190±4nm, which is similar in size to the R virus from *Ae. taeniorhynchus*. An *Iridovirus* has previously been reported from *Ae. vexans* larvae collected in Louisiana, but this virus invariably produced green iridescence (Chapman et al. 1966). This is only the second known *Iridovirus* which produces orange iridescence. In both cases these viruses have been found in species of mosquitoes from which viruses producing green iridescence are also known. This fact suggests that R type viruses may also occur in some or all of the other species of *Aedes* and *Psorophora* in which iridoviruses with green or blue iridescence have been found. Efforts should be made to further characterize and to determine the distribution of the "R" and "T" types of virus so that their evolutionary relationships can be better understood.

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RAPID COUNTING METHODS FOR MOSQUITO LARVAE

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A careful balance of larval numbers with water surface, water volume and diet is required for survival and uniform, optimal growth and development of mosquito larvae reared for experimental purposes (Gerberg 1970). Bar-Zeev (1962) used 2 sizes of suction tubes stopped with sintered glass filters to obtain either 2100 or 15,000 newly hatched *Aedes aegypti L.*, on the basis of packed volume. Morlan et al. (1963) dispersed newly hatched *A. aegypti* in 2 liters of water with a food mixer and counted 20 2-ml. samples to determine the number of larvae per ml. of the suspension. For large-scale production the larvae were dispersed in a modified agitator-type washing machine and dispensed automatically with a tipping-bucket dispenser. Similarly, Gerberg et al. (1968) dispersed newly hatched *Anopheles stephensi* Liston in 3 liters of water with an electric stirrer and counted 25 1-ml. samples to determine the larval concentration.

The method of Bar-Zeev (1962) is not readily adaptable to counting varying numbers of larvae, and those of Morlan et al. (1963) and Gerberg et al. (1968) require one-by-one counting of larvae in a number of samples to achieve an acceptable degree of accuracy. The present paper describes 2 supplementary methods developed in our laboratory to simplify larval counting.

PHOTOGRAPHIC COMPARISON METHOD. Photographs were made of Petri dishes containing 100, 200,....900, 1000 dispersed 1st instar larvae of *A. aegypti* and reprinted as a single, full-sized strip photograph of the larval concentrations in serial order (Figure 1). The strip-photograph (comparator) is placed on a dark bench or table, and the number of larvae in a Petri dish is estimated by moving the dish alongside the comparator until a matching photograph is found. Larvae are then added or withdrawn from the dish with a dropper, and a new match is made, until the desired number of larvae is obtained. If equal numbers of larvae are to be

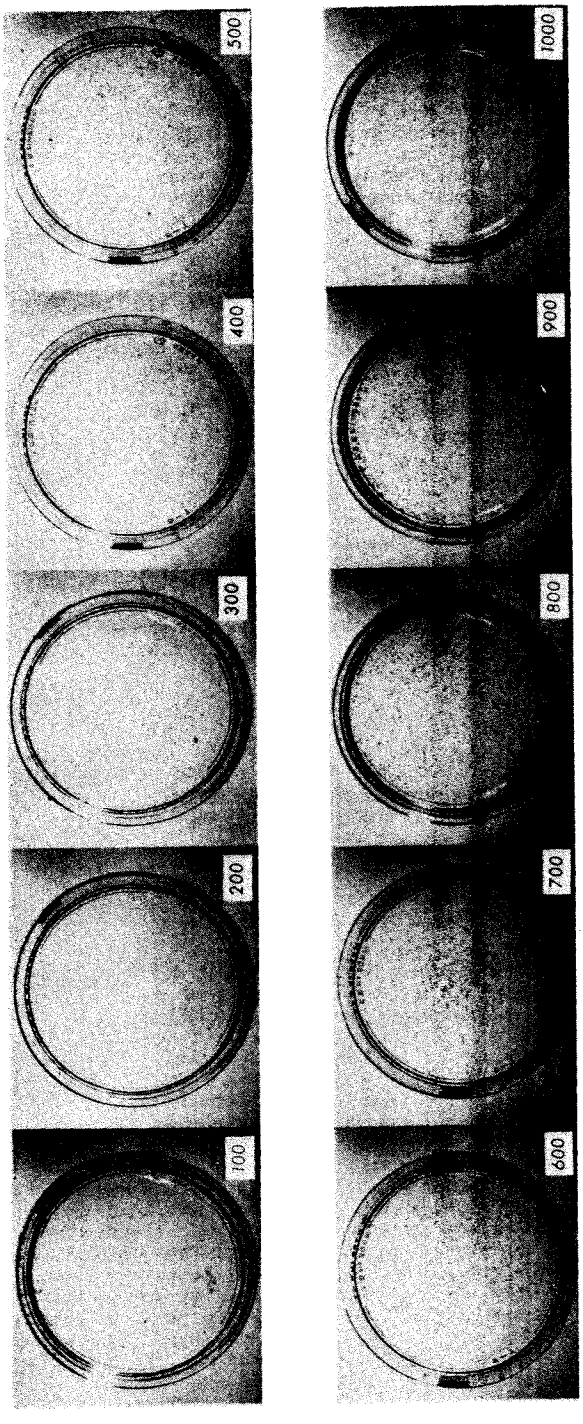

Fig. 1. Photographic comparator for use in counting 1st instar larvae of *Aedes aegypti*.

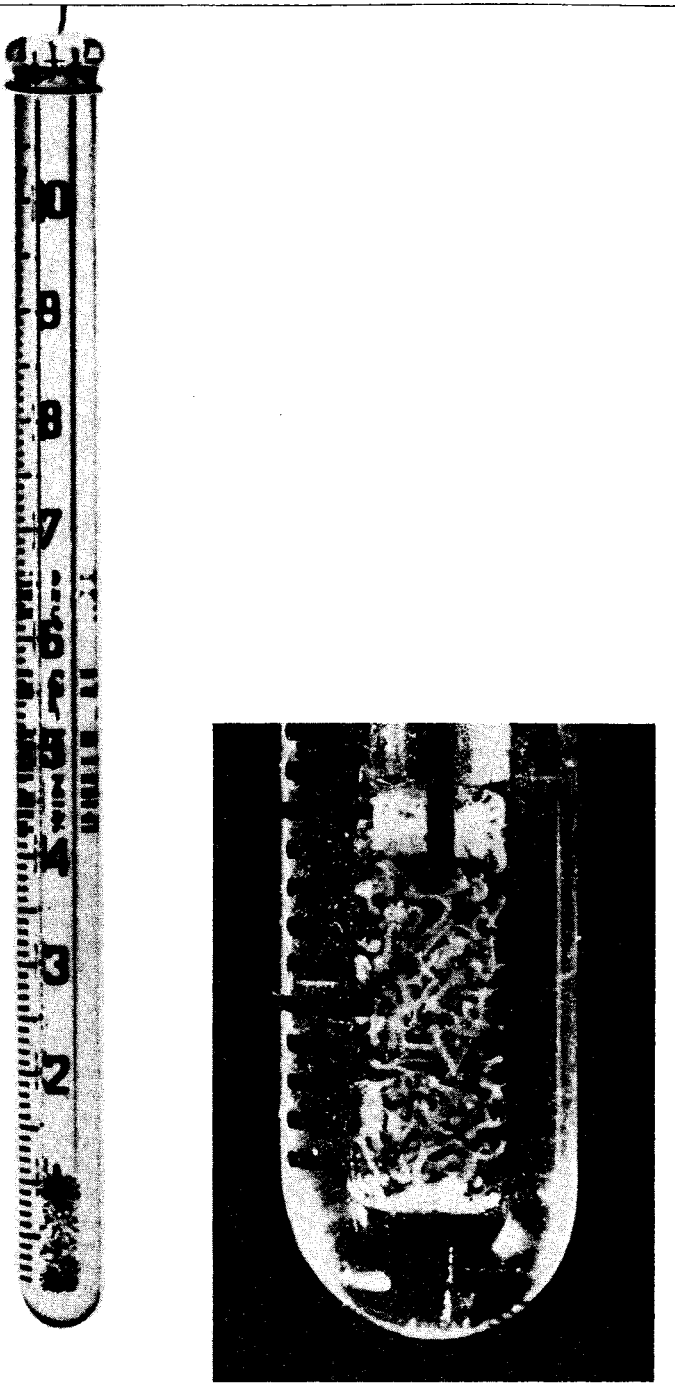

FIG. 2. First instar larvae of *Aedes aegypti* concentrated in 0.10 ml. of suspension in a hematocrit tube. Inset: Enlargement of the larval concentration.

distributed into rearing trays, one can use the comparator to determine the number of larvae in several 5-ml. samples of the larval stock and then dispense the larvae volumetrically from the stock into the trays. Uniform dispersion of the larval stock for sampling and distribution can be maintained by aeration, shaking or stirring.

VOLUMETRIC CONCENTRATION METHOD. This method makes use of the tendency of mosquito larvae to swim downward when concentrated in a narrow tube. Larvae from a concentrated larval stock are taken up in a Wintrobe pipet or Pasteur pipet, allowed to concentrate at its tip, and transferred to a hematocrit tube graduated in 0.01 ml. More larvae are added and water is withdrawn as necessary to concentrate the larvae as densely as possible in a pre-selected volume (Figure 2). The number of larvae concentrated in a particular volume tends to be constant for a given person, and thus the number counted can be regulated by adjusting the volume. If the larval stock is maintained evenly dispersed and the volume withdrawn is measured, the per-ml. concentration of larvae in the larval stock can be calculated.

RESULTS AND DISCUSSION. Fifty-five trials of the photographic comparison method were performed by 5 individuals who were asked to estimate 500 1st instar larvae of *A. aegypti*. One-by-one counts of the 55 lots provided an actual average of 563 larvae for an average error of 12.5%. The coefficient of variability among larval counts was 23%, and there was no significant difference among counts made by different individuals ($P>0.05$, F test).

Fifty-four trials of the volumetric concentration method were performed by 6 individuals to determine the number of 1st instar larvae of *A. aegypti* that could be concentrated in 0.10 ml. of suspension in a hematocrit tube. The average number of larvae concentrated by the 6 individuals varied from 104 to 276 (significant at the 1% level, F test). The coefficient of variability among larval concentrations produced by the same individual was 22%. Thus, the error associated with this method is comparable with that of the photographic comparator method if the average for each person making the counts is determined.

The foregoing degrees of error are regarded as acceptable for routine rearing purposes. In rearing experiments conducted in our laboratory with *A. aegypti, Anopheles albimanus* Wiedemann and *An. quadrimaculatus* Say we found that departures from optimal density comparable to the counting error did not result in deleterious undercrowding or overcrowding effects. The varying optimal larval population densities reported by different authors (see Gerberg, 1970) support this conclusion.

The advantages of the photographic comparison method and the volumetric concentration method over those previously available are chiefly practical. Both methods are simple and rapid, and neither requires expensive or hand-crafted equipment. In addition, both methods are readily adapted to counting different numbers of larvae for different purposes.

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THE CHALLENGE OF COPING WITH MOSQUITO LITERATURE

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The art of coping with literature one wants to see almost daily takes a bit of maneuvering on any researcher's part, especially if one is on a limited budget as most of us are.

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