OPERATIONAL AND SCIENTIFIC NOTES

A METHOD TO ESTIMATE POPULATIONS OF MOSQUITO LARVAE IN SHALLOW WATER[1]

CYRUS R. LESSER

Maryland Dept. of Agriculture, Pest Management Section, Rt. # 5, Box 281, Salisbury, Maryland 21801

In the course of a study to evaluate the effectiveness of marsh management techniques in reducing larval populations of *Aedes sollicitans* (Walker) in Delaware and Maryland salt marshes, a quantitative sampling technique was developed and is reported here. This technique combines the methodology of an area sampler and removal sampling and, while both methods are well established (Service 1976), it appears that their combination has not been reported before.

Area samplers are means of estimating density, and they provide a relatively easy way to obtain quantitative data. To be effective, most area samplers must either entrap all larvae enclosed within the sampler (Roberts and Scanlon, 1974), or provide a means of removing all confined larvae by siphoning or bailing. The main disadvantage of most area samplers is the relatively long time required per sample in the field: 15 to 20 minutes reported by Roberts and Scanlon (1974). The time factor becomes critical when many samples must be taken in the short length of time available for summer broods of *Aedes* or *Psorophora* spp. to demonstrate statistically accurate results. I found that by combining an area sampler and a removal sampling method, the length of time needed per sample in the field was greatly reduced, thereby permitting the collection of more samples.

The rationale of removal sampling is that by removing a known number of individuals from a static population, a decrease in the number caught in successive dips will result due to the decline in that population. Assuming all larvae have an equal chance of being captured, the number captured is proportional to the number present. Therefore, as successive catches are made more larvae are removed, thereby decreasing the catch in each successive dip, and eventually reducing it to zero. Any great deviation from this assumption—i.e. if successive catches increase instead of decrease—will invalidate this method for estimating the population.

Several methods are available to derive population estimates using the removal sampling technique. They vary in accuracy and complexity of calculation. The method which I found to be the most efficient in producing accurate estimates is the one proposed by Hayne (1949). It is one of the older methods and the simplest to calculate. Hayne (1949) suggested that the periodic catch (catch in dip 1, 2, 3 . . .) be plotted against the cumulative catch for all dips prior to the last (total catch for all dips up to and including the (n-1)th dip) on normal graph paper. The periodic catch is plotted on the Y axis and the cumulative catch is plotted on the X axis. The co-ordinate points are plotted on the graph and a line is fitted to the plotted data by eye. The population estimate is the point at which the line intercepts the abscissa (X axis).

The following data, obtained from a field population of *Ae. sollicitans*, were treated according to Hayne (1949). Five dips were taken from an area sampler enclosing 0.018 m^2 of water surface and the following counts were made: dip 1 = 21 larvae; dip 2 = 17 larvae; dip 3 = 9 larvae; dip 4 = 7 larvae; dip 5 = 2 larvae. These data were arranged as shown in Table 1. The coordinate points were plotted, a line fitted by eye to the plotted points, and the population estimate derived as shown in Fig. 1.

A more accurate way to plot the regression line of X on Y is to calculate a least squares regression line by solving the linear regression equation: $Y = a + bX$; where Y is the catch in the nth dip, a is the point of interception on the Y axis when $X = 0$, b is the slope (which is also the numerical probability of capture), and again X is the cumulative catch for all dips till the

Table 1. Format used to arrange field data in estimating population size by the removal sampling method.

Dip. No.	Y periodic catch for the nth dip	X cumulative catch for all dips till the (n–1)th dip
1	21	0
2	17	21
3	9	38
4	7	47
5	2	54

[1]Maryland Dept. of Agriculture Contribution No. 10.

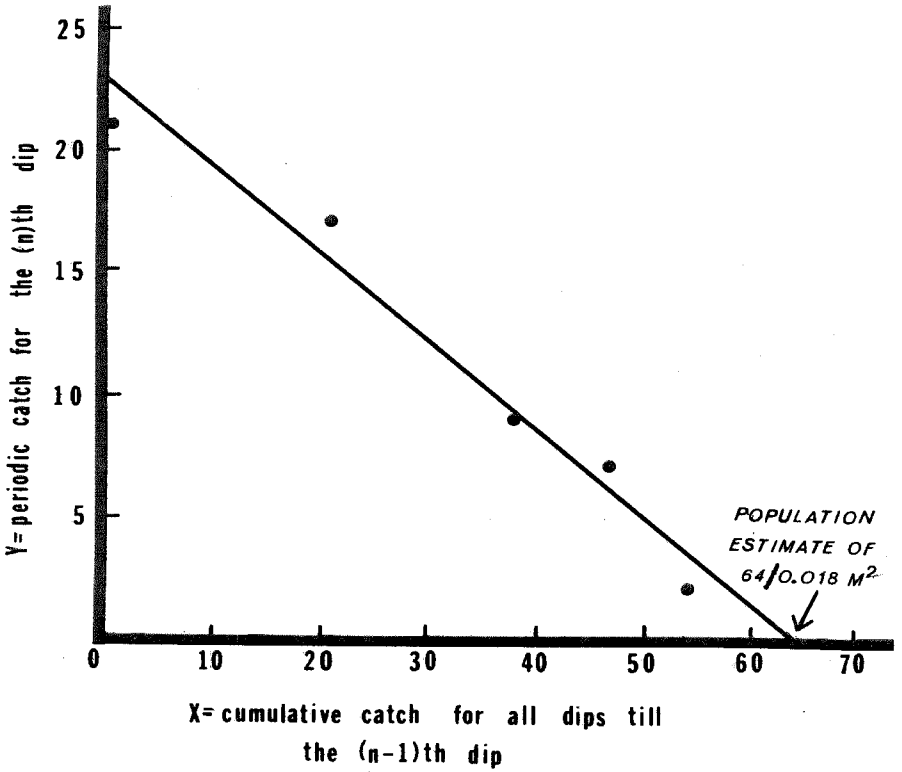

Fig. 1. Graphical method of estimating population size by the removal method.

(n-1)th dip. However, in most instances a line plotted by eye gives a population estimate in close agreement with the estimate given by a calculated line.

The technique of combining an area sampler and the removal sampling method (Hayne, 1949) was evaluated by introducing known numbers of *Ae. sollicitans* and *Culex pipiens* L. larvae into area samplers and calculating the populations within the quadrats. By subjecting 5 known populations of *Ae. sollicitans* to this method, an average accuracy of 96% was obtained in estimating the populations. In a 2nd series of tests using 5 known populations of *Cx. pipiens*, an average accuracy of 93% was obtained. These tests were conducted under laboratory conditions in 4 in. of water and results may vary with field populations. However, the results indicate that the method is accurate.

The equipment needed for this technique is simple, inexpensive, and readily available. It consists of: (1) a metal cylinder of sturdy construction which will enclose a known surface area of water (an empty 3 lb. coffee can with both ends removed makes an excellent sampler in water that is 4 inches deep or less), (2) a white enamel dipper or other device for dipping larvae from within the metal cylinder, and (3) normal graph paper with 10 lines to the inch. In practice, the cylinder is set in a breeding site at random by quickly thrusting it into the bottom and firmly seating it to insure that no larvae escape or enter the quadrat. If insufficient water is contained within the quadrat for dipping, additional water can be introduced by dipping from the immediate vicinity, but this

water must be carefully strained to prevent the introduction of additional larvae. After a sufficient length of time to allow the larvae to resurface (2-3 minutes for summer broods of *Ae. sollicitans*), the first dip is taken and the number of larvae dipped is recorded on a 3 × 5 in. card or other suitable paper. A total of 3 (or preferably 5) dips per sample site, with the number caught in each dip recorded, is sufficient to provide adequate data for an accurate population estimate. The field data are then used to calculate a population estimate for the sample site by the removal sampling method described above. The length of time required per sample site in the field is 5-10 minutes depending upon larval density, availability of larval escape habitat, etc. An additional 1-2 minutes is required to calculate the population for each sample site by using the method of Hayne (1949), or 3-5 minutes to calculate the regression line by the linear regression equation.

One question which frequently confronts the field investigator conducting a sampling program is the number of samples to be taken. The number of sample sites visited will depend upon area size, area of water surface providing larval habitat, time available for sampling, and the degree of precision required (as the sample size increases, results will more truly reflect the actual population, thereby increasing accuracy). When the desired number of samples has been taken and the individual population estimates for all sample sites derived, an average value for the population estimate should be calculated and expressed as the number of larvae per surface area enclosed by the area sampler. It is then desirable to calculate a coefficient of variation (C.V. = standard error × 100/ population estimate), confidence intervals, standard error, etc., as described in statistical texts such as Steel and Torrie (1960). If high values for the coefficient of variation or standard error are found, it is indicative that the sample mean is not accurately reflecting the true population mean, and the sample size should therefore be increased.

The average population estimate can be expressed in various ways. The simplest approach is to express the data in terms of density of larvae per area enclosed by the area sampler, or to multiply by the appropriate conversion factor to get larval density per square foot, square meter, etc. It is possible to derive an estimate of the larval population on a given area of marsh, woodland pool, etc. To do this, it is necessary to estimate or measure the surface area of water which provides breeding habitat and multiply by the average density figure.

To date, this technique has been used exclusively on salt marshes, where the substrate is mud and peat which facilitates rapid and firm seating of the area sampler. In a woodland pool or swamp habitat, seating the sampler may be more difficult due to the presence of sticks and leaf mats. If this type of situation is encountered, it will be necessary to have a sampler with a reinforced, sharpened lower edge, or one with saw-like teeth on the lower edge as suggested by Service (1976).

References Cited

Hayne, D. W. 1949. Two methods for estimating populations from trapping records. J. Mammalogy 30: 399–411.

Roberts, D. R. and J. E. Scanlon. 1974. An area sampler for collecting mosquito larvae in temporary woodland and field pools. Mosquito News 34: 467–468.

Service, M. W. 1976. Mosquito ecology: field sampling methods. Applied Sciences Publ., Ltd. London. 583 pp.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Inc. New York. 481 pp.

OBSERVATIONS ON LARVAL *AEDES AEGYPTI* (L.) AS SCAVENGERS

SUSAN MCIVER AND ROMAN SIEMICKI

Department of Microbiology and Parasitology FitzGerald Building, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 1A1

In our routine rearing of *Toxorhynchites brevipalpis* Theobald, individual larvae are placed in styrofoam bowls containing tap water. *Aedes aegypti* (L.) larvae are used as food and added in abundance, so that there are usually 10-20 in each bowl. On several occasions one of us (R.S.) observed that *Ae. aegypti* larvae were apparently feeding on dead *Tx. brevipalpis* larvae.

We performed the following experiment to determine (1) if the rate of disintegration of *Tx. brevipalpis* larvae, an indicator of both feeding and natural decomposition, is faster with or without *Ae. aegypti* larvae and, (2) to determine whether the *Ae. aegypti* larvae feed on dead *Tx. brevipalpis* larvae even in the presence of powdered larval food.

Fourth instar *Tx. brevipalpis* larvae were killed by freezing. Upon thawing each larva was exam-