EFFICIENCY OF THE ENFIELD SAMPLER FOR QUANTITATIVE ESTIMATES OF LARVAL AND PUPAL MOSQUITO POPULATIONS

G. PRITCHARD AND P. J. SCHOLEFIELD

Department of Biology, University of Calgary, Calgary, Alberta, Canada T2N 1N4

ABSTRACT. Inconsistencies in population estimates of temporary pond *Aedes* spp. led to tests of the efficiency of the Enfield sampler. With the samplers in place for 24 hr, only 41% of stage I *Ae. vexans* were collected after 1 hr. This was largely due to stage I larvae remaining below the trapping vessel. However, after 24 hr no more than 6% of the larvae are below the trapping vessel. The largest potential source of error is operational inefficiency when stage I larvae are present, and considerable care must be exercised when handling these samples.

INTRODUCTION

Two principal methods have been used to estimate population sizes of the aquatic stages of *Aedes* mosquitoes: mark-recapture (Service 1968), and some sort of area sampler (Welch and James 1960, Iversen 1971, Roberts and Scanlon 1974, Enfield and Pritchard 1977). Lesser (1977) has advocated a method which combines the area sampler with catch per unit effort methods, on the grounds that the area sampler alone is too time consuming.

The Enfield sampler (Enfield and Pritchard 1977) consists of 2 parts (Fig. 1). A PVC pipe is rapidly lowered into the water and pushed into the substrate, and larvae are removed from the enclosed column of water by means of a trapping vessel which fits inside the pipe. When set, the trapping vessel has a ring-shaped hole through which larvae and pupae can pass readily to the water surface, but only with difficulty can they return. Mosquitoes are trapped in the vessel by closing the gap, and the contents are then poured through a cylindrical stainless steel filter into a plastic cup. The cup fills with water and excess leaves through the filter, with larvae and pupae being retained in the cup.

When first used in the field in 1974 (Enfield and Pritchard 1977), the samplers were left in place for only 20 min, because in this time 95% or more of larvae of *Aedes vexans* (Meig.) in any stage had been recovered in laboratory tests. Furthermore, a pond could be sampled in a fairly short period of time with only a few samplers, by moving them to new locations after 20 min. However, the results of this sampling program suggested that the samplers were occasionally inefficient, taking fewer larvae on days when the population was predominantly in stage I or on cold days. In cold water or in stage I, larvae can obtain much of their oxygen

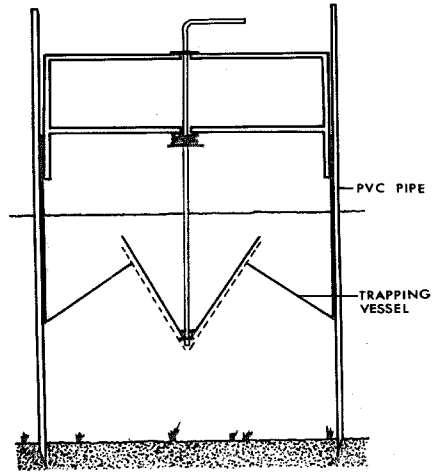

Figure 1. The Enfield Sampler (Enfield and Pritchard 1977).

requirements directly from the water and need not rise to the surface as frequently as must later stages or on warm days. Accordingly, in 1975, more samplers were used and they were left in place for 60 min when stage I larvae were present and for 40 min otherwise.

In 1976 enough sampling vessels were available so that the whole pond could be sampled with 1 set, and samplers were left in place for at least 60 min on each sampling occasion. However, the results still suggested that stage I larvae were not being taken in the same proportion as were later stages, since the estimates were lower than for later stages.

During July 1977, the trapping vessels were emptied after 1 hr, replaced in the pipes, and emptied again after 24 hrs. The results (Table 1) showed that sampling efficiency, particularly for stage I larvae, was low after 1 hr.

These results were used to correct the data obtained in 1976 and although the procedure is dubious because the correction terms were obtained from a different population under different conditions one year later, the better fits of the regression lines were reassuring. However, even when these correction terms were applied to population estimates collected in May 1977, stage I larvae were still greatly underestimated (Slater and Pritchard 1979).

One solution to this problem might simply have been to leave the samplers in position for the full 24 hrs. However, the proportion of the sample population that is actually retrieved may still not be 100%, nor is it necessarily constant for all stages under all conditions, nor would we be aware of handling errors. Therefore, further tests were carried out in 1979.

METHODS AND MATERIALS

One problem is the movement of larvae and pupae into or out of the trapping vessel. Can larvae survive for 24 hr without moving to the surface and thereby not enter the trapping vessel? And once in the trapping vessel can they pass back between the funnels and so escape the trap? In order to answer these questions samplers were set up in the field, cleared of larvae, and known numbers of larvae were placed either in the pipe below the trapping vessel or in the trapping vessel itself. Generally 15 larvae were placed in each of 5 samplers on 20 occasions over a 1 month period in August and September. The trapping vessels were emptied at 24 hr intervals after the introduction of larvae until no more mosquitoes were taken. We had no control over the environmental conditions that prevailed on any given day and so could not cover all of the possible combinations of stage, temperature and site of introduction to the sampler, but all larval stages of *Ae. vexans* were introduced into the pipes, while stage IV of *Ae. vexans* and stages I

Table 1. The efficiency of the trapping vessel after 1 hr in capturing *Aedes vexans*.

	Numbers captured after 1 hr and after 24 hr									
	Instar I		Instar II		Instar III		Instar IV		Pupa	
Date	1 hr	24 hr	1 hr	24 hr	1 hr	24 hr	1 hr	24 hr	1 hr	24 hr
20 July 77	240	354								
22 July 77	5	1	353	91						
25 July 77			16	3	57	5	640	85		
27 July 77			1	0	16	4	518	18		
29 July 77					4	1	24	3	211	64
Total	245	355	370	94	77	10	1182	106	211	64
Proportion after 1 hr	0.41		0.80		0.89		0.92		0.77	

and IV of *Culiseta inornata* (Williston) were introduced to the trapping vessel.

RESULTS AND DISCUSSION

The results after adding larvae to the pipe before introducing the trapping vessel, are shown in Table 2. The maximum efficiency of the samplers is that number of mosquitoes trapped after 24 hr expressed as a percentage of the total number of mosquitoes that were ultimately taken from the trap. The minimum efficiency is the proportion of the number put into the sampler that were trapped after 24 hr. These latter values assume that all mortality occurring in the traps was due solely to the traps themselves, an unlikely event, especially since we have previously estimated daily mortality rates in *Ae. vexans* populations of 10–20% (unpublished report, Alberta Environment) and the differences between minimum and maximum efficiencies are generally within this range. The maximum efficiencies are, therefore, considered to be more realistic, although these are based on the assumption that no mosquitoes are lost during handling, a problem to which we shall return. It will be noted that there are no differences between the maximum efficiencies of the sampler for different larval stages, and that these efficiencies are acceptably high (better than 94%).

However, a few larvae (up to 6%) are not in the trapping vessels at the time of emptying after 24 hr, although they do appear later. If larvae are not all being caught after 24 hr, either they are able to spend more than 24 hr in the water below the trapping vessel or they are able to move back into the pipe after visiting the surface. The first alternative seems unlikely given the low oxygen conditions that develop under the trapping vessels. Reiter (1978) has shown that the critical dissolved oxygen concentration at which cutaneous respiration adequately compensates for the absence of siphonal gas exchange at 27°C was as low as about 30% for stages II and IV larvae of *Anopheles stephensi* Liston, rising to only about 50% for stage IV larvae at 32°C. We have examined the dissolved oxygen concentration necessary for survival of stages I, II, and IV larvae of *Ae. vexans* and stage I and II *Cs. inornata* in stoppered bottles with no access to the air, and only at 100% dO_2 did larvae survive for 24 hr whether at 8°C, 13°C, or 21°C. At 75% dO_2 larvae generally died within 20 hr and at 50%, within 6 hr. Dissolved oxygen concentrations in the pond were generally about 80%, but under the trapping vessels they rapidly dropped to less than 10%. It seems unlikely, therefore, that larvae would not visit the surface at least once during a 24 hr period, and this leaves movement back down through the trapping vessel as the explanation for less than 100% efficiency.

The results of the experiments in which larvae were introduced into the top of the sampler with the trapping vessel in place are shown in Table 3. Since all larvae

Table 2. Efficiency of the Enfield sampler after 24 hr. *Aedes vexans* larvae added to the pipe before insertion of the trapping vessel.

Date	Mean Temp °C	Stage	N*	Maximum Efficiency**	Minimum Efficiency**
9/10 Sept 79 23/24 Aug 79	11.4	I	10	96.8% (79.7 ± 3.9)	65.8% (54.2 ± 4.4)
12/13 Sept 79	16.2	II	19	98.3% (82.6 ± 2.3)	88.3% (70.0 ± 2.6)
25–27 Aug 79	13.5	III	13	94.4% (76.3 ± 3.2)	75.0% (60.0 ± 1.8)
27–30 Aug 79	13.5	IV	17	96.0% (78.5 ± 2.3)	82.7% (65.4 ± 2.7)

* Number of sample units; 15 larvae in each.
** (Angular Transformation ± Standard Error).

Table 3. Efficiency of the Enfield sampler after 24 hr. Larvae added to the trapping vessel.

Date	Mean Temp °C	Stage	N*	Maximum Efficiency**	Minimum Efficiency**
Culiseta inornata					
31 Aug 79	16.2	I	5	98.3% (82.4 ± 4.0)	56.3% (48.6 ± 5.1)
21/24 Sept 79	11.3	IV	10	99.5% (86.0 ± 2.0)	97.1% (80.1 ± 3.1)
Aedes vexans					
20 Sept 79	11.9	IV	14	98.2% (82.2 ± 3.4)	90.5% (72.0 ± 4.0)
24 Sept 79	11.1	IV	10	100%	95.8% (78.1 ± 3.2)

* Number of sample units; 15 larvae in each.
** (Angular Transformation ± Standard Error).

were trapped at the start of the experiment, anything less than 100% maximum efficiency means that larvae have swum down into the pipe and were below the trapping vessel at the time of emptying. The fact that fewer than 2% apparently did this may suggest that, in the experiments in which larvae were introduced below the trapping vessel, some larvae had in fact not visited the surface during the 24 hr period. Differences between the results of the 2 experiments are, however, not statistically significant.

The minimum efficiencies in Table 3 include errors due to larvae swimming out of the trapping vessel, removal by predators, and handling. Errors due to larvae swimming out should be the same in both maximum and minimum calculations, while predation is probably low. The low recovery of stage I larvae of *Cs. inornata* contrasts with the recovery rate for stage IV larvae of *Cs. inornata* and *Ae. vexans*. This, along with the low minimum efficiency of stage I larvae of *Ae. vexans* in the 1st experiment (Table 2), suggests that some of these small larvae may be lost when the trapping vessels are emptied, either through leaks in the closing mechanism, sloppy operator technique, or failure to wash all larvae out of the vessel. This operational inefficiency would, therefore, appear to be the most important source of error in the use of this type of sampler. A further difficulty with stage I larvae is that they may be missed in counting back in the laboratory, and so samples containing stage I larvae should be counted under the microscope and great care exercised to ensure that none are missed.

CONCLUSIONS

In order to recover better than 94% of the mosquito larvae or pupae trapped by the Enfield sampler the following precautions should be taken:

1. The trapping vessels should be checked regularly to ensure that the top funnel seats firmly when closed, but leaves a sufficiently large opening for mosquito larvae and pupae to pass when open. (The mechanism used by Roberts and Scanlon (1974) is probably better than ours in this regard).
2. The PVC delimiting pipe must be well seated in the substrate; a series of saw-teeth around the base aids this operation.
3. The samplers should be left in place for 24 hr. (This is the maximum period of time between samples for a summer *Aedes* population).
4. When emptying the vessels, the operator must ensure that all water passes through the filter.
5. The trapping vessel should be washed out with water from a bottle carried for this purpose or with excess that flows through the filter; a thorough check should be made to ensure that no mosquitoes remain.
6. If mosquitoes are not to be counted very soon after sampling, predatory insects should be removed from the cups.

This can be done at the time of sampling if a sieve of appropriate mesh is inserted in the concentrating cylinder.

7. When stage I larvae are present, samples should be carefully sorted under a microscope.

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BACILLUS SPHAERICUS SPORE FROM SRI LANKA DEMONSTRATING RAPID LARVICIDAL ACTIVITY ON *CULEX QUINQUEFASCIATUS*

R.S.B. WICKREMESINGHE[1] AND C. L. MENDIS[2]
Medical Research Institute, Colombo, SRI LANKA

ABSTRACT. A strain of *Bacillus sphaericus* isolated from coconut husk pits in Sri Lanka proved to be rapidly larvicidal to *Culex quinquefasciatus,* the local vector of Bancroftian filariasis at a dilution of 10^3 organisms per ml. A distinctive feature of this organism was that it contained a parasporal inclusion attached to the spore and was of a serotype hitherto unrecorded.

INTRODUCTION

Filariasis in Sri Lanka is endemic in the western and southwestern coastal belt of the island encompassing 1,200 km^2 and inhabited by about 2.5 million people. The human infection rate is about 1%.

The etiological agent of the disease is *Wuchereria bancrofti,* and the vector is a local strain of *Culex quinquefasciatus.* Mosquito breeding sites include inter-alia husk pits, discarded receptacles and catch pits. Husk pits are water filled excavations in the soil in which coconut husks are deposited and allowed to putrefy before the coir is prepared. The presence of numerous husk pits in this area renders them an important breeding site of *Cx. quinquefasciatus* (Lambrecht 1974).

[1] Medical Microbiologist.
[2] Medical Entomologist.