LAKE VERA REVISITED: PARITY AND SURVIVAL RATES OF ANOPHELES PUNCTIPENNIS AT THE SITE OF A MALARIA OUTBREAK IN THE SIERRA NEVADA FOOTHILLS OF CALIFORNIA

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Abstract. Parity and survival rates of Anopheles punctipennis were studied at the site of a 1952 outbreak of malaria in the Sierra Nevada foothills of California with the purpose of estimating blood feeding frequency and survivorship of such populations. Anopheles punctipennis was the dominant species in landing collections conducted for 20 consecutive nights in August and September 1990. The mean parity rate of An. punctipennis was 0.82. The gonotrophic cycle was estimated to last three days based on time series analysis of the number of nulliparous and parous mosquitoes collected each day. Survivorship was estimated to be 0.79 per gonotrophic cycle and 0.92 per day. Ovarian dilatation data indicated that some females had completed eight gonotrophic cycles prior to being collected and 45% of the females completed two or more cycles. The high prevalence of multiparous individuals, high parity rate, long survivorship and a short three-day gonotrophic cycle estimate indicates that this population is long lived and has a capacity to be an efficient vector of malaria.

In 1952, an outbreak of malaria occurred in visitors to summer camps and residences at Lake Vera in the foothills of the Sierra Nevada in California. This outbreak was remarkable in that a single infected individual spent a weekend at the lake and served as the source for 35 subsequent cases.1,2 This suggested that one or more Anopheles species at Lake Vera were highly efficient vectors. Both An. punctipennis and An. freeborni were abundant when the outbreak was investigated in late August 1952.3, 4, 5 Although An. freeborni has been considered the principle vector of Plasmodium vivax in western North America,6 An. punctipennis, a vector of minor importance,5, 6 may have played a prominent role in transmitting malaria during the 1952 outbreak.3, 4, 5

During the 1980s, additional cases of mosquito-borne autochthonous malaria occurred in California in areas where An. punctipennis was abundant and An. freeborni were few or absent and where available larval habitat was more typically associated with the larvae of An. punctipennis than An. freeborni.6, 7

In spite of the potential role of An. punctipennis as a malaria vector, little is known about its biology; particularly, factors that contribute significantly to its biological capacity to serve as a vector including adult female survivorship, abundance, blood feeding frequency, and host range.8 The objective of the current study was to study the survivorship of anophelines at Lake Vera and determine which species were using potential larval habitats and would be attracted to potential human hosts.

MATERIALS AND METHODS

Study site. Lake Vera is a small artificial lake 10 km north of Nevada City in Nevada County, California. The lake is located in a transitional forest consisting of Ponderosa pine, incense cedar, live oak, and manzanita at an elevation of 800 m on the west slope of the Sierra Nevada.2, 3, 4 Lake Vera was shallow at the time of this study and approximately 30% of the surface was covered with mats of floating algae. The lake is fed and drained by Rock Creek that is lined by typical riparian vegetation consisting of overstory willow, California Bay, and young sycamore, while the banks of the creek are covered by blackberry vines. No mats of algae or emergent vegetation were found in deeply shaded portions of Rock Creek. Residence facilities for a major summer camp are found on the north, east, and south sides of the lake, and seasonally used recreational cabins are found on the west side. Two horses were present in a corral at the north side of the lake, providing a potential blood meal source.

Collections. Collections of anopheline larvae were made from Lake Vera and Rock Creek from August 23 to November 6, 1990. Standard dippers were used to collect the larvae. The larvae were reared to adult stage for identification of species because An. punctipennis and An. freeborni cannot be reliably distinguished as larvae.9

Adult mosquito landing collections were made using three collectors for 20 consecutive evenings from August 25 to September 13, 1990. Collections were made approximately 50 m from the northwest edge of the lake, from sunset to 1 hr after sunset. A previous study had indicated that peak host seeking activity of An. punctipennis occurred shortly after sunset.9 Mosquito collections were also attempted from natural and artificial resting sites and with CO₂-baited Centers for Disease Control and Prevention (Atlanta, GA) traps but were unsuccessful and were discontinued.

Mosquitoes from the landing collections were dissected to determine their physiological state and gonotrophic age and classified as nulliparous or parous based on the condition of ovarian trichoeoles.10 In 328 individuals, the trichoeoles of one ovary were examined and the other ovary was stained, dissected, and number of dilatations on the pedicle of the ovarioles was counted.11 The maximum number of dilatations on three or more ovarioles were assumed to indicate the number of gonotrophic cycles completed.

Parity rates were based on the number of parous and nulliparous and total number of mosquitoes in the collections. The number of nulliparous females collected per day was regressed over time to determine whether the rate of recruitment of nulliparous mosquitoes remained constant over the study period.12

The numbers of nulliparous, parous, and total mosquitoes collected per day were analyzed using a time series model...
TABLE 1
Number of female mosquitoes in evening landing collections at Lake Vera, Nevada County, California, August 25 to September 13, 1990

<table>
<thead>
<tr>
<th>Mosquito species</th>
<th>Number collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anopheles punctipennis</td>
<td>693</td>
</tr>
<tr>
<td>Culiseta incidens</td>
<td>20</td>
</tr>
<tr>
<td>Culiseta particeps</td>
<td>18</td>
</tr>
<tr>
<td>Anopheles freeborni</td>
<td>3</td>
</tr>
<tr>
<td>Aedes sierrensis</td>
<td>3</td>
</tr>
<tr>
<td>Aedes increpitus</td>
<td>1</td>
</tr>
</tbody>
</table>

FIGURE 1. Number and parity rates of Anopheles punctipennis from human landing collections made at Lake Vera, Nevada County, California, August 25 to September 13, 1990.

to estimate the duration of the gonotrophic cycle with the raw data filtered using an autoregressive iterative moving average model to eliminate spurious yet seemingly significant cross-correlations. The three filtered time series were then cross correlated at time lags of zero to eight days. Cross-correlations were considered to be statistically significant if the correlation coefficient exceeded $\pm 2/\sqrt{n}$. Statistically significant cross correlation coefficients between time series with the same time lag were considered to indicate the duration of the gonotrophic cycle.

Survivorship per gonotrophic cycle was estimated from the ratio of the total number of parous mosquitoes collected to the total number of mosquitoes collected one gonotrophic cycle previously. The daily survivorship probability was estimated using the method of Davidson.

RESULTS

Of the 3,106 larvae collected, 705 (22.8%) were reared and identified as adults; 696 An. punctipennis and nine An. franciscanus. Larvae were collected both from algal mats in the lake and from the edges of Rock Creek below the lake. First instar larvae were collected from Lake Vera on November 6, indicating that gonotrophically active females were present at Lake Vera this late in the year.

The number of adult female mosquitoes of six species collected are presented in Table 1. Anopheles punctipennis was the most abundant species in 20 consecutive landing collections made from August 25 to September 13, 1990 (Table 1) with only three adult An. freeborni collected during the study period.

Some An. punctipennis from the landing collections were somewhat atypical in appearance, having reduced numbers of white scales on the costa. These individuals were, however, subsequently confirmed to be An. punctipennis in an isozyme electrophoretic study.

The number of An. punctipennis females collected ranged from 16 to 72 per day and the daily parity rates ranged from 0.74 to 0.91 per day (Figure 1). The average parity rate was 0.82.

Of individuals in which both tracheal skein and ovarian dilatation methods were used, 326 (99%) of 328 were classified as parous or nulliparous using both methods, indicating a high degree of consistency between the two methods. Multiple dilatations were observed in the ovaries of 46% of the dissected mosquitoes. Individual females had as many as eight dilatations per ovariole indicating completion of eight gonotrophic cycles prior to collection. The number of females in each age class is summarized in Figure 2.

There was a significant downward trend for the number of nulliparous females collected per day over the study period with the slope $b$ being significantly different from 0 (Y = 8.98 - 0.327X, $R^2 = 0.328$, b; $t = -2.96$, degrees of freedom [df] = 19, $P = 0.008$), but no significant trends were observed for the number of parous females and total females collected per day (parous: Y = 33.8 - 0.449X, $R^2 = 0.004$, b; $t = -1.04$, df = 19, $P = 0.312$; total: Y = 43.0 - 0.789X, $R^2 = 0.08$, b; $t = -1.63$, df = 19, $P = 0.12$). These results indicated that the rate of recruitment of nulliparous host seeking mosquitoes was not constant and that population parity rate estimates could not be used to establish a vertical estimate of survivorship per gonotrophic cycle.

Statistically significant correlation coefficients were observed between the number of parous females collected per day (time series parous) and the number of nulliparous females collected per day (time series nulliparous), and between the total number of females collected per day (time series total) and the nulliparous time series with time lags
AN. PUNCTIPENNIS PARITY AND SURVIVAL RATE IN CALIFORNIA

FIGURE 2. Parity class and number of ovarian dilatations in *Anopheles punctipennis* from human landing collections made at Lake Vera, Nevada County, California, August 25 to September 13, 1990.

![Figure 2](image)

FIGURE 3. Cross-correlation coefficients for time lags of 0–7 days between the time series for the number of parous, number of nulliparous and the total number of *Anopheles punctipennis* females collected per day in human landing collections made at Lake Vera, Nevada County, California, August 25 to September 13, 1990. The arrow indicates significant cross-correlation coefficients at a time lag of three days between the time series for the number of nulliparous and parous, and the number of nulliparous and total number of *An. punctipennis* females collected each day.

![Figure 3](image)

of three days (Figure 3). The highest cross-correlation coefficient between time series parous and time series total was observed with a time lag of three days, though this coefficient was not statistically significant. These results suggest that the duration of the gonotrophic cycle was approximately three days.14

Based on the duration of the gonotrophic cycle being three days, survivorship per gonotrophic cycle was estimated to be 0.78. Daily survivorship was estimated to be 0.92.

DISCUSSION

The absence of *An. freeborni* in the larval collections and low numbers in the landing collections indicate a low population during the study period. This finding contrasts with the earlier reports of high *An. freeborni* abundance in late August 1952, following the malaria outbreak.3,4 The reason for the absence of *An. freeborni* in the present study is not known.

The presence of first instar *An. punctipennis* larvae in Lake Vera in early November indicated that gonotrophically active females were present at the lake this late in the year. This is consistent with the findings of Washino and Bailey18 who collected *An. punctipennis* larvae in mid November.

The high parity rate, survivorship, and prevalence of multiparous mosquitoes in this study suggests that the *An. punctipennis* population at Lake Vera is long lived.

The 0.82 parity rate estimate from the current study was considerably higher than the 0.19–0.35 parity rate estimates rates reported for *An. punctipennis* collected in landing collections in the San Joaquin Valley and adjacent lower foothill areas of California.9 While the parity rate could not be used directly as a vertical estimate of survivorship, the large difference in parity rates suggests that the *An. punctipennis*
population at Lake Vera may have been substantially longer lived than in the San Joaquin Valley and the adjacent foothill area.

The survivorship estimate of 0.92 per day is considerably higher than the estimates of 0.74–0.76 and 0.84–0.90 obtained for An. freeborni and Aedes melanimon in the Sacramento Valley of California, respectively, using a similar time series analysis,19,20 and comparable with the 0.88–0.91 estimates for Aedes communis obtained using mark-release-recapture methods at high elevation in the Sierra Nevada.21

The three-day gonotrophic cycle estimate for An. punctipennis at Lake Vera is shorter than those reported for other North American Anopheles mosquitoes and California mosquitoes. The gonotrophic cycle of An. freeborni in the Sacramento Valley, An. quadrimaculatus sensu lato in Maryland, and An. quadrimaculatus species C1 in Florida were reported to be 4–6, 4–5, and five days, respectively.18,19,22 In California, the gonotrophic cycle of Ae. melaninon, Ae. dorsalis, and Culex tarsalis were reported to be five days during the summer.18,19,24

The case reproductive rate of 35 in the 1952 outbreak suggests that the source was fed on by very high numbers of anophelines, a small percentage of which survived long enough to transmit P. vivax malaria or that the mosquito population was extremely efficient at transmitting malaria. The high survivorship estimate from the present study suggests that more than 43% of infected An. punctipennis would survive a 10-day extrinsic incubation period26 and be capable of transmission. Likewise, a three-day gonotrophic cycle would result in individuals being capable of transmitting malaria to several individuals within a relatively short period. The percent of females surviving long enough to take one, two, three, and four potentially infectious bites would be 37%, 29%, 22%, and 17%, respectively. This is consistent with the Lake Vera An. punctipennis population being very efficient at transmitting malaria.

Acknowledgments: We thank the Sacramento-Yolo County Camp Fire Council for allowing us access to Camp Minaluma. We also thank Ann Donatelli, Mike Gurney, and Peter Redowski for assisting in the collections and Emma Elliott for collecting and technical assistance.

Financial support: This research was supported in part from USDA Research Grant CR 806771-02, RF-4148A, and by funds from the University wide Mosquito Research Program of the University of California.

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