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A Simple Method for Sampling the Immature Stages of *Aedes togoi*

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Abstract: A simple and accurate method was developed for sampling the immature stages of *Aedes togoi* in rock pools. As the distribution of this mosquito is contagious in water, the number of larvae and pupae caught by the conventional dipping method, in which surface water is collected by a dipper, is variable among samplings. But by stirring well the water in a jar, the distribution pattern became uniform, and nearly the same number of larvae and pupae could be collected by any dipping. The ratio of the number of immature stages of mosquitoes caught by a dipper to the whole number of them in the jar was exactly equivalent to the ratio of the volume of the dipper to that of the water in the jar.

Key words: *Aedes togoi*, Sampling method, Distribution pattern

The sampling method is very important in observing the seasonal changes of immature stages of *Aedes togoi* in rock pools on the sea shore. Usually samples of immature mosquito population in water are obtained by a dipper in the field. As Wada and Mogi (1974) and Service (1976) indicated that different instars of immature mosquitoes may remain at water surface for varying periods, and samples by dipping surface water may frequently be biased for particular instars. Another bias is due to the habit of *Ae. togoi* larvae and pupae to frequently make a cluster in breeding water, and many samplings have to be taken inadvertently to get accurate estimates. Moreover, this mosquito larvae and pupae submerge for some time after they are disturbed by dipping, and therefore at least a few minutes are required before the next dipping is made. Thus, the conventional dipping method is not suited for sampling immature *Ae. togoi*.

Nakamura *et al.* (1988) counted all larvae and pupae of *Ae. togoi* in the whole water pumped up from a rock hole on the coral reef for the seasonal abundance of this mosquito in Okinawa. Though correct data can be obtained by their method, the practical application may be troublesome owing to much time needed in the survey.

Dixon and Brust (1972) described that larval density could be estimated from the water volume in the pool and the number of mosquito larvae caught by a dipper with known capacity, when water in the pool was stirred prior to sampling. By stirring water

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well, the distribution pattern of mosquito larvae and pupae would be changed from contagious state to uniform one. If the distribution pattern of mosquito larvae and pupae is uniform in water, the same number of larvae and pupae would be expected theoretically in any dipping. Previous to the study on the seasonal abundance of *Ae. togoi* in the field, this idea was examined for the applicability in the lavoratory and the sampling efficiency was compared with the conventional dipping method.

The mosquito of *Ae. togoi* used in this experiment was the laboratory strain originated from Abunze, Fukue island, Nagasaki. Six hundreds of each instar larvae and pupae were released in an earthen jar of 12cm diameter with 2000ml water and small amount of dry yeast for the food of mosquito larvae. The jars were placed near the window in a room with natural light. The water temperature was 22.5℃ at the time of this experiment.

The water in the jar was stirred well with a soup ladle of 100ml, and dipped out inadvertently 3 times. The number of larvae and pupae was counted in each dip. After the 3rd dipping, larvae and pupae were put back into the jar, and this handling was repeated 5 times. For comparison, the surface water was dipped out 3 times by the soup ladle at an interval of 15 minutes. This interval was long enough for the larvae and pupae to rise to water surface and to become quiet. The former dipping method is called the "stir and dip" method and the latter the conventional dipping method in this paper.

Table 1 shows the number of each instar larvae and pupae caught by 3 dips in both sampling methods. Three dips by 100ml soup ladle collected 300ml water, which was equivalent to 0.15 of 2000ml water in the jar. As the distribution of mosquitoes was regarded to be uniform after stirring the water, it was expected that 90 larvae in each instar or pupae, which were equivalent to 0.15 of the number of the whole larvae or pupae in the jar, would be caught. The actual number caught was 102.8 in 1st instar larvae, 96.0 in 2nd instar larvae, 88.4 in 3rd instar lavae, 103.2 in 4th instar larvae and 97.0 in pupae, respectively. There was not any significant difference between these values and the expected ones ($P<0.05$, t-test). It appears that the "stir and dip" method could sample *Ae. togoi*

Table 1. Total numbers of larvae and pupae caught by three dips of stirred water ("stir and dip" method) and of surface water at 15 minutes intervals (conventional dipping method).

	"Stir and dip" method*	Conventional dipping method*
1st instar larva	102.8± 15.1[a]	356.0± 46.7[a]
2nd instar larva	96.0± 8.6[a]	452.2±101.7[a]
3rd instar larva	88.4± 9.8[a]	380.4± 50.1[a]
4th instar larva	103.2± 11.0[a]	180.4± 43.5[b]
Pupa	97.0± 19.8[a]	165.4± 29.4[b]

* Mean±S.D. of 5 replications.
Means followed by the same letter within a vertical column were not significantly different ($P=0.05$) according to Duncan's New Multiple Range Test.

population in the jar with remarkable accuracy. The conventional dipping method, in which the surface water was dipped quickly, caught more 1st-3rd instar larvae than 4th instar larvae or pupae.

Different sampling efficiency among different instars of *Culex tritaeniorhynchus* by the conventional dipping method was reported by Wada and Mogi (1974). Shogaki and Makiya (1970) stated that the bias in sampling efficiency in immature stages of *Culex pipiens pallens* among the developing stages might be due to differences in vertical distribution of different instars. Service (1976) mentioned that the degree of aggregation of both *Aedes cantans* and *Anopheles gambiae* larvae and pupae differs between various larval instars and pupae. The different degree of aggregation might cause different sampling efficiency by the conventional dipping. In *Ae. togoi*, that the size and density of larval and pupal cluster seems to affect the sampling efficiency by the conventional dipping method.

According to Service (1976), many samples usually have to be taken to get a reliable estimate value of the population, as the numbers caught cause greatly differ in different samples in the immature population with contagious distribution. Therefore, the number of larvae and pupae of *Ae. togoi* caught by the conventional dipping method will be considirably different among samples in natural breeding sites, as the immature stages make up clusters. On the other hand, in the "stir and dip" method, nearly the constant numbers of larvae and pupae are sampled, and the variance in the samples is small. It is suggested that accurate estimating values are obtained with less effort in shorter time by this sampling method.

Table 2 shows the number of larvae and pupae caught only by the first dip in the two methods mentioned above. There was a large difference in sampling efficiency between 1st-3rd instar larvae and 4the instar larva or pupa by the conventional dipping method as in the results of 3 dips. Also variability in the numbers caught by each dipping was large. On the other hand, a single dip by the "stir and dip" method yielded the result parallel with that of 3 dips. The difference was not significant between the number caught by each dip and the expected value that was 30 ($P<0.05$, t-test). This means that if water is stirred well, even one dip would be enough to estimate the density of immature mosquitoes in a breeding site.

Table 2. Numbers of larvae and pupae caught only by the first dips of stirred water ("stir and dip" method) and of surface water (conventional dipping method).

	"Stir and dip" method*	Conventional dipping method*
1st instar larva	38.4± 5.8[a]	153.2± 61.0[a]
2nd instar larva	33.4± 6.3[a]	279.0± 76.8[ab]
3rd instar larva	33.2± 8.9[a]	214.0± 65.8[b]
4th instar larva	30.2±14.1[a]	61.6± 33.5[c]
Pupa	35.6± 8.0[a]	38.6± 15.4[c]

* Mean±S.D. of 5 replicatins.
Means followed by the same letter within a vertical column were not significantly different ($P=0.05$) according to Duncan's New Multiple Range Test.

Results in this paper show that the "stir and dip" method is very simple and very useful in sampling the *Ae. togio* population in rock pools. If the volume of water in a breeding pool is known, the population size of immature stages could be estimated accurately.

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