POPULATION SIZE AND SURVIVORSHIP OF ADULT Aedes triseriatus IN A SCRAP TIREYARD IN NORTHERN INDIANA

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ABSTRACT. Population size and probability of daily survival of adult Aedes triseriatus were measured in a 0.03 ha section of scrap tireyard in South Bend, Indiana, using single-release, multiple-recapture methods. Average population size estimates ranged from 1,349 to 4,492 females (999 males in one experiment), demonstrating that population densities of Ae. triseriatus can reach levels in tireyards far exceeding densities in woodlots. The probability of daily survival was equivalent between males and females (0.78-0.82). When laboratory-reared small and large mosquitoes were released, small mosquitoes of both sexes were recaptured at lower rates than large mosquitoes, suggesting that experimental populations of small and large mosquitoes behave differently once released into the field, but that mark-recapture methods may not be sensitive enough to detect differences in survivorship.

INTRODUCTION

Discarded tires provide habitat for some larval mosquitoes (Beier et al. 1983), including Aedes triseriatus (Say), a major vector of La Crosse encephalitis (LAC) virus in the midwestern USA. This virus has been isolated from larvae recovered from tires (Watts et al. 1974) and epidemiological studies link sites of documented human cases of LAC to proximity to discarded tires (Hedberg et al. 1985). Approximately 200,000,000 tires are discarded annually in the U.S. (Deese et al. 1981), and thousands of tires are routinely imported into the U.S. from other countries for reprocessing (Reiter and Sprenger 1987). Often, the discarded tires stockpiled at auto salvage yards and other areas create the potential for the development of enormous mosquito populations.

Presumably, scrap tireyards have much larger Ae. triseriatus populations than woodlots because of the greater concentration of larval breeding sites (tires vs. tree holes) in the former, yet estimates of the population size of adult Ae. triseriatus in tireyards are lacking. In contrast, there are extensive data on population density estimates for Ae. triseriatus adults in woodlots, ranging from 126 to 294 females and 279 males/ha (Sinsko and Craig 1979, Haramis and Foster 1983, Walker et al. 1987).

Several recent publications document variation in body size of adult mosquitoes (Fish 1985) and suggest that smaller individuals have reduced survival and blood feeding success, and decreased parous rates (Haramis 1985, Hawley 1985, Nasci 1986a, 1986b, 1988). Although Haramis (1983) reported a correlation between parous rate and body size in a tireyard population of Ae. triseriatus in northern Indiana, Landry et al. (1988) found no such relationship in a southern Wisconsin woodland population. Lower parous rates of small mosquitoes may imply that these individuals have decreased survivorship because parous rate is correlated with mean age in a population (Davidson 1954). Mori (1979) and Walker et al. (1987) attempted to examine the relationship between probability of daily survivorship and body size in adult mosquitoes using mark-recapture experiments, but their results were equivocal.

In the present study, mark-recapture experiments were conducted with Ae. triseriatus to estimate their population size and probability of daily survival in a scrap tireyard in northern Indiana, and to measure the influence of body size on probability of daily survival.

MATERIALS AND METHODS

Study site: The study site was a commercial auto salvage yard in South Bend, St. Joseph County, Indiana, where thousands of discarded tires are stockpiled. This yard has been the site of previous studies on Ae. triseriatus (e.g., Beier et al., 1983, Berry and Craig 1984). All experiments were conducted in an entirely shaded 0.03 ha section at the northern boundary of the yard during the summers of 1984 and 1985. This boundary was formed by a large concentration of tires and a natural hardwoods (ca. 10 ha) of mainly sugar maple (Acer saccharum Marsh), black oak (Quercus velutina Lam.) and American beech (Fagus grandifolia Ehrh.).

Mark-recapture of wild mosquitoes: On June 30 and July 2, 1984, Ae. triseriatus females were collected at the site with a large, battery-powered aspirator (Nasci 1981) and brought to the laboratory, where they were counted and transferred to holding cages, provided with honey- and water-soaked pledgets, and maintained at 21°C, 16:8 L:D and 70-80% RH. Mosquitoes (726, omitting dead and weak individuals) were marked on July 2 with a colored fluorescent dust (Day Glo) following the methods of Sinsko and

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Craig (1979), held overnight and released on July 3 at a single location between the tires forming the border and a pile of ca. 100 tires situated 3 m north of the border. Mosquitoes were recaptured from vegetation or as they flew about the collector with the large aspirator on four consecutive days, July 4–7, from each of four compass points (15 minutes per point) at the edge of this tire pile. Captured mosquitoes were kept alive in collecting bags, returned to the laboratory, killed by freezing, sorted at low magnification under long-wave ultraviolet light to distinguish recaptures from wild-caught individuals and counted.

Mark-recapture of laboratory strain—adult size unregulated: In June, 1985, Ae. triseriatus (WALTON strain, in colony since 1969, originating from South Bend, Indiana) mosquitoes were reared in the laboratory at the University of Notre Dame according to the methods of Munstermann and Wasmuth (1985). The larval diet consisted of beef liver powder suspended in water and delivered ad lib. Male and female pupae were separated, counted, allowed to emerge inside cages and maintained as before. Mosquitoes were marked with colored fluorescent dust on July 11, held overnight and released on July 12. Mosquitoes were recaptured as before on days 3, 4, 5, 7, 10, 11, 14, 18 and 20 postrelease, treated and examined as previously described and additionally were grouped according to sex.

Mark-recapture of laboratory strain—small and large adults: In this experiment Ae. triseriatus (WALTON strain) were reared in July and August, 1985, according to the “1/2X” (underfed) or “2X” (well-fed) diet protocols of McCombs (1980)\(^3\), in order to generate small and large size classes of adults. Larvae were reared at 21°C in pans of water (100 larvae in 1 liter) and were fed a liver powder suspension (4 g/liter) every other day. Underfed larvae were given 3, 4, 5, 7, 7 and 7 ml of food at consecutive feedings; whereas well-fed larvae were given 12, 16, 20, 28 and 28 ml of food at consecutive feedings. It was necessary to provide the former group one additional feeding to boost adult production and shorten development time. Pupae were separated by sex, counted and adults allowed to emerge into cages. Adults were marked with group-specific colored fluorescent dusts as before and released by the tire pile on August 16, 1985. The released mosquitoes numbered: underfed (1–13 days old postemergence), 1,461 females and 2,194 males; well-fed (1–9 days old), 716 females and 765 males. Recaptures were made on days 3, 4, 5, 6, 7, 10 and 15 postrelease, and collections were processed as before.

In order to characterize the relative size of underfed and well-fed mosquitoes, the wings of 20 mosquitoes of each sex from each group were measured from the beginning of the costal scales to the end of the wing, excluding the fringe, with an ocular micrometer at 20×. The average wing lengths (SEM) of the experimental groups (n = 20) were: underfed males, 2.73 (0.03) mm; underfed females, 3.21 (0.05) mm; well-fed males, 3.32 (0.02) mm; and well-fed females, 4.19 (0.04) mm.

To test whether marking affected survival, 50 mosquitoes of each sex from both size classes were either marked with fluorescent dust or left unmarked, placed into plastic cages, provided honey and water, and held at 21°C and 70–80% RH. The number alive and dead after 30 days was recorded. There were no significant differences between marked and unmarked males or females, within size groups, still alive after 30 days (2 × 2 contingency tables, \(P > 0.05\)).

Data analysis: The probability of daily survival was calculated from recapture data by regressing the number recaptured, transformed by \(\log_{10} (x + 1)\), on the day of recapture; the antiloglo of the slope of the regression line is the estimated probability (Charlwood et al. 1986, Walker et al. 1987). The regression coefficient was tested for significance under the null hypothesis that it was not less than zero (i.e., one-tailed test). Population size was estimated for each day of recapture with the Lincoln index, modified for low recapture rate and compensated for mortality (Service 1976) as \(P = (as'(n - r + 1))/((r + 1))\), where \(P = \text{estimated population density on day } t\), \(a = \text{number of mosquitoes released}\), \(s = \text{estimated probability of daily survival}\), \(t = \text{sampling day postrelease}\), \(n = \text{total number captured (marked and wild) on day } t\) and \(r = \text{number of marked individuals recaptured on day } t\). Recapture rates for small and large mosquitoes were compared with 2 × 2 contingency tables.

**RESULTS**

Mark-recapture of wild mosquitoes: The overall recapture rate was 13.9% (101 recaptured/726 females released); 1,109 unmarked mosquitoes were collected. The slope (–0.1059) of the regression line was significantly less than 0 (\(r = -0.94, P < 0.05\)) and yielded a probability of daily survival of 0.78 (Fig. 1). The average population size estimate (females only) for the four successive recapture days was 4,492 (Table 1).

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Fig. 1. Results of regression of log_{10} (number recaptured + 1) on day postrelease for marked, wild *Aedes triseriatus* females released on July 3, 1984, into an Indiana tireyard.

Table 1. Number of marked *Aedes triseriatus* females recaptured (726 released on July 3, 1984) and corresponding population estimates in an Indiana tireyard.

<table>
<thead>
<tr>
<th>Days post-release</th>
<th>Mosquitoes captured</th>
<th>Lincoln Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Marked</td>
<td>Wild</td>
</tr>
<tr>
<td>1</td>
<td>39</td>
<td>295</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>289</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>200</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>325</td>
</tr>
</tbody>
</table>

Mark-recapture of laboratory strain-adult size regulated: The overall recapture rate for males was 11.1% (233 recaptured/2,095 released) and for females was 16.4% (248 recaptured/1,510 released). Five-hundred unmarked males and 685 unmarked females were collected.

The slope (-0.1045) of the regression line for male recaptures was significantly less than 0 (r = -0.94, P < 0.01) and yielded a probability of daily survival of 0.79 (Fig. 2A); the slope (-0.0834) of the regression line for female recaptures was significantly less than 0 (r = -0.91, P < 0.05) and yielded a probability of daily survival of 0.82 (Fig. 2B). The average population size estimate for males was 999 (8 recapture days, excluding day 18 when no recaptures were made), and for females was 1,349 (9 recapture days) (Table 2).

Mark-recapture of laboratory strain-small and large mosquitoes: Large females were recaptured at a significantly higher rate (38/716, 5.3%) than were small females (15/1,416, 1.0%) (X^2 = 34.82, P < 0.005). Large males were also recaptured at a significantly higher rate (21/765, 2.8%) than were small males (18/2,194, 0.8%) (X^2 = 15.59, P < 0.005). The recapture regression equations were: small males, Y = 0.9119 - 0.0697X (r = -0.52, P > 0.05); small females, Y = 0.9971 - 0.1015X (r = -0.66, P > 0.05); large males, Y = 1.1494 - 0.0977X (r = -0.89, P < 0.05); large females, Y = 1.1083 - 0.0692X (r = -0.71, P < 0.05). Because the slopes to the regression lines for small males and females were not significantly less than 0, they could not be compared by t-test with the slopes from the large-sized groups' regression lines to determine whether probability of daily survival differed between the experimental populations. Therefore, recapture data of large and small mosquitoes were combined to calculate probability of daily survival (Figs. 3A and B). The slope for males, -0.0962, was significantly less than 0 (r = -0.74, P < 0.05) and yielded a probability of daily survival of 0.80. The slope for females, -0.0781, was significantly less than 0 (r = -0.72, P < 0.05) and yielded a probability of daily survival of 0.84.

Population size estimates were calculated based upon recaptures of males and females
Table 2. Number of laboratory-reared, marked *Aedes triseriatus* recaptured (2,095 males and 1,510 females released on July 12, 1985) with corresponding population size estimates.

| Mosquitoes captured | Males | Females | | | |
|---------------------|-------|---------|-------|-------|
| Days post-release   | Marked | Wild | Total | Lincoln Index | Marked | Wild | Total | Lincoln Index |
| 3                   | 127    | 23    | 150   | 191     | 112    | 37    | 149   | 285     |
| 4                   | 42     | 42    | 84    | 800     | 77     | 46    | 123   | 422     |
| 5                   | 20     | 35    | 55    | 1,078   | 13     | 41    | 54    | 1,754   |
| 6                   | 16     | 79    | 96    | 1,727   | 15     | 87    | 102   | 2,166   |
| 7                   | 16     | 151   | 167   | 1,688   | 15     | 179   | 194   | 2,490   |
| 8                   | 4      | 49    | 55    | 1,484   | 8      | 39    | 47    | 812     |
| 9                   | 6      | 67    | 73    | 700     | 4      | 107   | 111   | 2,218   |
| 10                  | 1      | 17    | 17    | 323     | 2      | 74    | 76    | 1,191   |
| 20                  | 1      | 37    | 38    |         | 2      | 75    | 77    | 822     |

**DISCUSSION**

Assumptions of reliability of Lincoln index:

Several assumptions underlie single-release, multiple-recapture experiments (Southwood 1978, Blower et al. 1981). We assumed that: 1) marking does not affect behavior or longevity; 2) marks are permanent; 3) marked individuals mix randomly with unmarked individuals, and sampling is random with respect to marked and unmarked individuals; 4) sampling intervals are short relative to longevity of marked individuals; and 5) gain and loss to the population owing to recruitment, emigration, or death must be minimal or accountable. In this study, assumptions 1–4 above were probably well satisfied. However, assumption 5 is difficult to meet as it cannot be controlled by the experimenter, and its violation may greatly affect Lincoln index estimates.

Our estimates of population size of *Ae. triseriatus* in the scrap tireyard varied among years, among experiments and within experiments among recapture days. The female population size estimate from 1984 (wild mosquitoes) is probably very reliable because the recapture rate was high and population size estimates among the four consecutive days of recapture were similar, well within an order of magnitude of each other (Table 1). Similarly, estimates from early 1985 (laboratory mosquitoes with ad lib. larval diet) are probably also reliable because the recapture rates for males and females were high. However, the estimates among successive recapture days varied by almost an order of magnitude for both sexes (Table 2). This may have resulted from higher variation in the number of unmarked mosquitoes caught compared with 1984, suggesting that assumption 5 did not hold well for the first experiment of 1985. Further, the recapture duration was longer than in 1984, and consequently the dynamics of the wild popula-
Table 3. Number of laboratory-reared, marked *Aedes triseriatus* recaptured (2,959 males and 2,132 females released on August 16, 1985) with corresponding population size estimates.

<table>
<thead>
<tr>
<th>Days post-release</th>
<th>Marked</th>
<th>Wild</th>
<th>Total</th>
<th>Lincoln Index</th>
<th>Marked</th>
<th>Wild</th>
<th>Total</th>
<th>Lincoln Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
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<td>78</td>
<td>95</td>
<td>6,687</td>
<td>30</td>
<td>120</td>
<td>150</td>
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<tr>
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<td>8</td>
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<td>54</td>
<td>6,377</td>
<td>12</td>
<td>120</td>
<td>132</td>
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<td>133</td>
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<td>19,783</td>
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<tr>
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<td>5</td>
<td>32</td>
<td>37</td>
<td>4,314</td>
<td>1</td>
<td>63</td>
<td>64</td>
<td>23,680</td>
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<tr>
<td>7</td>
<td>1</td>
<td>14</td>
<td>15</td>
<td>4,716</td>
<td>2</td>
<td>29</td>
<td>31</td>
<td>6,182</td>
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<tr>
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<td>67</td>
<td>4,954</td>
<td>1</td>
<td>103</td>
<td>104</td>
<td>7,626</td>
</tr>
</tbody>
</table>

...
Aedes triseriatus have a higher probability of daily survival than do small Aedes triseriatus because of low recapture rates in the small-sized group and high variation in their recapture. Recapture rates were significantly lower for the small individuals, as Mori (1979) and Walker et al. (1987) found, suggesting that experimental populations of small and large mosquitoes behave differently. Either a large number of the small individuals rapidly died off or dispersed quickly away from the study site. These two possible explanations are not separable with the present experimental design; if body size does affect probability of daily survival of adult mosquitoes, then mark-recapture experiments may not be technically sensitive enough to measure the effect.

The relationship between body size and survivorship of mosquitoes is controversial because various studies have drawn different conclusions based on different approaches (Landry et al. 1988). Frequently, parous rate has been used as an index of survival for constructed size-classes of mosquitoes (Haramis 1983; Hawley 1985; Nasci 1986a, b, 1988; Landry et al. 1988). However, the correlation of parous rate and longevity may not be good when the oviposition interval is long and variable. Walker et al. (1987) reported Ae. triseriatus to have an average life expectancy of 11.6 days, and the time from first blood meal to first oviposition ranged from 8 to 17 days. Consequently, at least half of the Ae. triseriatus females in that study died before ovipositing. In such a situation, the nulliparous and parous fractions of the population would contain a wide range of young to old mosquitoes, and the age distributions of the nulliparous and parous fractions would overlap considerably. In field populations of Ae. triseriatus, nulliparity persists during the season (Scholl et al. 1979). This suggests a net trend of recruitment of nulliparous which makes parous rate an underestimator of survival rate (Holmes and Birley 1987). Regression, time series and cross-correlation methods (Holmes and Birley 1987) may provide the resolution required to relate directly parous rate of different size cohorts to probability of daily survival.

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