microhabitat.

4. Monitoring of the population dynamics and estimation of field survivorship of immature C. variipennis at Saltville, VA.

Developmental times, developmental thresholds and thermal constants were determined for larval and pupal C. variipennis using constant photoperiod (14L:10D) and temperatures (20, 23, 27, 30, 35°C). Developmental threshold temperatures (°C) and thermal constants (°days) for larvae and pupae were 9.6°C and 387°days (larval stage), and 9.6°C and 30°days (pupal stage) respectively. Egg development was too variable for meaningful threshold values to be calculated. Erratic development of eggs may have been due to asynchronous embryonic development and/or eclosion. Because the relationship of temperature and insect development is sigmoidal, I suspect that some development of larval/pupal C. variipennis probably can occur below the 9.6°C threshold value that I have calculated. However, this value probably is valid for determining heat accumulations for larval/pupal development in the field. After monitoring the dynamics of a field population at Saltville, I found accumulated heat units ranged from 366-376°days between successive generations during the spring and summer. These values are in fair agreement with values calculated from my laboratory studies (larval + pupal stage = 417°days). Thus,

I believe that these values may allow for reasonable forecasting of the peak summer midge emergences at Saltville. However, my values differed somewhat from values calculated for New York populations of C. variipennis. Therefore, similar research from other regions may be warranted before incorporating such values into prediction models intended for use in bluetongue-endemic areas. Heat accumulations required for completion of immature development of C. variipennis at Saltville were found to be much greater (831°days) for the overwintering generation. This is probably a result of shortening daylength, triggering physiological diapause in these insects.

Larval/pupal distribution within the microhabitat was found to change throughout the year. During the warmer months of the year most larvae were found to inhabit the surface mud at or near the waterline. Pupae were found farther above the shoreline, which is probably a reflection of the fact that most Culicoides spp. cannot pupate successfully underwater. Insects overwintered in the larval stage, mostly as 3rd instars. During autumn, larvae began to burrow into the shoreline mud. In winter, when the pond and underlying mud froze, most larvae were recovered far from the shoreline, most likely being moved passively in the course of ice crystal formation. Although overwintering

larvae possessed glycerol (anti-freeze), episodes of heavy larval mortality were noted to occur within the habitat following periods of abrupt freezing (late December) and thawing (mid-February). In early March, most larvae appeared to have molted to 4th instars and migrated above shoreline in preparation for pupation and adult emergence in April. Larvae collected during the summer were found to be smaller than those collected during the autumn and winter. This phenomenon was attributed to elevated temperatures, lowered organic content of ingested mud and the possible effects of crowding during the summer. The distribution of early instar larvae and pupae during the summer appeared relatively constant throughout the 24h period. However, there occurred subtle shifts in the diurnal distribution of later instars within the microhabitat. This diel migration was restricted largely to the surface cm of mud. Although much of the late instar larval population remained at shoreline throughout the 24h period, a substantial portion (ca. 50%) appeared to have moved offshore during the day. At night, most late instars (ca. 75%) were densely aggregated at the shoreline. This movement was not correlated with diel changes in substrate temperature. Examination of larval guts revealed that the majority of feeding at shoreline occurred during the night, with little

shoreline feeding occurring in the mid-afternoon. Although impossible to attribute direct cause-and-effect in the circadian movements of C. variipennis larvae, my data suggest that such daily movements may be related to the partitioning of space and resources by 3rd and 4th instar larvae, as well as the pre-pupational behavior of mature 4th instars.

The population dynamics of immature C. variipennis at Saltville were monitored by regular sampling of a major breeding habitat. The sampling design took into account differences in the distribution of different lifestages in order to avoid systematic error in the over- or under-sampling of an age-class. I documented a total of 4 generations (an overwintering generation and 3 summer generations) for 1983-84. Emergence of the overwintering generation occurred from mid to late April. Subsequent generations emerged during mid-June, late July/early August, and from late September to early October. Larval populations were highest in July and August but larval development was too rapid at that time to monitor early instar survival. However, estimates of stage survivorship could be calculated for those portions of the year where cohort development was of sufficient length with respect to the weekly sampling interval. Not surprisingly, I found

differences in the stage survivorship at different times of the year. The survivorship curve for the overwintering generation approached that of a Type II survivorship curve, where there occurs constant fall-off in numbers from one age-class to the next (i.e. constant mortality rate). The survivorship curves for the first summer generations of 1983 and 1984 were more indicative of Type I survivorship curves, where mortality exerts little effect on younger age-classes (i.e. the larval stages) but wields its influence most acutely upon the older age-classes (in this case, the pupal stage). The excellent survival of C. variipennis larvae probably accounts for the enormous densities (up to 30 larvae/ml) which I have frequently observed at Saltville during late summer. Factors contributing to sustained survival of the larvae probably include the abundance of food, habitat stability and the paucity of larval predators and parasites. Although ducks were often observed to feed in the littoral zone of Saltville ponds, their role in larval mortality is not clear. The biological "check" operating in this system to prevent logarithmic population growth of this insect appears to affect itself at or near metamorphosis. The exact nature of this "check" is uncertain at this time but, from observations made at the study site, it appears that insect predators (e.g. ants and

carabid beetles) may play a role in the summer mortality of metamorphosing C. variipennis.

Literature Cited

Akey, D. A., H. W. Potter and R. H. Jones. 1978. Effects of rearing temperature and larval density on longevity, size, and fecundity in the biting gnat Culicoides variipennis. Ann. Entomol. Soc. Amer. 71: 411-418.

Andrewartha, H. G. and L. C. Birch. 1954. The Distribution and Abundance of Animals. Univ. Chicago Press. 782 pp.

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

Battle, F. V. and E. C. Turner, Jr. 1971. A systematic review of the genus Culicoides (Diptera: Ceratopogonidae) of Virginia. Insects of Virginia No. 3. Va Tech Res. Div. Bull. 44. 129pp.

Baust, J. G. and R. E. Lee. 1983. Population differences in antifreeze/cryoprotectant accumulation patterns in Antarctic insects. Oikos 40: 120-124.

Becker, P. 1958. The behavior of larvae of Culicoides circumscriptus Kieff. (Diptera: Ceratopogonidae) towards light stimuli as influenced by feeding, with observations on the feeding habits. Bull. Entomol. Res. 49: 785-802.

Bidlingmayer, W. L. 1957. Studies on Culicoides furens Poey at Vero Beach. Mosq. News 17: 292-294.

Blanton, F. S. and W. W. Wirth. 1979. The sand flies (Culicoides) of Florida (Diptera:Ceratopogonidae). Fla. Dept. Agic. and Cons. Ser. Contrib. No. 424. 204pp.

Boreham, M. M. 1981. A new method for sampling mangrove mud for Culicoides larvae and pupae, with notes on factors affecting its use. Mosq. News 41: 1-6.

Davidson, J. 1944. On the relationship between temperature and rate of development of insects at constant temperatures. J. Anim. Ecol. 13: 26-38.

Davis, E. L., D. L. Kline, J. F. Reinert, R. H. Roberts, and J. F. Butler. 1983. Development of immature

Culicoides mississippiensis (Diptera:Ceratopogonidae) in the laboratory. Ann. Entomol. Soc. Am. 76: 918-924.

Deevey, E. S. 1947. Life tables for natural populations of animals. Quart. Rev. Biol. 22: 283-314.

Downes, J. A. 1978. The Culicoides variipennnis complex: a necessary realignment of nomenclature (Diptera: Ceratopogonidae). Can. Entomol. 110: 63-69.

Dove, W. E., D. G. Hall and J. B. Hull. 1932. The salt marsh sand fly problem. Ann. Entomol. Soc. Amer. 25: 505-527.

Dzhafarov, Sh. M. 1964. Biting Midges (Diptera: Heleidae) of Transcacas. (Morphology, Biology, Ecology, Geographical Distribution, and Harmfulness, Control, Fauna of the Genera Culicoides, Leptoconops and Lasiohelea). Publishing House of the Academy of Science of Azerbajan SSR. 525pp. (translated from Russian for Agric. Res. Ser. USDA and NSF, Washington D.C. by Franklin Book Programs, Inc., Cairo. 1976.)

Enfield M. A. and G. Pritchard 1977. Estimates of population size and survival of immature stage of four species of Aedes (Diptera: Culicidae) in a temporary pond. Can. Ent. 109: 1425-1434

Foster, N. M., R. D. Breckon, A. J. Luedke and R. H. Jones. 1977. Transmission of two strains of epizootic hemorrhagic disease virus in deer by Culicoides variipennis. J. Wildl. Dis. 13: 9-16.

Foster, N. M. and R. H. Jones. 1979. Multiplication rate of bluetongue virus in the vector Culicoides variipennis (Diptera: Ceratopogonidae) infected orally. J. Med. Entomol. 15: 302-303.

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

Harker, J. E. 1961. Diurnal rhythms. Ann. Rev. Entomol. 6: 131-146.

Hensleigh D. A. and W. R. Atcheley. 1977. Morphometric variability in natural and laboratory populations of Culicoides variipennis (Diptera: Ceratopogonidae). J. Med. Entomol. 14: 379-386.

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

Hoff, G. L. and D. O. Trainer. 1979. Hemorrhagic diseases of wild ruminants. pp. 45-53. Chap. 4. Infectious Diseases of Wild Animals. Academic Press, NY.

Isaev, V. A. 1977. Seasonal migration of larvae of the blood-sucking midges, Culicoides odibilis Austen (Diptera: Ceratopogonidae). Med. Parazit. Moskva 46: 41-45.

Iversen, T. M. 1971. The ecology of a mosquito population (Aedes communis) in a temporary pool in a Danish beech wood. Arch. Hydrobiol. 69:309-332.

Jamnback, H. A. 1965. The Culicoides of New York State (Diptera:Ceratopogonidae). N. Y. State Mus. Sci. Serv. Bull. 399. 154 p.

Jamnback, H. and W. J. Wall. 1958. A sampling procedure for Culicoides melleus (Coq.)(Diptera: Heleidae) with observations on the life histories of two coastal Culicoides. Mosq. News 18: 85-89.

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

Jones, R. H. 1967. An overwintering population of Culicoides in Colorado. J. Med. Entomol. 4: 461-463.

Jones, R. H., A. J. Luedke, T. E. Walton and H. E. Metcalf. 1981. Bluetongue in the United States, an entomological perspective toward control. World Anim. Rev. 38: 2-8.

Kettle, D. S. 1977. Biology and bionomics of blood-sucking ceratopogonids. Ann. Rev. Entomol. 22: 33-51.

Kettle, D. S. and J. W. H. Lawson. 1952. The early stages of British biting midges Culicoides Latreille (Diptera: Ceratopogonidae) and allied genera. Bull Entomol. Res. 43: 421-67.

Kettle, D. S., R. H. Nash, and B. A. Hopkins. 1956. Field tests with larvicide againt Culicoides impunctatus Goetgh. in Scotland. Bull. Entomol. Res. 47: 553-73.

Kitaoka, S. 1982. Effects of rearing temperature on length of larval period and size of adults in Culicoides arakawae and Culicoides maculatus (Diptera: Ceratopogonidae). Natl. Inst. Anim. Health Q. (Jpn.) 22: 159-162.

Kline, D. L., J. C. Dukes and R. C. Axtell. 1975. Salt marsh Culicoides (Diptera: Ceratopogonidae): comparison of larval sampling methods. Mosq. News 35: 147-150.

Lamb, R. J., G. H. Gerber and G. F. Atkinson. 1984. Comparison of developmental rate curves applied to egg hatching data of Entomoscelis americana Brown (Coleoptera: Chrysomelidae). Environ. Entomol. 13: 868-872.

Linley, J. R. 1966. Field and laboratory observations on the behavior of immature stages of Culicoides furens Poey (Diptera: Ceratopogonidae). J. Med. Entomol. 2: 385-391.

Linley, J. R. and G. M. Adams. 1972. Ecology and behavior of immature Culicoides melleus (Coq.)(Diptera: Ceratopogonidae). Bull. Entomol. Res. 62: 113-127.

Linley, J. R. 1976. Biting midges of mangrove swamps and saltmarshes (Diptera: Ceratopogonidae). pp. 335-376. In: Marine Insects. North Holland Publ. Co., Amsterdam.

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

Madder, D. J., G. A. Surgeoner and B. V. Helson. 1983. Number of generation, egg production and developmental time of Culex pipiens and Culex restuans (Diptera: Culicidae) in Southern Ontario. J. Med. Entomol. 20: 275-287.

Marchant, R. and H. B. N. Hynes. 1981. Field estimates of feeding rate for Gammarus pseudolimnaeus (Crustacea: Amphipoda) in the Credit River, Ohio. Freshwater Biol. 11: 27-36.

Mogi, M., I. Miyagi and B. D. Cabrera. 1984. Development and survival of immature mosquitoes (Diptera: Culicidae) in Phillippine rice fields. J. Med. Entomol. 21: 283-291.

Mullens, B. A. 1982. The bionomics of Culicoides variipennis variipennis (Coquillet)(Diptera: Ceratopogonidae) in central New York State, with particular reference to vector competence. Ph.D. Dissertation. Cornell Univ., Ithaca, NY. 137pp.

Mullens, B. A. and D. A. Rutz. 1983a. Development of immature Culicoides variipennis at constant laboratory temperatures. Ann. Entomol. Soc. Am. 76: 747-751.

Mullens, B. A. and D. A. Rutz. 1983b. Voltinism and seasonal abundance of Culicoides variipennis (Diptera: Ceratopogonidae) in central New York State. Ann. Entomol. Soc. Am. 76: 913-917.

Mullens, B. A. and D. A. Rutz. 1984. Age structure and survivorship of Culicoides variipennis (Diptera: Ceratopogonidae) in central New York State, USA. J. Med. Entomol. 21: 194-203.

Parker, M. D., D. H. Akey and L. H. Lauerman. 1977. Microbial flora associated with colonized and wild populations of the biting gnat Culicoides variipennis. Entomol. Exp. Appl. 21: 130-6.

Ray, C. E., B. N. Cooper, and W. S. Benninghoff. 1967. Fossil mammals and pollen in a late Pleistocene deposit at Saltville, Virginia. J. Paleontol. 41: 609-22.

Rieb, J-P. and D. Guinier. 1981. Analyse statistique de la distribution horizontale des larves de Culicoides (Dipteres, Ceratopogonides) d'un gite fluviatile. Bull. Ecol. 12 (4): 313-326.

Rowley, W. A. 1967. Observations on larval habitats and the winter bionomics of some common species of Culicoides (Diptera: Ceratopogonidae) in the central Columbia Basin. Mosq. News 27: 499-505.

Slobodkin, L. B. 1962. Growth and regulation of animal populations. Holt, Rinehart and Winston, New York. 184 pp.

Southwood, T. R. E. 1978. Ecological Methods. Halstead Press, N. Y. 524pp.

Southwood, R. R. E., G. Murdie, M. Yasuno, R. J. Tonn, and P. M. Reader. 1972. Studies on the life budget of Aedes aegypti in Wat Samphaya, Bangkok, Thailand. Bull. Wld. Hlth. Org. 46: 211-226.

Taylor, F. 1981. Ecology and evolution of physiological time in insects. Amer. Nat. 117: 1-23.

Tauber, M. J. and C. A. Tauber. 1976. Insect seasonality: diapause maintainence, termination and post-diapause development. Ann. Rev. Entomol. 21: 81-107.

Weerekoon, A. C. J. 1953. On the behavior of certain Ceratopogonidae (Diptera). Proc. R. Entomol. Soc. Lond. (A) 28: 85-92.

Williams, R. W. 1960. A new and simple method for the isolation of freshwater invertebrates from soil samples. Ecology 41: 573-574.

Wirth, W. W. 1977. A review of the pathogens and parasites of the biting midges (Diptera: Ceratopogonidae). J. Wash. Acad. Sci. 67: 60-75.

Wirth, W. W. and R. H. Jones. 1957. The North America subspecies of Culicoides variipennis (Diptera: Heleidae). USDA Bull. 1170. 35pp.

Zimmerman, R. H. 1981. Seasonal abundance and host preference of the Culicoides of Virginia: with emphasis on the ecology of Culicoides variipennis (Diptera: Ceratopogonidae). Ph.D. Dissert., V.P.I. S.U., Blacksburg, VA 192pp.

Zimmerman, R. H., S. J. Barker, and E. C. Turner. 1982. Swarming and mating behavior of a natural population of Culicoides variipennis (Diptera: Ceratopogonidae). J. Med. Entomol. 19: 151-156.

Zimmerman, R. H., and E. C. Turner, Jr. 1984. Dispersal and gonotrophic age of Culicoides variipennis (Diptera: Ceratopogonidae) at an isolated site in southwestern Virginia, USA. J. Med. Entomol. 21: 527-535.

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