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Studies on the Population Estimation for Insects of Medical Importance : III. Sequential Sampling Technique for *Culex tritaeniorhynchus summorosus* Larvae in the Paddy-Field.*

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Abstract

The distributions of the numbers of *Culex tritaeniorhynchus summorosus* larvae per dip in respective paddy-fields were proved to be of an aggregated type and to be fitted well to the negative binomial distributions with a common k. It was also proved that the sequential sampling method based on this distribution pattern can properly be used to classify the larval density of each of paddy-fields examined, into one of the three density levels, low, moderate, and high, at a given statistical reliability by a relatively small number of dips, and in turn to compare the breeding situation of the paddy-fields in question.

Introduction

The ecological knowledge of *Culex tritaeniorhynchus summorosus*, which is the main vector of Japanese encephalitis in Japan, is indespensable for clarifying the epidemiology of the disease. Since the onset of ecological studies of *C. t. summorosus* in 1965, it has been felt that the immediate requisite is to develop a reliable method of estimating the mosquito density, particularly in the immature stages.

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As an approach to it, the sequential sampling method, which will be reported here, was used to learn the relative density of the mosquito in a paddy-field. The method was proved to be very useful to classify the larval density of each of paddy-fields into one of the three density levels, low, moderate and high, at a given statistical reliability by a relatively small number of dips.

Before going further, we wish to express our sincere appreciation to Professor Nanzaburo Omori of the Department of Medical Zoology, Nagasaki University School of Medicine, for his constant encouragement and valuable criticism throughout the course of this study.

Place and method

Mosquito larvae (including pupae) were collected by a dipper of 15cm in diameter and 3cm in depth with a wooden handle of 1.2m in length, in paddy-fields of Mogi, near Nagasaki City, situated in a small basin of approximately 1.5km x 0.4km, from June to October, 1968. At the outset a collector stood at a point on the side of a paddy-field, and took a dip on the water surface, which was thought to be most favorable for the breeding of the larvae, within the reach of the dipper. Then, the collector moved by about 5m along the side and took a dip in the same way. In each paddy-field, the dipping was made ten times which were thought to be necessary to determine the distribution pattern of the numbers of *summorosus* larvae in a paddy-field.

Results obtained

Distribution pattern of C. t. summorosus *larvae within paddy-field*

The number of paddy-fields examined for the larvae in Mogi is given in Table 1. Among the 221 paddy-fields, 152 had no *summorosus* larvae and the remaining 69 had one or more larvae of this mosquito.

Letting the number of *C. t. summorosus* larvae per dip in a paddy-field be x, then mean ($\bar{x}$), standard deviation (s), and coefficient of variation (CV) are calculated as follows :

$$\bar{x}=\frac{\sum x}{n}, \quad (1)$$

$$s=\sqrt{\frac{\sum(x-\bar{x})^2}{n-1}}, \quad (2)$$

and

$$\mathrm{CV}=\frac{100s}{\bar{x}}, \quad (3)$$

where n is the number of dips taken in each paddy-field being equal to 10 in this case.

Table 1. Number of paddy-fields examined by ten dips for *C. t. summorosus* larvae in Mogi, from June to October, 1968.

No.	Date	Number of paddy-fields		
		with larvae	without larvae	total
1	Jun. 15	7	2	9
2	Jun. 22	1	11	12
3	Jul. 13	12	7	19
4	Jul. 19	8	1	9
5	Aug. 3	7	7	14
6	Aug. 10	3	14	17
7	Aug. 17	4	10	14
8	Aug. 23	2	16	18
9	Aug. 30	7	11	18
10	Sep. 7	2	13	15
11	Sep. 16	5	12	17
12	Sep. 21	4	13	17
13	Sep. 28	3	16	19
14	Oct. 8	2	8	10
15	Oct. 12	2	11	13
Total		69	152	221

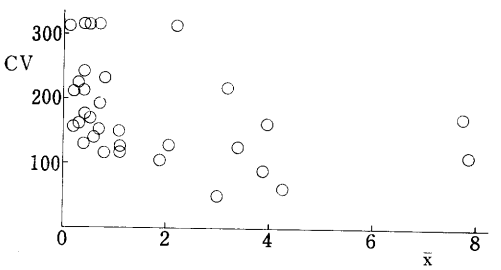

Fig. 1. Relation between the mean ($\bar{x}$) and coefficient of variation (CV) of the numbers of *C. t. summorosus* larvae per dip in each paddy-field.

In Fig. 1, the relation between mean ($\bar{x}$) and coefficient of variation (CV) is shown for the 69 paddy-fields in each of which one or more larvae were found (see Table 1).

Although there appears to be a slight tendency of decreasing CV with the increase of $\bar{x}$, there is a clear fact that the coefficient of variation is in every case large, being between 50 and 310.

It is required to establish the nature of the distribution pattern of *C. t. summorosus* larvae in the paddy-field before applying the sequential sampling technique. For this reason, the distribution pattern of the larvae was analyzed by using the numbers of larvae collected per dip.

When a distribution is considered random, Poisson distribution is often applied as a theoretical model. In the Poisson distribution, the probability of observing x count, $P(x)$, is given as

$$P(x)=e^{-m}m^x/x! \qquad (4)$$

where m is the population mean. It is a property of the Poisson distribution that the mean is equal to the variance. Here, the relation between the sample mean ($\bar{x}$) and sample variance (s^2) of the numbers of larvae per dip in each paddy-field is illustrated in Fig. 2 both on log scale. The sample variance, s^2, is given by

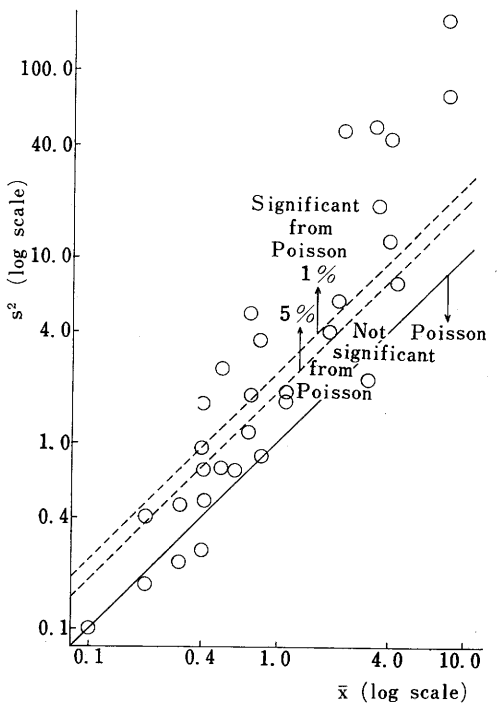

Fig. 2. Relation between the mean ($\bar{x}$) and variance (s^2) for the numbers of *C. t. summorosus* larvae in each paddy-field, showing whether or not the difference from Poisson distribution is significant at 5% or 1% level (for further explanation see text).

$$s^2=\frac{\Sigma(x-\bar{x})^2}{n-1} \quad (5)$$

In the Poisson distribution, the value of χ^2 is approximately given (Andrewartha, 1961) as

$$\chi^2=\frac{\Sigma(x-\bar{x})^2}{\bar{x}}$$

$$=\frac{(n-1)s^2}{\bar{x}} \quad (6)$$

with $(n-1)$ degrees of freedom. Here, χ^2 (P=0.05) with 9 degrees of freedom is 16.9. If we take logarithm of the both sides of expression (6), substitute 16.9 for χ^2 and 10 for n, and rearrange them, we have

$$\log s^2 = \log 1.88 + \log\bar{x}.$$

Thus, a linear relation holds between $\bar{x}$ and s^2 both in log scale for significant departure from the Poisson distribution at 5% level, as shown in Fig. 2. In the same way, we have the following equation for significant departure at 1% level :

$$\log s^2 = \log 2.41 + \log\bar{x},$$

which is also drawn in Fig. 2.

It is apparent from Fig. 2 that the Poisson distribution can not be rejected when $\bar{x}$ is as small as about 0.4 or less, but the departure from the Poisson distribution becomes greater with the increase of $\bar{x}$, and when $\bar{x}$ is 1.0 or more, the departure is significant at 5% or 1% level in most cases. In other words, the distribution is not random, but of an aggregated type when $\bar{x}$ is large. Non-significance from the Poisson distribution when $\bar{x}$ is small seems to be due to the low expectation of the occurrence of the larvae in individual dips.

Insect counts in respective samples in the field are often fairly well fitted by a negative binomial distribution, which is one of aggregated-type distributions. The frequency distribution of the negative binomial is given by expanding the expression $(q-p)^{-k}$, where $q-p=1$, $p=m/k$, m is population mean, and k is a positive exponent. In the following, it will be tried to examine the goodness-of-fit of the population of *summorosus* larvae in respective paddy-fields with the negative binomial with a common k. The calculations used here are based on Bliss and Owen (1958).

Two statistics, x' and y', are calculated from sample mean $(\bar{x})$ and sample variance (s^2) of a component sample by ten dips in each paddy-field:

$$x'=\bar{x}^2-s^2/n, \quad (7)$$

$$y'=s^2-\bar{x}, \quad (8)$$

where n is sample size. Their expectations are given exactly by

$$E(x')=m^2, \quad (9)$$

$$E(y')=m^2/k. \quad (10)$$

Thus $(y'-x'/k)$ has zero expectation, and the variance of this expression is given to order $1/n^2$ by

$$V=2m^2(m+k)^2(k(k+1)-$$
$$(2k-1)/n-3/n^2)/(n-1)k^4. \quad (11)$$

The invariance w $(=1/V)$ is of the nature of a weight, being given by

$$w=0.5(n-1)k^4/(k(k+1)-$$
$$(2k-1)/n-3/n^2)m^2(m+k)^2. \quad (12)$$

To estimate a common value for k from a set of samples of size n taken from populations with different means, we replace m by $\bar{x}$, m^2 by x', and k by an empirical trial value k', then we have $1/k_c$ as an estimate of $1/k$ as follows :

$$1/k_c=\Sigma(wx'y')/\Sigma(wx'^2). \quad (13)$$

In the expression (13), $1/k_c$ is obtained as the slope of a linear regression of y' on x', the regression line being constrained to pass through the origin ($x'=0$, $y'=0$). The empirical trial value k' is obtained by

$$1/k'=\Sigma y'/\Sigma x', \quad (14)$$

or

$$k'=g/\Sigma(y'/x'), \quad (15)$$

were g is the number of samples of size n. If k_c should differ appreciably from its trial

value k', the calculations are repeated by replacing k' by k_c. The homogeneity of k in the different samples can be tested by comparing the observed value of

$$\chi^2=\Sigma(wy'^2)-(\Sigma(wx'y'))^2/\Sigma(wx'^2) \quad (16)$$

with the tabular χ^2 distribution with $(g-2)$ degrees of freedom.

In the present data, there are rather few individual counts of *C. t. summorosus* larvae, as seen from Fig. 1 or 2, in each component sample, i.e., by ten dips in each paddy-field. In such a case, the component samples are grouped into approximately equal intervals on the basis of the total count X in each paddy-field. Then an average x' and y' is determined for each grouping interval as follows :

$$x'=(\Sigma X^2-\Sigma\Sigma x^2)/fn(n-1), \quad (17)$$

$$y'=(n\Sigma\Sigma x^2-\Sigma X^2-(n-1)\Sigma X) /fn(n-1), \quad (18)$$

where f is the number of samples in each grouping interval, ΣX the total count in the f samples, ΣX^2 the sum of their squared totals, and $\Sigma\Sigma x^2$ the pooled total sum of squares of the individual counts x. The weight for each x' and y' is given by expression (12) multiplied by f. A trial estimate of k equivalent to that in expression (14) may be computed from the totals of each term in the numerators of x' and y' in expressions (17) and (18). And expression (15) may be modified to

$$k'=\Sigma f/\Sigma(fy'/x'). \quad (19)$$

Now, for the present data of *C. t. summorosus* larvae in 69 paddy-fields, the value of a common k will be estimated. The value of k', a trial estimate of k, was calculated as 0.4283 by expression (14), and as 0.2756 by expression (19). The harmonic mean of the two trial values was 0.3354, which was used as the first trial value of k'. By using expression (13) we had the weighted estimate, $k_c=0.3413$, which did not differ appreciably from the first trial value. The observed value of χ^2 with 4 degrees of freedom (the number of grouping intervals was 6 in the present data) was calculated by expression (16) as 2.5537, the probability of which was between 0.50 and 0.75. Thus, a common k was justified, and its value was estimated at 0.3413.

Method of classifying larval density

The sequential sampling technique can be used for classifying a population into one of three pre-defined density levels, based on the accumulated numbers in each paddy-field. This is particularly useful for comparing local and seasonal differences in mosquito density in connection with different environmental situations. The utility value of this procedure is that it involves a flexible sample size and the expenditure of time and effort is generally much less than would be expected in usual procedure of estimating the relative density of larvae. Wada (1965) applied this technique to Canadian mosquito larvae, of which distribution follows a negative binomial with a common k, but the value of k differs with mosquito species. So, the calculation was necessary by using a different value of k to apply the sequential sampling technique. The procedure given by Morris (1954) was mainly followed by

Table 2. Density levels for sequential sampling of *C. t. summorosus* larvae in each paddy-field.

Density level	Mean No. of larvae per dip*
Low	- 0.23 (- 0.10)
Moderate	0.23 - 5.09 (0.50 - 2.50)
High	5.09 - (12.50-)

* In parentheses critical mean number of larvae is given for H_0 and H_1 hypotheses (for further explanation see text).

the present application.

The first step for the sequential sampling technique is to set up density levels of *C. t. summorosus* larvae. In Table 2, three density levels, low, moderate and high, were set up, based on the mean numbers of larvae per dip in respective paddy-fields (see Fig. 1). The density level "high" may be regarded as the indication of the mosquito production being actively going on, "low" may indicate the suppression state in breeding, and "moderate" is the intermediate situation between the two.

The next step is to set up alternative hypotheses, H_0 and H_1. To distinguish low and moderate density levels, H_0 is that the number of larvae per dip is 0.23 or less, and H_1 is that the number is 0.23 or more; to distinguish moderate and high H_0 and H_1 are that the number is less than 5.09 and more than 5.09, respectively. In H_0 and H_1 hypotheses, there are two types of error, α and β which are the probabilities of rejecting H_0 and H_1 at the respective critical densities shown in Table 2. For example, in the relation of low versus moderate density levels α is the probability of rejecting H_0, or accepting H_1, when the mean number of larvae per dip is 0.10, and β is the probability of rejecting H_1, or accepting H_0, when the mean number is 0.50. The values of the constants at the critical densities under these hypotheses are shown in Table 3, based on the negative binomial distribution with

Table 3. Values of the constants at the critical densities (see Table 2 and text) under the hypotheses of H_0 and H_1, based on the negative binomial distribution of the numbers of *C. t. summorosus* larvae per dip, with a common k of 0.3413.

Constant	Low vs. moderate density		Moderate vs. high density	
	H_0	H_1	H_0	H_1
Mean$=kp$	0.1	0.5	2.5	12.5
$p=kp/k$	0.2930	1.4650	7.3249	36.6247
$q=1+p$	1.2930	2.4650	8.3249	37.6247
Variance$=kpq$	0.1293	1.2325	20.8123	470.3088

a common k, of which value have already been estimated at 0.3413 in the earlier section.

Then, the acceptance and rejection lines for sequential sampling are calculated by

$$d=bn+h_0 \quad (20)$$

and

$$d=bn+h_1, \quad (21)$$

where d is the cumulative number of larvae in the first n dips. The slope of the lines, b, is

$$b=k\log(q_1/q_0)/\log(p_1q_0/p_0q_1), \quad (22)$$

where q_0 and q_1 are the values of q, under the hypotheses of H_0 and H_1, and p_0 and p_1 are those of p (see Table 3). The intercepts of the lines on the d-axis are

$$h_0=\log B/\log(p_1q_0/p_0q_1), \quad (23)$$

$$B=\beta/(1-\alpha) \quad (24)$$

and

$$h_1=\log A/\log(p_1q_0/p_0q_1), \quad (25)$$

$$A=(1-\beta)/\alpha. \quad (26)$$

Here, the same probability level was taken for both α and β, and acceptance and rejection lines were computed for low versus moderate levels and for moderate versus high levels, when α,β is 0.1, 0.2, 0.3, and 0.4. The calculated values of the constants in the lines given by expressions (20) and (21)

Table 4. Values of the constants, b, h_0, and h_1 in acceptance and rejection lines given by expressions (20) and (21).

Value of b	Value of $h_1(=-h_0)$ when α, β is 0.1	0.2	0.3	0.4
Low versus moderate density classes				
0.2284	2.2788	1.4378	0.8788	0.4205
Moderate versus high density classes				
5.0929	21.7367	13.7143	8.3822	4.0112

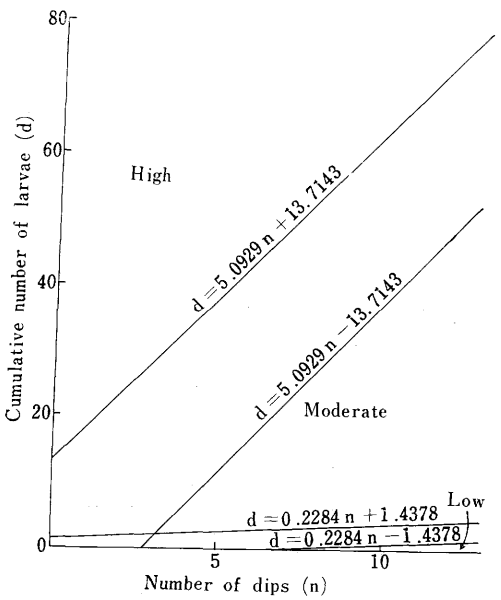

Fig. 3. Acceptance and rejection lines for sequential sampling of *C. t. summorosus* larvae in the paddy-field when α, β is 0.2.

are presented in Table 4.

As an example, the acceptance and rejection lines, when α, β is 0.2, for sequential sampling of *summorosus* larvae in the paddy-field is illustrated in Fig. 3. This graph may be used in the field to decide how far dipping should be continued at each paddy-field to determine the density level within the accepted probability limit of 0.2. It is helpful to visualize each pair of lines as an enclosing band from which the plotted points must escape before the density level is determined. For example, suppose that the numbers of larvae per dip are 0, 1, and 1 in the first three dips, and we plot the cumulative number of 0, 1, and 2 on the number of dips of 1, 2, and 3, respectively. The plotted points are within the bands, so we continue to dip. If we get 2 larvae in the fourth dip, then the cumulative number of 2 + 2 = 4 is plotted on dip 4. This point is shown to have escaped from the bands and to have fallen into the moderate zone, so dipping is discontinued. Thus, the density is classified into moderate. If the plotted points escape into the area above the higher band, the density is classified into high, and if below the lower band, the density into low. The table for sequential sampling prepared from the acceptance and rejection lines for α, β of 0.1, 0.2, 0.3, and 0.4, as shown in Table 5, may be more convenient for the use in the field than the graph like Fig. 3.

The operating characteristic curves are not essential in the application of the sequential plan, but are useful aids in understanding how the plan operates at different population levels. They are obtained by plotting the probability ($L(p)$) of accepting H_0 hypothesis for any value of the population mean of kp. Here, $L(p)$ is calculated from

$$L(p)=(A^h-1)/(A^h-B^h), \qquad (27)$$

$$p=[1-(q_0/q_1)^h]/[(p_1q_0/p_0q_1)^h-1], \qquad (28)$$

where A and B are as defined by expressions (26) and (24), and h is a dummy variable which may be assigned convenient values. The operating characteristic curves are presented in Fig. 4 for α, β of 0.1, 0.2, 0.3, and 0.4 respectively. The left-hand curves are

Table 5. Table for sequential sampling of *C. t. summorosus* larvae in the paddy-field, prepared from the acceptance and rejection lines.

No. of dips(n)	Cumulative number of larvae											
	$\alpha, \beta = 0.1$			$\alpha, \beta = 0.2$			$\alpha, \beta = 0.3$			$\alpha, \beta = 0.4$		
	Low	Moderate	High	Low	Moderate	High	Low	Moderate	High	Low	Moderate	High
1			27-			19-			14-		1	10-
2			32-			24-			19-	0	1-6	15-
3			38-			29-		2-6	24-	0	2-11	20-
4			43-		3-6	35-	0	2-11	29-	0	2-16	25-
5			48-		3-11	40-	0	3-17	34-	0	2-21	30-
6		4-8	53-		3-16	45-	0	3-22	39-	0	2-26	35-
7		4-13	58-	0	4-21	50-	0	3-27	45-	0-1	3-31	40-
8		5-19	63-	0	4-27	55-	0	3-32	50-	0-1	3-36	45-
9		5-24	68-	0	4-32	60-	0-1	3-37	55-	0-1	3-41	50-
10	0	5-29	73-	0	4-37	65-	0-1	4-42	60-	0-1	3-46	55-

Continue to dip until the cumulative number falls into one of the three density levels of low, moderate and high.

for low versus moderate density level, and the right-hand for moderate versus high. When the mean, kp, is 0.1, the probability of accepting H_0 (low density level) is 0.9, 0.8, 0.7, or 0.6, and therefore the probability of accepting H_1 (moderate density level) is 0.1, 0.2, 0.3, or 0.4, according to α, β of 0.1, 0.2, 0.3, or 0.4. Of course, the smaller the error probability of α, β is, the more correct the decision of density level is. For example,

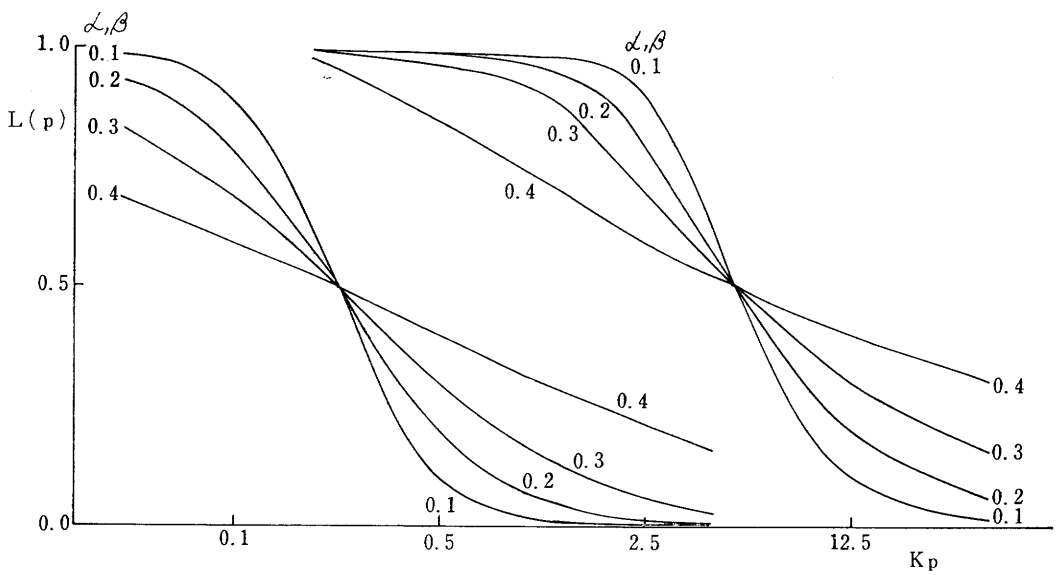

Fig. 4. Operating characteristic curves for low versus moderate density classes (left) and for moderate versus high (right), showing the probabilities of accepting H_0 hypothesis, $L(p)$, on the mean number of *C. t. summorosus* larvae per dip, kp, when α, β is 0.1, 0.2, 0.3, and 0.4.

for kp of 2.5 (moderate density level), when α, β is 0.1 the probabilities of accepting low, moderate and high density levels are respectively nearly zero, 0.9 and 0.1. But, for the same value of kp of 2.5, when α, β is 0.4, the probabilities of accepting the three density levels are nearly 0.22, 0.38, and 0.40, respectively.

Table 6 gives the results of the application of sequential sampling technique by

Table 6. Results of the application of sequential sampling method to the data on the number of *C. t. summorosns* larvae in each dip in respective 221 paddy-fields in Mogi, 1968, showng frequency distributions of paddy-fields with respective density levels determined by sequential method and, for comparison, by averaging the numbers of larvae in 10 dips, together with the mean numbers of dips required to determine the density level.

Density level*	Mean No. of larvae per dip	No. of paddy-fields					
		by averaging in 10 dips	by sequential method when α, β is				
			0.1	0.2	0.3	0.4	0.4**
Low	0-0.23	188	152	161	183	198	198
Low-moderate			42	31	2	0	0
Moderate	0.23-5.09	31	20	26	27	20	20
Moderate-high			6	2	2	0	2
High	5.09-	2	1	1	2	3	1
Total		221	221	221	221	221	221
% incorrect determination***			0.5	1.4	4.1	9.5	9.5
Mean No. of dips required		10.00	9.69	7.39	4.45	2.00	1.99

*When density levels were not determined by the end of the first 10 dips (the first 3 dips in **) by sequential sampling method between low and moderate levels and between moderate and high levels, they were designated as low-moderate and as moderate-high, respectively (for further explanation see text).
*** Incorrect determination means moderate to high density levels when the level by averaging is low, low and high when moderate, and low to moderate when high.

using Table 5 to the data in respective 221 paddy-fields shown in Table 1. In Table 6 are given the frequency distributions of paddy-fields with respective density levels determined by the sequential technique and, for comparison, by averaging the numbers of larvae in 10 dips. As ten dips were taken in each paddy-field, sometimes, especially when α, β is as small as 0.1 or 0.2, the cumulative number of larvae did not fall into any of the three density levels by the sequential sampling technique by the end of the 10 dips, and remained to be intermediate between low and moderate levels or between moderate and high levels. In such cases, it was designated as low-moderate or moderate-high density level. For comparison, the frequency distribution of paddy-fields, provided that only 3 dips were taken in each paddy-field, is also given in Table 6.

It is apparent from Table 6 that when α, β is smaller, the frequency of low-moderate or moderate-high density level is higher, but the percentage of incorrect determination of density levels is smaller, where incorrect determination means moderate to high density levels when the level by averaging the numbers of larvae in 10 dips is low, low and high when moderate, and low to moderate

when high. On the other hand, the smaller the values of α and β is, the larger the mean number of dips required for the determination of density level by the sequential sampling technique. Thus, when α, β is larger, the error in density determination is larger but the mean number of dips required is smaller, and vice versa. It depends on the object of investigation what value of α, β we must take.

A disadvantage of the sequential sampling technique is that it is fairly troublesome in the field to count the number of larvae in respective dips and compare the cumulative number of the larvae obtained with the number in sequential table such as presented in Table 5. This may be overcome by fixing the number of dips in respective paddy-fields and using a tool similar to a plankton net which concentrates the larvae in each dip into a small bottle with some formalin for later counting. When α, β is 0.4, the number of dips required to classify the larval density by the sequential sampling technique in each paddy-field is almost always 3 or less, therefore even if the maximum number of dips in respective paddy-fields is limited to 3, the results of density classification will little change as seen in Table 6 and the time for the field work will be saved. If we use a concentrator for larvae, the saving of the time will more and more increase. Thus, the sequential sampling method in which α, β is 0.4 and the number of dips in each paddy-field is limited to 3 may very properly be used for some purposes.

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衛生害虫の個体数の推定に関する研究. III. 水田のコガタアカイエカ幼虫に対する逐次抽出法の適用.

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摘　　要

個々の水田におけるコガタアカイエカ幼虫の柄杓当りの個体数は集中分布を示し，共通の k を持つ負の2項分布に適合することを証明した．この分布型を基礎とした逐次抽出法は，個々の水田の幼虫密度を，ある一定の統計的確かさからしで，しかも比較的少ない掬いとり回数で，低，中，高の何れかに分類することができ，ひいては，個々の水田の幼虫の発生量を比較するのに役立つ．