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A SIMPLE TECHNIQUE FOR OBTAINING STANDARD NUMBERS OF NEWLY HATCHED MOSQUITO LARVAE

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INTRODUCTION. The mosquito *Aedes aegypti* L. is reared in many laboratories for studies in various fields, because of the relative ease with which it can be reared, and the important advantage that its eggs can be stored for several months. The key to success in obtaining the maximal number of normal-sized adults from a given number of newly hatched larvae is a proper ratio between the number of larvae in a rearing container and the amount of food given to the larvae (Shannon & Putnam 1934, Bar-Zeev 1956). If, for a given number of newly hatched larvae, there is too much food, mortality of larvae and pupae will result, mostly due to scum formed on the water surface (Christophers 1960). If there is too little food, the time from first to final pupation will be prolonged; many larvae which have not yet pupated will be lost, unless they are separated (Bar-Zeev & Galun 1961), and placed in fresh water with food. Furthermore, the resulting adults will be small (Bar-Zeev 1956). If the number of larvae per breeding container can be kept constant, the optimal amount of food for this number of larvae can be determined.

In mosquito investigations, it is important to rear the larvae under identical conditions and to obtain adults of uniform size. It has been shown by Harrison (1952) that small house flies are more susceptible than large ones to DDT deposits. This may also be true of mosquitoes, not only in regard to susceptibility to insecticides but also to a number of other factors.

A survey of the literature shows that the number of larvae given per breeding container is either counted (Hartzell *et al.*, 1958) which is time consuming, or estimated by eye by various means (Trembley 1955, McKiel 1957, Christophers 1960 and Kirkwood 1961) which obviously yields somewhat unreliable results. In this study various techniques were tested in order to achieve a more satisfactory method. The following has proved to be the most suitable.

MATERIALS AND METHODS. Eggs were submerged in clean water in a jar in the afternoon and left overnight (without adding food) to allow most larvae to hatch. The contents of the jar were then filtered through cloth held below a 40-mesh metal screen. The latter allowed the larvae to pass but retained most debris (egg shells, dead mosquitoes, etc.). The larvae were retained on the cloth and were then transferred to a beaker containing a little water, and filtered again through cloth placed in a 7 cm diameter funnel the stem of which had been cut off. Washing with a small quantity of water serves to concentrate the larvae at the bottom of the funnel, which is then placed on top of a beaker

(250 ml) containing water, so that a little enters the funnel from below.

The mixture of larvae and water in the funnel has a consistency somewhat similar to a heavy viscous oil which can be altered by adding or removing some water from the beaker. A specially constructed suction tube (Figure 1) is applied. It

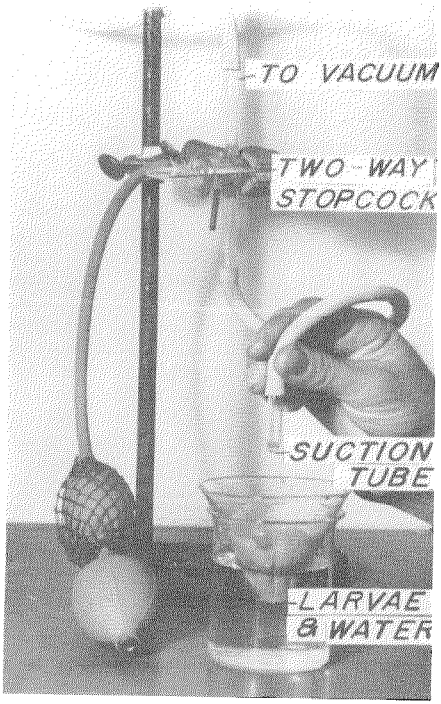

FIG. 1.—Apparatus for obtaining standard numbers of newly hatched mosquito larvae.

consists of an 8 mm inside diameter glass tube in the lower part of which is a sintered glass disc (extra coarse), 3 mm thick. From the sintered glass to the tip, the tube tapers over a length of 4 mm to an aperture at the tip of 1.7 mm diameter through which the negative pressure is applied. The upper end of the tube is connected by a rubber tube to a two-way stopcock of 5 mm bore. One way leads to a filte flask (which serves as a water trap) an thence to a water jet pump with a vacuun gauge. When water flows with the ta fully opened, a vacuum of about 28 inche of mercury is produced. The other exi connects the sucking tube to a rubber bul with one-way valves, by means of a rubbe tube (Figure 1).

The tip of the suction tube is applie to the mixture in the funnel; the two-wa stopcock is then opened to connect th tube with the water jet pump (full opened) and held there for about 10 sec onds—or longer, if the larval suspensio is dilute. This forces the mixture into th tube; excess of water passes through th sintered glass, whereas the larvae whic fill the lower portion of the tube, are re stricted below the sintered glass. Th column of water above the sintered glas reaches a certain height (depending o the concentration of the larvae in the fun nel) within a few seconds, and then con tinues to rise very slowly (about 1 mm per minute). The stopcock is then turne to connect the suction tube to the rubbe bulb, and the suction tube is removed. A small cluster of larvae usually adheres t the outside of the aperture of the suckin tube. This is removed with a scalpel, i order to obtain larvae filling the suctio tube up to the aperture only. The tube i then held above a small container of water and the rubber bulb is pressed to force th larvae out.

The reason for interrupting the suc tion and then removing the tube from th mixture is to prevent the cluster of larva which adheres to the outside of the device (the amount is not constant and depend on the concentration of larvae in the fun nel) from being sucked in by air. Th smallness of the aperture at the end of th suction tube reduces the cluster of larva formed to a minimum. When a large suction tube is used (see Figure 2) th number of larvae in the cluster is very small compared to the total number o larvae in the tube and can most probably be ignored.

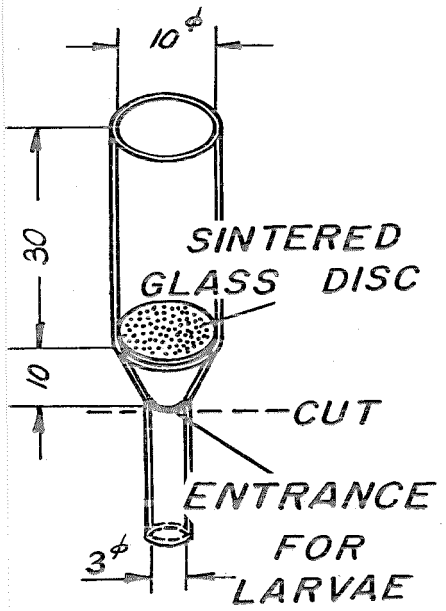

FIG. 2.—Ready-made funnel with sintered glass disc. Dimensions in millimeters.

When a large number of samples is taken, the density of the larvae in the funnel is continuously reduced. In this case, some water from the beaker (on which the funnel is placed) is poured out to restore approximately the original density of the mixture.

RESULTS AND DISCUSSION. In Table 1 the number of larvae per charge of the suction tube is recorded. Each group (A, B, etc.) in the table designates experiments carried out on different days, hence with different larval batches. The results show relatively small variations in the number of larvae obtained per charge. Only in two cases (3rd in group C and 1st in group I) did the deviations from the mean reach about 16 percent; in all others, they were smaller than 9 percent.

In another set of experiments, the effect of the density of the larvae in the funnel on the number obtained per filling was tested. This was carried out by first taking samples from a mixture in which the density of the suspension was extremely high (almost like a paste); some water was then added to the beaker on which the funnel is placed, and again samples were taken (the difference in the density of the larval suspension was obvious to the naked eye). Results are given in Table 2. The density of the larvae in the funnel does not affect the number obtained per filling.

In order to determine whether satisfactory results are also obtained with a larger suction tube, analogous experiments were carried out with a similar, but much larger suction tube taken from a ready-made funnel with sintered glass disc (micro, Büchner type) (Jenaer Glaswerk Schott & Gen, Landshut, Bayern, Germany) the stem of which was cut off as shown in Figure 2.

TABLE 1.—Number of larvae obtained per filling with different batches of larvae. (The figure in parenthesis gives the deviation from mean. A, B, . . . indicate different batches.)

A.—2080(63),	2250(107),	2224(81),	2155(12),	2160(17).
B.—2010(133),	2100(43),	2050(93),	2070(73),	2100(43).
C.—2080(63),	2090(53),	1795(348),	2180(37),	1980(163).
D.—2100(43),	2220(77),	2180(37),	2000(143),	2110(33).
E.—2180(37),	2120(23),	2210(67),	2010(133),	2050(93).
F.—2320(177),	2150(7),	2050(93),	2110(33),	2200(57).
G.—2260(117),	2070(73),	2125(18),	2320(177),	2170(27).
H.—2120(23),	1960(183),	2150(7),	2150(7),	2200(57).
I.—2510(367),	2335(192),	2285(142),	2230(87),	2240(97).

Mean=2143.
Standard deviation=119.8.
Standard error=18.1.

TABLE 2.—Number of larvae obtained per filling for very high and low larval densities in the funn

	No. of larvae/filling at high larval density	Deviation from mean		No. of larvae/filling at low larval density	Deviation from mean
A	2180	111	A	1950	130
	1975	94		2110	30
	1918	151		2180	100
B	2075	6	B	2140	60
	2225	156		1965	115
	2040	29		2137	57
Mean	2069	...		2080	...
Standard deviation	117.6	...		97.8	...
Standard error	49.0	...		40.7	...

The number of larvae obtained per filling was very high. In order not to have to count such large numbers, the following approximative method was used: the larvae from one filling were placed in a cylindrical vessel (36.5 cm in diameter and 40 cm high) containing 10 litres of water. The latter was agitated by means of a stream of air (80 litres/min) which entered the vessel through a metal spiral, having holes (0.5 mm in diameter and 4 cm apart), placed at the bottom of the vessel. Ten samples, each containing 100 ml of water were taken from the vessel and the number of larvae in each sample was counted (Table 3). The total number of larvae in the vessel was approximately 100 times the total count. Each group (A, B etc. in the table) denotes samples taken on a different day, hence from different larval batches. This table also gives the maximum and minimum counts obtained in each set of ten samples. The variations indicate that a relatively large number of aliquots should be counted to obtain a working accuracy.

The total number of larvae per filling of the large suction tube was between 14,000–16,000, i.e., a maximum deviation of about ± 7 percent from the mean, similar to the results obtained with the smaller suction tube. It is advisable to have the suction tube cleaned after use by sucking in an acid or any suitable cleaning solution and leaving the latter in the suction tube overnight to prevent dirt accumulation on or inside the sintered glass.

TABLE 3.—Average number of larvae per 10 aliquots each containing 100 ml. of water *

Group	Average No. of larvae ± S.E.	Min. No. of larvae/ aliquot	Max. No. of larvae/ aliquot
A	146.2±4.3	138	158
	143.4±4.1	120	168
	144.7±4.4	123	167
B	151.6±2.5	138	163
	153.5±3.4	139	170
	156.1±3.5	138	170
C	161.8±4.1	142	187
	155.8±3.7	136	176
	163.9±3.2	148	181
D	153.2±2.7	134	165
	151.0±5.8	111	171
	153.2±3.9	138	167
E	163.8±3.1	153	174
	158.1±2.9	144	174
	164.7±4.2	153	184

* Total number of larvae contained in the larg suction tube is the No. given in col. 2 x 100.

The technique described is now being used in our laboratory. It has the advantage of being simple, accurate enough for practical purposes, and rapid. One operator can prepare many aliquots within a few minutes. The size of the suction tube can be made so as to suit the approximate number of larvae per container. The number of larvae is then determined by counting a few samples and the optimal amount of food required for such a number is finally determined. The method de

cribed may be useful also with other pecies of mosquitoes.

In the present experiments, about 17 ours elapsed from the time the eggs were laced in water until newly hatched larvae vere used. Although no food was added o the water, it may be assumed that some rowth of the larvae occurred. Such rowth, although infinitesimal (because of ack of food), may vary under different onditions (density of the larvae, rate of atching, temperature, purity of water tc.) and thus may cause some variations n different batches. It might, therefore, e preferable to use a stimulus for hatch- ng, such as hatching the eggs under acuum or in boiled water to reduce the xygen tension (Burgess 1959), and then o use larvae which are only a few hours ld.

SUMMARY. A dense mixture of larvae nd water is sucked by means of a water t pump into a suction tube. The larvae re concentrated by a sintered glass disc ituated at the lower portion of the tube. tandard numbers (depending on the vol- me of the suction tube below the sintered lass) of newly hatched mosquito larvae re obtained.

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