LARVAL POPULATION ESTIMATES OF *AEDES COMMUNIS* USING GIEMSA MARKING

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ABSTRACT. A modified Lincoln index was used to estimate larval populations of *Aedes communis* in 5 woodland snow pools in the Pocono Mountains of northeastern Pennsylvania. One thousand fourth instars were removed from each pool, stained with Giemsa, and replaced within 1–3 hr. A second sample was collected within 18 hr. Population estimates ranged from 8,416 to 194,000 among the 5 pools. Regression analysis indicates that larval population size can be predicted from measurements of the surface area of the pools.

INTRODUCTION

Aedes communis (DeGeer) is a holarctic mosquito species that commonly develops in vernal ponds at higher altitudes in the northeastern USA. While this species frequently can be found in association with other univoltine *Aedes* mosquitoes, it is not unusual to find pure populations of *Ae. communis* in ponds of small to moderate size (Haufe and Burgess 1956, Iversen 1971). In the Pocono Mountains of northeastern Pennsylvania several such ponds were observed in 1968 during a study on larval succession (Wills and Fish 1973). When revisited in 1983, these same ponds were again observed to contain pure populations of *Ae. communis.*

Fourth instar *Ae. communis* form extremely dense aggregations covering an estimated 1 m^2 of water surface area (Hocking 1953). Accurate sampling to estimate larval population size at this time is difficult, if not impossible, using a standard dipping technique (Siverly and DeFoliart 1968, Service 1976). Samples collected within the aggregation will overestimate and samples missing the aggregation will underestimate the true population size (Hocking 1953). Random samples of the habitat yield a mean count with an exceedingly high variance.

Larval mosquito population estimates are necessary to assess the significance of vernal ponds as sources of biting mosquitoes and to predict the magnitude of adult populations that may affect man. Reliable larval estimates are also desirable in assessing the effectiveness of mosquito control activities.

The control of *Ae. communis* may be desirable for both medical and economic reasons. Jamestown Canyon virus (California serogroup) recently has been reported to cause serious disease in humans (Diebel et al. 1983) and in New York State it has been isolated from *Ae. communis* more frequently than from any other mosquito species (Grayson et al. 1983). In addition to its vector potential, *Ae. communis* is a serious daytime pest of many recreation areas in the mountainous regions of the northeast.

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MATERIALS AND METHODS

The Pocono Mountains of northeastern Pennsylvania form a series of elevated plateaus and shallow valleys where vernal ponds are numerous. Hickory Run State Park, Carbon County (41°02′N, 75°42′W, elev. 500 m) contains vernal ponds at densities frequently in excess of 1/ha. Five ponds of different sizes that supported pure populations of *Ae. communis* were selected for study. Synchronous development of this species resulted in a brief time period when the total population in each pond was in the last instar. At this time (April 23, 1983), 1,000+ larvae were removed from the dense aggregation occurring in each pond with a fine mesh net.

Larvae were counted in lots of exactly 1,000 at each site and placed in a 5 liter bucket of pond water for staining. Samples of excess larvae were saved for species identification. The larvae were then exposed to 100 ppm Giemsa stain (Fisher Scientific Co.) for 1–3 hr, rinsed in clear pond water and returned to their respective pools. To insure mixing of marked mosquitoes with the original population, the entire aggregation was disturbed by walking through the pool while the marked larvae were being replaced.

Approximately 18 hr after the mosquitoes were marked and returned to their respective pools, approximately 500 larvae were removed from each pool and examined for Giemsa stain. Larvae stained with Giemsa exhibit an intense blue color that is easily observed with the unaided eye when placed against a white background. The number of blue larvae recaptured from each pool was determined as was the number of unmarked larvae in the sample. Total population estimates for each pool were calculated using a modified Lincoln index (Bailey 1951), also known as the Petersen estimate (Begon 1979). This population estimate employs the formula:

$$N = r\left(\frac{n + 1}{m + 1}\right)$$

where r = the number of initially marked mosquitoes, n = the sample size, and m = the number of marked mosquitoes in the sample.

The dimensions of each pool were determined by pacing the length and width. Water depth also was determined to obtain total water volumes. Regression analysis was used to determine relationships between larval population size and the surface area and water volume of the habitat.

RESULTS AND DISCUSSION

The modified Lincoln index population estimates for *Ae. communis* larvae in the 5 study pools ranged from 8,416 to 194,000 (Table 1). To relate these population estimates to the measured physical parameters of the larval habitats, regression analysis of larval populations upon either surface area or water volume was significant (P = < .001). However, surface area was determined to be a more accurate predictor of population size (Y = 22047 + 478.7X, r = 0.96) than water volume (Y = 29191 + 679.8X, r = 0.92) (Fig. 1).

Larval populations of univoltine snow pool mosquitoes are particularly conducive to size estimates using Giemsa stain with simple ratio formulas such as Lincoln index since this method is likely to meet nearly all of the assumptions upon which the accuracy of the estimate is based. These assumptions are: 1) all marks are permanent prior to recapture, 2) marking method has no influence upon the probability of recapture, 3) marking method has no influence upon mortality, 4) marked individuals are distributed at random among unmarked, and 5) there is no recruitment of new individuals into the population (no births or immigration) (Begon 1979). Assumptions #1 and #3 are satisfied from previous studies with the Giemsa self-staining technique (Joslyn et al. 1985). When applied at the proper dosage, Giemsa stain marks all larvae, remains visible throughout the life of the mosquito, and causes no detectable mortality in the larval (or adult) stage. Since marked and unmarked larvae cannot be distinguished prior to sampling, assumption #2 is met. Random mixing of marked larvae within the larval population was assured by the methods previously described to meet assumption #4. Since *Ae. communis* is univoltine and larvae were the subjects of study, assumption #5 is automatically met.

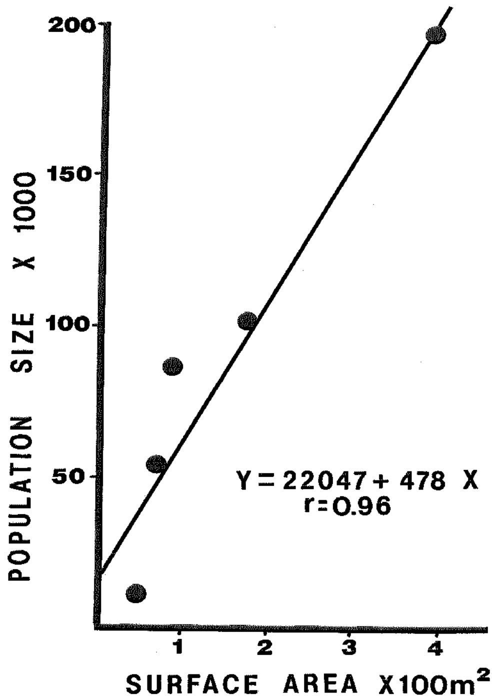

Fig. 1. Regression analysis of estimated population size of *Aedes communis* upon surface area of larval habitat.

Although marking larvae and using recapture indices to estimate larval abundance may seem tedious for routine larval surveys, such techniques may be necessary when reliable population estimates are required for mosquito species that are highly aggregated in the larval stage. In addition to *Ae. communis,* the larvae of many other mosquito species have aggregated distribution patterns, including *Ae. sollicitans*

Table 1. Lincoln index population estimates of larval *Aedes communis* and habitat parameters for 5 snow pools.

Pool number	Marked larvae	Unmarked larvae	Population estimate	Surface area (m^2)	Water volume (m^3)
1	7	398	50,750	25.1	11.3
2	60	445	8,416	55.8	16.8
3	5	501	84,500	73.7	33.3
4	1	386	194,000	156.5	142.6
5	3	387	97,750	368.3	221.8

(Walker), *Ae. taeniorhynchus* (Wied.) *and Ae. cantator* (Coq.) (Service 1976). Recapture methods are also useful for plant-associated habitats (phytotelmata) (Fish 1983) from which mosquito larvae are often difficult to extract. The Giemsa staining technique employed in this study provides an easy method of marking mosquito larvae for recapture analysis.

The relationship between larval population size and some easily measurable physical parameter of the aquatic habitat is important to establish when population estimates of mosquitoes over a large geographic area are needed. Habitat surface area is a more accurate predictor of larval population size than total water volume for *Ae. communis* (Fig. 1). This might suggest that surface area is a more important indicator of either food resources or previous egg abundance than water volume. Surface area measurements might be employed to provide estimates of an initial adult population of *Ae. communis* emerging from many small snowpools in a given area when it is not possible to estimate larval populations in each pool.

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