ESTIMATION OF SURVIVAL AND GONOTROPHIC CYCLE LENGTH OF *CULICOIDES VARIIPENNIS* (DIPTERA: CERATOPOGONIDAE) IN CALIFORNIA

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ABSTRACT. The use of a time series analysis to estimate the survival rate and gonotrophic cycle length of *Culicoides variipennis* at 2 California sites is described. Collections were made daily for 28 days in Yolo County (northern California) and for 25 days in Riverside County (southern California) in July and August of 1989, respectively, using CO_2-baited suction traps. The time series analysis of these collections yielded a gonotrophic cycle length estimate of 3 days. Stage-specific and daily survivorship estimates 0.242 and 0.623, respectively, were determined for the northern California site. The time series method was found unsuitable for estimating the gonotrophic cycle length or daily survivorship at the southern California site.

INTRODUCTION

Of the numerous factors influencing vectorial capacity, survivorship is one of the principal parameters that determines the ability of hematophagous insect populations to transmit disease agents. Survival rate is expressed on a daily basis or over the length of the gonotrophic cycle. Estimates of the survival rate can be obtained from calculation of the mean parous rate (Birley 1984), mark-release-recapture studies (Milby and Reisen 1989) and through time series estimation of the proportion of individuals surviving oviposition (Birley and Boorman 1982).

The biting midge, *Culicoides variipennis* (Coq.), is well known as a vector of bluetongue virus (BTV) in North America (Foster et al. 1963). Stage survivorship estimates using gonotrophic age data from ovarian dissections have been reported previously for this species in New York State (Mullens and Rutz 1984). However, methodological difficulties in estimating survival rates of *Culicoides* spp. exist (Mullens 1985a). For example, trap type and placement may influence the reproductive stage of *Culicoides* captured (Zimmerman and Turner 1984, Mullens 1985b, Anderson and Linhares 1989).

The technique of time series estimation (Birley and Boorman 1982) has been used successfully to determine the survival rates of different species of *Culicoides* in Israel and Africa (Braverman et al. 1985). This technique has also been applied to mosquitoes (Birley and Rajagopalan 1981) and blackflies (Birley et al. 1983). Survival rate estimates using time series analysis have been similar to those obtained using mark-release-recapture (Milby and Reisen, 1989), suggesting that, under the proper set of conditions, this method can be useful. Using survival rate estimates, the expectation of infective life has been calculated for *Culicoides* and BTV to assess the potential of different species to transmit BTV (Birley et al. 1984, Braverman et al. 1985).

Based on serological surveys (Osburn et al. 1981, Jessup et al. 1984), southern California appears to have a higher seroprevalence of BTV in domestic and free-ranging ruminants than does northern California, and this may be related to the different survivorships of *C. variipennis* in these regions. The objective of this study was to evaluate the time series analysis in estimating the survivorships and the lengths of the gonotrophic cycles in 2 discrete field populations of *C. variipennis* in California. The effects of trap location in determining these 2 estimates were also examined.

MATERIALS AND METHODS

Study site 1: The study site in northern California consisted of a flat grassland and prairie oak ecosystem encompassing 45 ha near Davis, CA (Fig. 1A). This area contained 2 enclosures accommodating Canada geese (*Branta* sp.), mallards (*Anas* sp.) and cranes (*Grus* sp.), and one enclosure accommodating 10–15 black-tailed deer (*Odocoileus* sp.). The avian enclosures were semienclosed, allowing free migration of animals. The deer were maintained according to animal use and care protocols of the University of California, Davis. Of the 3 ponds in this ecosystem, 2 ponds in the avian enclosures were heavily contaminated with guano and had gently sloping sides bereft of vegetation. The third pond contained thick mats of vegetation on its edge. The possible presence of *C. variipennis* in the ponds was not determined, but their physical characteristics were consistent with optimal *C. variipennis* breeding habitat (Mullens 1989). The site was surrounded by tilled or fallow fields to the north, west and east and by a flowing creek to the south.

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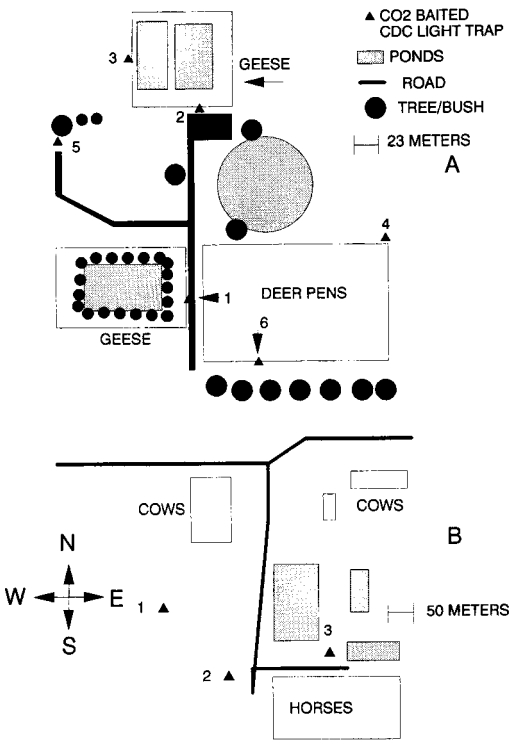

Fig. 1. Trap locations at the northern (A) and southern (B) California sites.

Study site 2: The study site in southern California was a dairy in the Chino basin of western Riverside County (Fig. 1B). The terrain was level to somewhat hilly. This area contains one of the largest aggregations of dairies in the United States, with over 250,000 milking cows in a 75-km^2 area. Most dairies in the area contain waste water ponds with *C. variipennis* larvae in varying amounts and the edges devoid of vegetation (Mullens 1989). Many penned cattle and horses were located within 100 m of the site.

Biting midge collection: Adult *C. variipennis* were collected with CDC-type miniature suction traps, each with a small incandescent light and approximately 1 kg dry ice (CO_2) as an attractant. Six traps were set out at the northern California site, 3 located near (≤5 m) and 3 located away (≥15 m) from water (Fig. 1A). Three traps were set out at the southern California site; one located at a dairy wastewater pond containing a high population of immature *C. variipennis*, one located 50 m southwest of the ponds on level ground and one located 100 m west of the ponds on a hill ca. 15–20 m higher elevation than the pond (Fig. 1B). Traps were set out daily ca. 2 h before sunset and retrieved 2 h after sunrise. Insects were collected alive into mesh bags (northern California) or into saline (southern California) and were processed by trap to 70% ethanol daily for later identification and sorting. Traps were operated from August 1 to August 28, 1989 (28 days), in northern California and from July 18 to August 11, 1989 (25 days), in southern California. Daily maximum and minimum temperatures as well as RH were recorded at both sites using a hydrothermograph.

Parity determination: The parity rate of females in each trap per night was determined using parous pigment methods (Dyce 1968, Akey and Potter 1979). Females were categorized as unfed nulliparous and parous based on the presence or absence of parous pigment on the abdomen. All females in each collection from both sites were age graded at UC Davis (TMW); in some large collections (exceeding 300 females), at least 100 randomly selected females were aged and the parity rate from this sample extrapolated to the total number of females for each trap.

Calculation of stage-specific survivorship and gonotrophic cycle length from a sequential collection of insects has been described previously (Birley and Boorman 1982, Braverman et al. 1985). Briefly, one assumes: 1) the collections of biting midges can be correctly sorted into nulliparous and parous conditions, 2) the sampling method is unbiased, and 3) the duration of the gonotrophic cycle and survival rate are independent of age; mortality and dispersal are not distinguishable. Conditions 1 and, to a lesser extent, 3 were considered valid. Unbiased sampling from a population may be difficult if not impossible (Mullens 1985a). CDC traps tend to overestimate host-seeking parous and nulliparous numbers while underestimating the gravid and blood-fed segments of the population (Anderson and Linhares 1989).

Survivorship and gonotrophic cycle length calculations: Stage-specific survivorship (S) was estimated by the following formula (Birley and Boorman 1982):

$$P_t = S * T_{t-u}$$

where P_t is the total number of parous insects caught at time t, u is the gonotrophic cycle length, T_{t-u} is the total number of insects (parous and nulliparous) caught one cycle previously, and S is the stage-specific survivorship (or survivorship per gonotrophic cycle). To estimate u, the parous time series is lagged by t-1, t-2, . . . t-k intervals where $k = (t)^{1/2} + 10$, and t is the length of the trapping period in days. For each interval lag, the parous time series is correlated with the unlagged total time series. The lag providing the highest cross-correlation co-

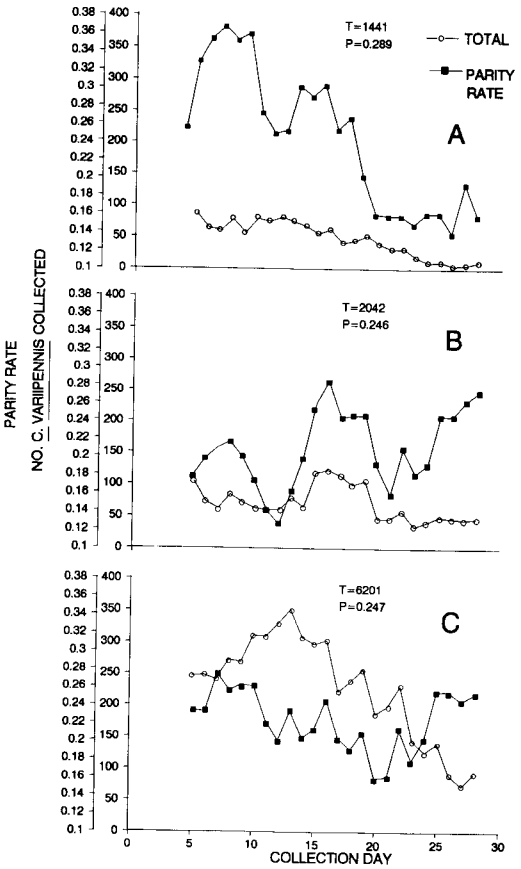

Fig. 2. Five-day running means of the total numbers per trap and running mean parity rates for traps 1–3 (A–C) northern California. T is the cumulative total caught in trap and P is the mean parity rate.

efficient is interpreted as the best estimate of u. This method is particularly applicable in populations where the recruitment rate varies rapidly.

Recently, this method was modified by adding a filter to the parous and total time series (Holmes and Birley 1987). At times, significant cross-correlation coefficients can be the result of spurious day-to-day correlations that may arise from sequential trappings. To reduce these correlations, Holmes and Birley (1987) proposed a filter whereby parous and total time series are autoregressed by a lag of 1 day. The cross-correlation is then repeated using the filtered series; and for time series of length n, cross-correlation coefficients exceeding the interval $\pm 1.96(n)^{1/2}$ are considered significant.

Data analysis: Analyses were done with the MINITAB statistical package using the CC function and filtering through the Auto-Regressive Iterative Moving Average (ARIMA 1,0,0) model. Gonotrophic cycle length (u) was determined by evaluating the plotted cross-correlations of the filtered time series and taking the lag peak with the highest coefficient as the best estimate of gonotrophic cycle length. Best estimates of stage-specific survivorship were determined using regression of the parous and total time series through the origin after lagging the parous series by u. Best estimates of daily survivorship were calculated by taking the uth root of stage-specific survivorship. Determinations of association between the numbers per parity category and trap placement were made using the Pearson chi-square test (a = 0.05).

RESULTS

Northern California: There was a progressive decline in the numbers of *C. variipennis* collected in all traps toward the end of the study (Figs. 2 and 3). Traps 3 and 4 caught over half

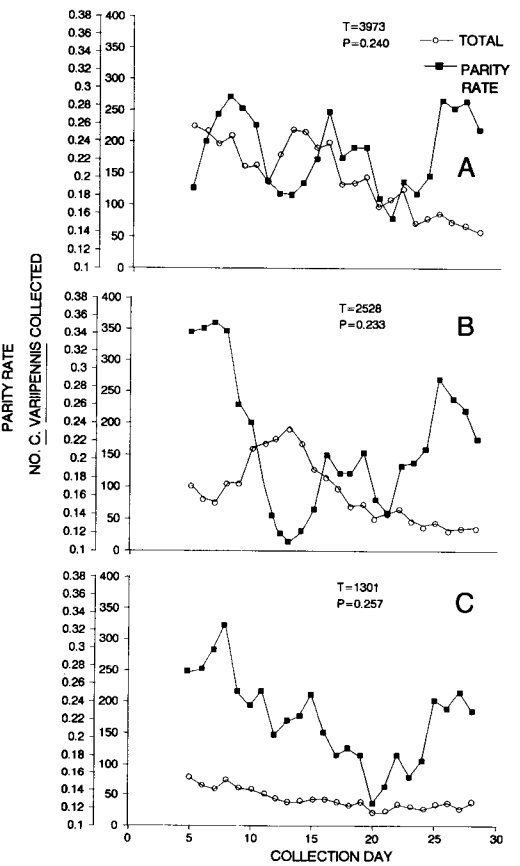

Fig. 3. Five-day running means of the total numbers per trap and running mean parity rates for traps 4–6 (A–C) in northern California. T is the cumulative total caught in trap and P is the mean parity rate.

of the total number while mean parity rates for each trap were similar and ranged from 0.233 to 0.289. The mean parity rate for pooled traps was 0.247. The 5-day running means of the parity rates for the individual traps revealed peaks occurring on days 6–8, 15–18 and 25–28. No stable relationship between the number of *C. variipennis* collected and the parity in the traps was observed. For the traps with the highest numbers, however, the numbers tended to be higher during the periods of lower parity.

Survivorship was estimated for individual (Fig. 4A–F) as well as pooled (Fig. 7A) traps. Unfiltered data resembled filtered data for all traps except that unfiltered peaks in correlation were not as distinct. For filtered data, significant correlation peaks were observed at lag 3 only for traps 3, 4, and pooled traps. Nonsignificant peaks were observed at lag 3 for traps 2, 5 and 6, and at lag 2 and 6 for trap 1. These results suggested that the best estimate of the gonotrophic cycle length in the northern California population was 3 days. Stage-specific (S) and daily survivorship estimates (DS) calculated based on a 3-day gonotrophic cycle for each trap ranged from 0.169 to 0.288 and 0.553 to 0.660, respectively. Pooled traps had a similarly calculated S and DS of 0.242 and 0.623, respectively.

Minimum and maximum temperatures ranged from 6.9 to 17.2°C and 26.7 to 38.3°C, respectively. Minimum and maximum RH ranged from 17.3 to 44.3 and 54.9 to 90.3, respectively. Water level in all ponds progressively receded to expose ca. 1 m of previously submerged shoreline at the end of the study period. Significant differences existed among the traps in the total numbers of parous and nulliparous *C. variipennis* caught ($P < 0.01$); however, there was no association between the numbers in each category caught near (≤5 m) versus far away (≥15 m) from water ($P > 0.05$).

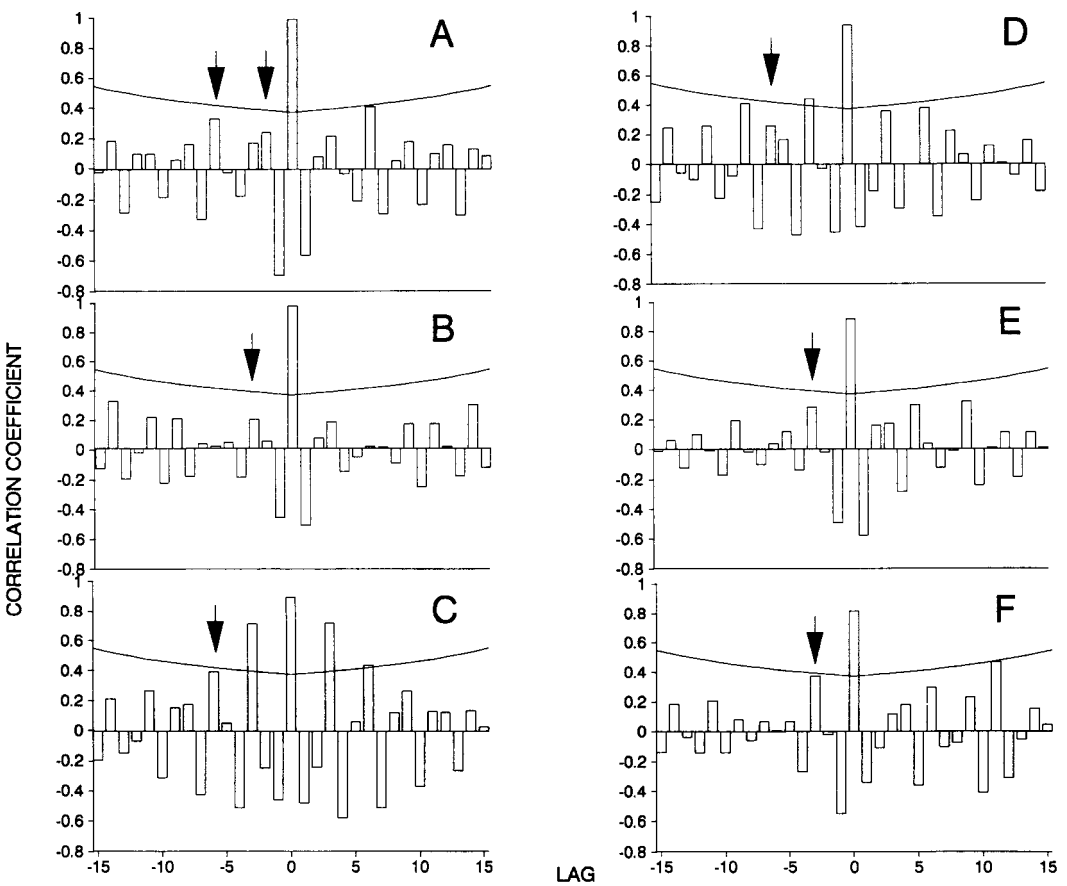

Fig. 4. Correlation coefficients vs. lag for each trap at the northern California study site. Curved lines represent the 5% level of significance. Arrows indicate possibly significant peaks. A–F represent traps 1–6.

Southern California: Trap 1 caught the most *C. variipennis*, followed by trap 2 then trap 3 (Fig. 5A–C). The mean parity rates for the individual traps ranged from 0.172 to 0.718 with an overall parity rate of 0.328 calculated for all traps. Five-day running mean parity profiles differed among the 3 traps with no similarity in occurrence of peak parity. Total numbers and parity were inversely related in traps 1 and 2, but not in trap 3.

Survivorship was estimated for each individual (Fig. 6A–C) trap as well as pooled (Fig. 7B) traps. Filtered data revealed peaks at lag 2 (trap 1 and pooled traps), lag 3 (trap 3) and lag 5 (traps 1–3 and pooled traps); all were statistically not significant. Stage-specific survivorship estimates calculated for lag 2 and 5 for the individual and pooled traps ranged from 0.273 to 0.286 (trap 1), 0.130 to 0.174 (trap 2), 0.713 to 0.756 (trap 3) and 0.312 to 0.338 (pooled traps). Similarly calculated daily survivorship estimates ranged from 0.522 to 0.778 (trap 1), 0.36 to 0.705 (trap 2), 0.844 to 0.946 (trap 3) and 0.566 to 0.805 (pooled traps).

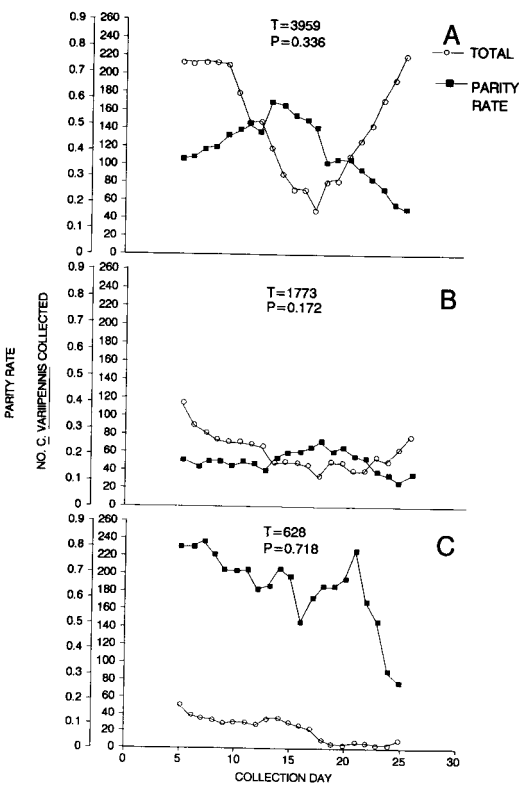

Fig. 5. Five-day running means of the total numbers per trap and running mean parity rates for each trap in southern California. A–C correspond to traps 1–3. T is the cumulative total caught in trap and P is the mean parity rate.

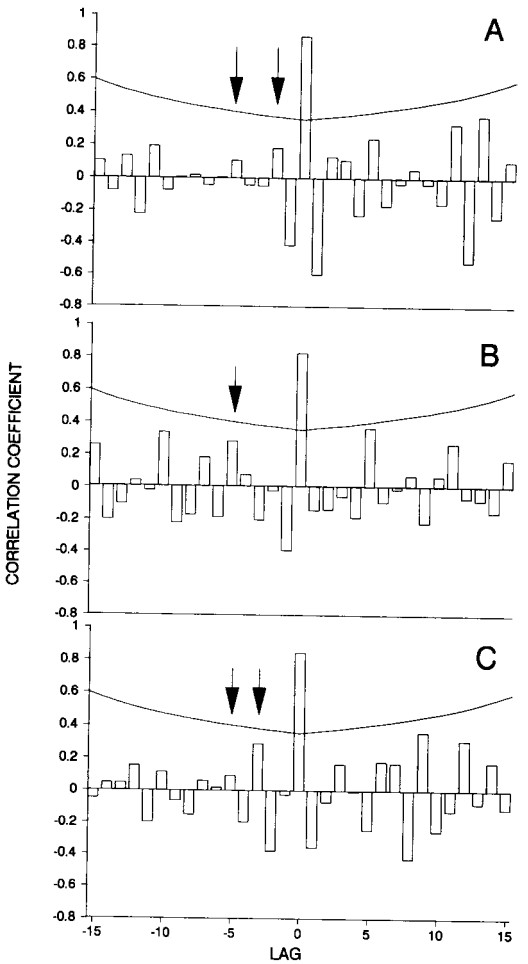

Fig. 6. Correlation coefficients vs. lag for each trap in southern California. Curved lines represent the 5% level of significance. Arrows indicate possibly significant peaks. A–C represent traps 1–3.

High and low temperatures ranged from 34.4 to 39.4°C and 12.8 to 18.3°C, respectively. Maximum and minimum RH ranged from 60 to 100% and 25 to 40%, respectively. The water level near trap 3 was decreasing until 1 August (day 15) when overflow from the western pond increased the water level fairly rapidly until about 7 August (day 21). There was a significant association between trap placement and parity rates, with a higher parity rate in insects caught near rather than away from water ($P < 0.0005$).

DISCUSSION

There were distinct differences in the results between the northern and southern California

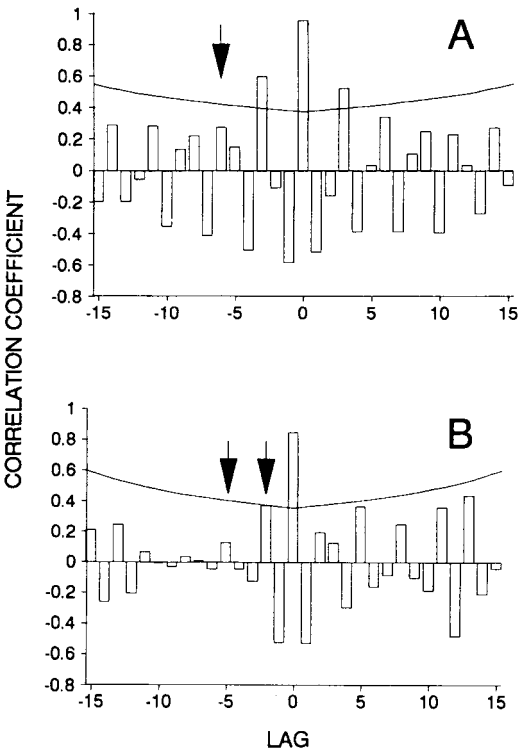

Fig. 7. Correlation coefficients for pooled traps in northern (A) and southern (B) California. Curved lines represent the 5% level of significance and arrows indicate possibly significant peaks.

sites in both the numbers and parity of *C. variipennis* trapped, estimates of gonotrophic cycle length and the survivorship. In northern California, absolute numbers caught, overall and running mean parity rates for the different traps encompassed a much narrower range than in southern California. This may have been influenced by several factors. First, the topography at the northern California site was more level and uniform, thus resulting in a more even or greater dispersal of CO_2 and a more similar range of attraction for the traps. Second, while water sources (ponds) at the northern site appeared suitable as breeding sites for *C. variipennis*, it was not known whether the ponds held larvae or were being used for oviposition. In contrast, the southern California pond was known to harbor high densities of *C. variipennis* larvae. Third, the northern California site did not have a large number of *C. variipennis* breeding sites in the immediate vicinity though the numbers of this species captured were high. The southern California site, in contrast, was in an area known to have a very high density of dairy wastewater ponds, many of which were excellent *C. variipennis* breeding sites. A high level of local dispersal and influx of individuals from other larval habitats could have masked trends at this study site.

At the southern California site, trap 3 clearly had the highest mean parity rate (0.718). This trap was located in a basin adjacent to a pond, and dispersal of CO_2 may have been restricted to that small basin. Mullens and Rutz (1984) suggested that a trap located adjacent to a pond site was biased in collecting parous *C. variipennis*. Another study in the Chino basin of Riverside Co. (Mullens 1985b) also showed a higher parity rate near a wastewater pond compared with the hills located away from it. It appears that the trap located adjacent to the pond was primarily collecting parous females that had recently oviposited and were again responding to CO_2. This interpretation is supported by the fact that the numbers of females in this trap declined rapidly with the rising water level (and presumably decreasing the attractiveness of the habitat for oviposition), which eventually resulted in the water's edge concurring in the emergent vegetation. Mullens and Rodriguez (1989) demonstrated a substantial increase in oviposition in experimental dairy wastewater ponds following reductions in the water level. The period of high water in the pond also corresponded with the lower collections in traps 1 and 2, but in both cases, collections started to decline well before the decrease in water level. As expected, the parity and numbers in traps 1 and 2 were inversely related, and a period of about 3.5–4.5 wk between emergences is reasonable for *C. variipennis* in these habitats during the summer (Mullens and Lii, 1987).

Mullens and Schmidtmann (1982) showed that ca. 3 days were required for oogenesis at a constant temperature of 22°C. A 4-day gonotrophic cycle length was assumed for *C. variipennis* by Mullens and Rutz (1984) including 1 day prior to the initiation of post-emergence host-seeking activity. Our findings of a 3-day gonotrophic cycle length occurring from host seeking to host seeking in northern California is consistent with these results. The overall parity levels at the site were slightly lower than other California studies (Nelson and Scrivani 1972, Mullens 1985b, Linhares and Anderson 1989); and coupled with a shorter estimate of gonotrophic cycle length, the daily survival estimates are somewhat less than those that have been reported previously in California (Linhares and Anderson 1989) and New York (Mullens and Rutz 1984). Possibly, different environmental conditions, availability and type of bloodmeal source as well as oviposition substrate may have been responsible for these differences.

With a 3-day estimated gonotrophic cycle in northern California, an individual female would have to survive at least 4 cycles (12 days) before being able to transmit bluetongue virus. This is based on a 10–14 day laboratory extrinsic incubation period for the virus in *C. variipennis* (Foster and Jones 1979). Using the calculated pooled-trap stage specific survival rate of 0.242, a cohort of 1,000 females would have only 3 individuals available to transmit the virus at the end of 4 cycles. Using the highest (0.288) and lowest (0.169) calculated estimate of stage specific survivorship with the same initial cohort of 1,000 insects would yield 6 and 0 individuals, respectively, available to transmit bluetongue. However, other factors not examined in this study will determine the ability of an insect to transmit a virus. Mullens and Rutz (1984) revealed that stage-specific survivorship in *C. variipennis* varies according to the parity status of the insect; our study did not differentiate between uniparous and multiparous insects. Furthermore, the extrinsic incubation period of bluetongue virus will vary according to season and local climatic conditions. Also, gonotrophic cycle length is probably not the same for all individuals within a population. The fact that over 90% of the deer in the study site have seroconverted to bluetongue while on the northern California study site demonstrates that virus transmission is occurring.

Correlations were not statistically significant in the southern California site. Data from the different traps yielded different peaks at 2, 3 and 5 days. A 2-day gonotrophic cycle (trap 1) may be too short for *C. variipennis*, even though cycle lengths of 3 or 5 days are plausible. The drastically different parity rates in the different traps resulted in equally substantial differences in the survivorship estimates. These differences were probably influenced by immigration of *C. variipennis* from nearby breeding sites and/or biases associated with trap location as noted earlier with trap 3. The nature of these dairy wastewater habitats is such that irregular perturbations, like water level fluctuations, could be expected to create a mosaic of *C. variipennis* populations with different patterns of emergence in a given area. Likewise, the more stable inverse relationship between the total numbers and parity at the southern California site suggests consistent sampling of a coherent population, at least for traps 1 and 2.

Basing gonotrophic cycle or daily survivorship estimates on individual trap collections in southern California, at least over the period of time used (25 days), is dubious. Birley and Boorman (1982) suggested pooling traps that had a high initial cross-correlation between pars and nullipars; however, in the case of the southern California site, this would be of little use. Perhaps a more reasonable basis for pooling trap collections would be to observe the similarity between the traps in the running mean parity rates as was done in northern California. Alternate methods of survivorship determination may be required, such as at the southern California site.

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