Estimating Biomass of Mosquito Populations[1]

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ABSTRACT   The slope of the line relating dry weight to stage of development does not vary among five species of mosquitoes belonging to all three subfamilies of Culicidae and both tribes within the subfamily Culicinae. Population or community biomass may then be estimated from a general mosquito equation adjusted for the mean weight of a given species' 4th instar.

A number of studies have considered population sizes in mosquito communities, especially those breeding in container habitats (Corbet 1964, Kitching 1971, Frank et al. 1977, Fish and Beaver 1979, Seifert 1980, Lounibos 1981, 1983). Few of these studies, however, have considered community or population biomass. Istock et al. (1976) estimated population biomass of the pitcher-plant mosquito, *Wyeomyia smithii* Coq., by measuring the total length of larvae and relating biomass to total length by a fifth-order equation. Istock's method resulted in a high degree of accuracy ($r^2 = 0.996$) but is applicable only when a means for measuring each larva is readily available and where census replacement regimes are not required or desireable. The object of the current report is to examine the relationship between biomass and stage of development among a variety of unrelated mosquitoes and propose a means for estimating biomass of populations in the field without resorting either to a microscope or to destructive sampling.

Materials and Methods

This study considers five species of mosquitoes, all container breeders, representing all three subfamilies of mosquitoes and both tribes in the Culicinae: the tree-hole mosquitoes, *Toxorhynchites rutilus* Coq. (subfamily Toxorhynchitinae), *Anopheles barberi* Coq. (subfamily Anophelinae), *Orthopodomyia signifera* Coq. (subfamily Culicinae, tribe Culicini), and *Aedes triseriatus* (Say) (subfamily Culicinae, tribe Culicini), and the pitcher-plant mosquito, *Wyeomyia smithii* (subfamily Culicinae, tribe Sabethini). Various instars of the former four species were collected from automobile tires during December 1978 at the Tall Timbers Research Station, Leon County, Fla.; larval and pupal weights of *W. smithii* were based on mosquitoes collected during July 1978 from pitcher plants near Wilma, Liberty County, Fla.; actual population biomass was based on *W. smithii* collected during September 1981 from the same locality.

Larvae and pupae were dried onto fragments of glass cover slip. All instars and pupae of *T. rutilus* were weighed individually. For all other species, 4th instars and pupae were weighed individually; but, to achieve measurable weights of younger stages, 100 1st instars, 20 2nd instars, or 5 to 10 3rd instars were pooled on a given cover slip. When larvae were pooled, a conscious effort was made to place the smallest larvae of a given instar on a single cover slip, the next smallest on another, and so on up to the largest. The intention was to maintain as much variation as possible which otherwise would be obscured by random pooling of individuals. After drying, all manipulation of cover slips was carried out with watchmaker's forceps. The larvae with cover slips were weighed to the closest 0.5 μg on Mettler or Cahn balances, the larvae were scraped off, and the cover slip fragment was tared.

Results and Discussion

Among all species, mean biomass increased exponentially with stage of development (Fig. 1). Because variances are clearly heterogeneous, no effort was made to run regressions on the raw weights. Nonetheless, several relationships are apparent. First, there is distinctly greater variation among 3rd and 4th instars than among pupae; second, the increase in log weight is approxi-
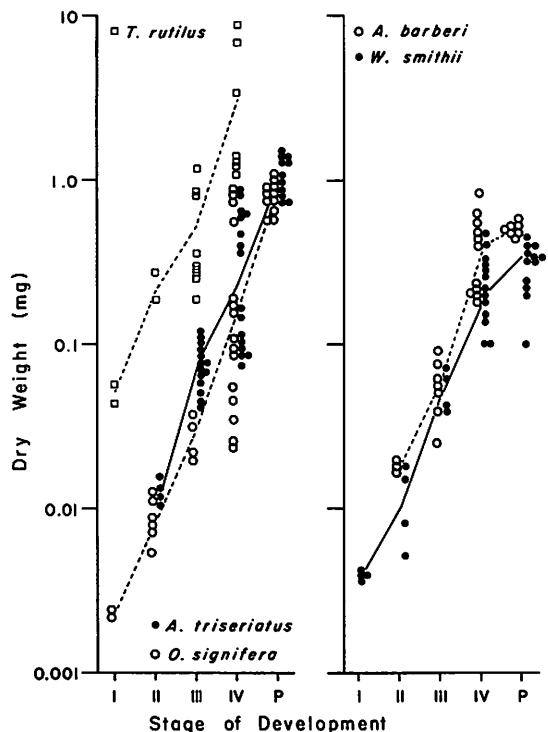

FIG. 1.   Dry weights of various stages of development of tree-hole and pitcher-plant mosquitoes collected in 1978. Lines connect means for each stage.

mately a linear function of stage; finally, there is a striking similarity in the slope of the weight-stage relationship among all species. Analysis of covariance of mean log weights and stages of development reveals no significant differences among slopes ($F_{4,12} = 3.08$; $P > 0.05$) but highly significant differences among heights of regression lines ($F_{4,16} = 56.4$; $P < 0.001$).

The greater variation among larval than pupal weights is not surprising. Since larvae were collected in the field, they likely represent individuals which have just recently molted to a given instar as well as those which have been in that instar for a longer period of time. The latter larvae would have had ample opportunity for feeding and growth.

The linear relationship between log weight and stage of development is consistent with earlier observations among a variety of insects (see pp. 54–44 in Wigglesworth [1967]). Further, the similar slopes of the weight-stage relations (Fig. 1) among a diverse representation of species does suggest a conservative relationship among the Culicidae in general. To investigate this point, I started by deriving an equation relating weight to instar for tree-hole mosquitoes; I then tested the ability of this equation to predict population biomass by using it to estimate the population biomass of independently collected samples of pitcher-plant mosquitoes. First, I adjusted the log weights of each of the tree-hole species by

$$\text{adjusted LW}_i = \text{LW}_i - \text{LW}_{\text{iv}} \qquad (1)$$

where LW_i is the log weight of the ith stage of development (pupae being scored as 5). When plotted on a common coordinate system (Fig. 2), the adjusted log weights of tree-hole mosquitoes fall on a straight line and cluster tightly together, illustrating more clearly the common slope of the weight-stage relationship. The equation for the common line,

$$\text{adjusted LW}_i = -2.54 + 0.649i \qquad (2)$$

thus represents the relationship for mosquitoes in general which is changed to that of an individual species by adding back in a species-specific constant, namely, the
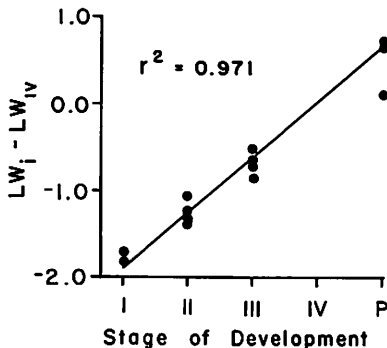
mean log weight of the 4th instar, so that for *W. smithii*, where $\text{LW}_{\text{iv}} = 2.346$,

$$\text{predicted LW}_i = -2.54 + 0.649i + 2.346 \qquad (3)$$

To assess the value of equation 3 in predicting population biomass, *W. smithii* were collected from leaves at Wilma, Fla., in September 1981. The larvae from each leaf were staged by instar, pooled within each leaf, dried en masse, and weighed. Estimated population biomass was then calculated by using the tree-hole mosquito equation (equation 3) and the mean weight of 4th instars from the 1978 collection 3 years earlier. The 1978 and 1981 collections were made by different persons. The 1978 larvae were dried over silica gel for 72 h and weighed on a Cahn electrobalance; the 1981 larvae were dried over Drierite for 6 months and weighed on a Mettler microgram balance. The 1981 samples were collected by one person, coded and dried by a second person, and weighed and decoded by a third person. In this manner, I sought to make the prediction and test of population biomass as independent as possible.

Figure 3 shows that the correlation between actual and predicted population biomass was close, predicted biomass accounting for some 85% of the variation in actual biomass. Using actual mean instar weights of *W. smithii* from 1978 to predict 1981 biomass resulted in a higher coefficient of determination ($r^2 = 0.88$), as did an equation relating only 1978 *W. smithii* weights to stage of development ($r^2 = 0.87$). However, the improvement in predictive power from using strictly *W. smithii*-based weights as opposed to the more general equation (equation 3) is slight.
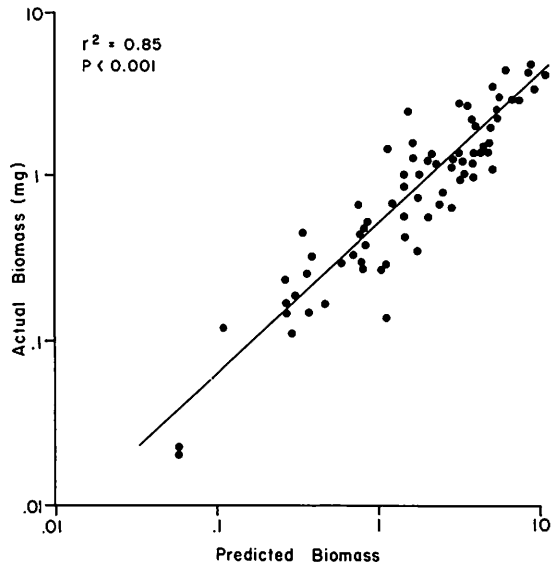
FIG. 3. Actual and predicted population biomass of *W. smithii*. Actual biomass is from *W. smithii* collected from the field in 1981; predicted biomass is calculated from the general tree-hole mosquito equation (equation 2) adjusted for mean weight of 4th-instar *W. smithii* collected from the same locality in 1978 (equation 3).

FIG. 2. Adjusted mean log weight of tree-hole mosquitoes. The mean log weight of the ith instar (LW_i) is adjusted by subtracting the mean log weight of the 4th instar (LW_{IV}) of the same species.

The generality of the growth constant among mosquitoes provides a means for estimating population biomass in the field. For most species of mosquitoes, an investigator with a little practice can learn to discriminate instars without the aid of a microscope. Although microgram balances are not part of one's normal field equipment, milligram balances are generally available at most universities and many other research laboratories. Since the weight of 4th instars lies in a range from 0.02 to 10 mg (Fig. 1), pooling of 50 or fewer 4th instars could bring them into the range of these balances. In any event, one need determine the mean log weight of 4th instars only once. One could then add the mean log weight of 4th instars to equation 2 to estimate the weight of other instars and, when summed over individuals, the biomass of the population. The close correlation between actual and predicted biomass (Fig. 3) indicates that determination of population size rather than limitations of equation 2 will be the major factor affecting the accuracy of biomass estimates.

The methods for field estimation of biomass presented here are not as accurate as direct determination of dry weight or as Istock's (Istock et al. 1976) determination based on length. The present method is, nontheless, a good predictor of mean weights, can be used to assess population or community biomass, and is based entirely on the numbers of each instar present. The wide taxonomic range represented by the species I studied suggests that a consistent pattern prevails among all mosquitoes, and perhaps among the rest of the lower Diptera as well.

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