# Studies on the Population Estimation for Insects of Medical Importance. IV. A Method for the Estimation of the Relative Density of Culex tritaeniorhynchus summorosus Larvae in the Whole Paddy-Fields of an Area.* 

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#### Abstract

The estimation method of the relative density of Culex tritaeniorhynchus summorosus larvae in the paddy-fields of an area as a whole was investigated, based on the variances of transformed variate $y=\log (x+1)$ within and between paddy-fields, where $x$ is the number of larvae per dip. As a result, it was shown that only one dip should be taken in each paddy-field, and instead, the number of paddy-fields examined should be increased, in order to estimate the relative density of larvae in the area in maximum efficiency. If one dip is taken in a paddy-field, the estimation precision becomes higher, of course, when the number of paddy-fields examined is increased. In the case of the same number of paddy-fields being examined, the higher the mean larval density is, the higher the estimation precision is.


## Introduction

In a previous paper (Wada et al., 1971), it was reported that a sequential sampling method is useful to classify the relative density of larvae of Culex tritaeniorhynchus summorosus which is the main vector of Japanese encephalitis in Japan, in each paddy-field into one of the three density levels, low, moderate and high, at a
given statistical reliability by a relatively small number of dips. The sequential sampling method can probably be used for the classification of the larval density also in the whole paddy-fields of an area based on the variation in the number of larvae between paddy-fields. However, not only the classification of the relative

[^0]density, but also the estimation of the relative density itself of summorosus larvae in the paddy-fields of an area as a whole is often required in ecological studies of this mosquito especially in relation to the epidemiology of Japanese encephalitis. The estimation method for this
purpose was inquired into in the present paper.
We are very grateful to Dr. N. Omori, the former professor of the Department of Medical Zoology, Nagasaki University School of Medicine, under whose guidance this study was carried out.

## Materials and method

Materials used to inquire into the method of estimating the relative density of C. $t$. summorosus larvae in the whole paddy-fields in an area were the same as used in the previous paper (Wada et al., 1971). Mosquito larvae (including pupae) were collected by a dipper of 15 cm in diameter and 3 cm in depth with a wooden handle of 1.2 m in length, in paddy fields of Mogi near Nagasaki City from June to October, 1968. At the outset a collector stood at a point on the side of paddy-field, and took a dip at the site which was thought to be most preferable for larvae within the reach of the dipper. Then, the collector moved by about 5 m along the side and took a dip in the same way. In each paddy-field, the dipping was made ten times, and the number of summorosus larvae was recorded in each dip. The number of paddy-fields thus examined was 221 in total.

The method for estimating the relative density of $C$. $t$. summorosus larvae or the mean number of larvae per dip in
paddy-fields of a particular area in maximum efficiency will be discussed, based on the variances of the number of larvae within and bstween paddy-fields. As the distribution of the number of larvae per dip was proved to follow the negative binomial distribution with a common $k$ (Wada et al., 1971), the transformation of $y=\log (x+1)$ was adopted (Bartlett, 1947) to normalize the data in the 221 paddy-fields in each of which ten dips were taken, where $x$ is the number of larvae in each dip. Firstly the variances of $y$ within and between paddy-fields, $\sigma_{A}^{2}$ and $\sigma_{B}{ }^{2}$, was estimated, and secondly the number of dips within each paddy-field that should be taken for maximum efficiency with a given cost was determined. When the number of dips in each paddy-field was thus fixed, the relation between the number of paddy-fields examined and the estimation error of the relative density of larvae in the paddy-fields in an area as a whole was given.

## Results obtained

Letting the number of paddy-fields be $b$, the number of dips in a paddy-field $a$, the transformed number of larvae in a
dip $y$, the total of $y$ in a paddy-field $t$, the grand total of $y$ in $b$ paddy-fields $T$, then the correction term, $C$, and the
total sum of squares, $S S A B$, are calculated by

$$
\begin{align*}
& C=\frac{T^{2}}{a b}  \tag{1}\\
& S S A B=\Sigma y^{2}-C \tag{2}
\end{align*}
$$

and the between paddy-fields sum of squares, $S S B$, and the within paddy-fields sum of squares, $S S A$, are given by

$$
\begin{align*}
& S S B=-\frac{1}{a} \Sigma t^{2}-C  \tag{3}\\
& S S A=S S A B-S S B \tag{4}
\end{align*}
$$

The between paddy-fields mean square is given by $S S B /(b-1)$, which is an estimate of $\sigma_{A}^{2}+a \sigma_{B}{ }^{2}$, and the within paddy-fields mean square is given by $S S A$ $/ b(a-1)$, which is an estimate of $\sigma_{A}^{2}$, where $\sigma_{B}^{2}$ is the population variance of between paddy-fields samples and $\sigma_{A}^{2}$ is that of within paddy-fields samples. Thus we have $s_{B}{ }^{2}$ as the estimate of $\sigma_{B}{ }^{2}$ and $s_{A}^{2}$ as that of $\sigma_{A}^{2}$ by the following:

$$
\begin{align*}
& s_{B}^{2}=\frac{S S B /(b-1)-S S A / b(a-1)}{a} \\
& s_{A}^{2}=S S A / b(a-1) \tag{6}
\end{align*}
$$

From the present data $s_{B}{ }^{2}$ and $s_{A}{ }_{A}$ were calculated, where $b=221$ and $a=10$, as

$$
\begin{aligned}
& s_{B}^{2}=0.0116 \\
& s_{A}^{2}=0.0152
\end{aligned}
$$

The optimum number of dips per paddyfield, $n_{a}$, is approximately given (Southwood, 1966) by

$$
\begin{equation*}
n_{a}=\sqrt{\frac{C_{B} s_{A}^{2}}{C_{A} s_{B}^{2}}} \tag{7}
\end{equation*}
$$

where $C_{B}$ is the cost of moving to another paddy-field and $C_{A}$ is the cost of taking a dip within paddy-field. As it is thought to be appropriate that the both costs, $C_{B}$ and $C_{A}$, are the same, by substituting the calculated values of ${ }_{s_{B}}{ }^{2}$ and ${ }_{\boldsymbol{s}}{ }_{\boldsymbol{A}}$ we have

$$
\begin{aligned}
n_{a} & =\sqrt{\frac{0.0152}{0.0116}} \\
& \fallingdotseq 1
\end{aligned}
$$

This means that only one dip needs to be taken in each paddy-field to estimate the mean number of larvae in a particular area in maximum efficiency.

The variance of the mean of $y, s_{y}^{2}$, is given by

$$
\begin{equation*}
s_{\frac{2}{y}}=\frac{s_{B}^{2}}{n_{b}}+\frac{{ }^{s_{A}^{2}}}{n_{b} \frac{n_{a}}{}} \tag{8}
\end{equation*}
$$

where $n_{b}$ is the number of paddy-fields. The number of paddy-fields which must be taken depends on the degree of precision required. The number of paddy-fields, $n_{b}$, which needs to be taken to estimate the mean of $y$ with the variance of $d^{2}$, i. e., the standard error of $d$, can be calculated from

$$
\begin{equation*}
\frac{s_{B}^{2}}{n_{b}}+\frac{{ }_{s_{A}^{2}}^{2}}{n_{b} n_{a}}=d^{2} \tag{9}
\end{equation*}
$$

Here, $n_{a}=1$ and $s_{B}^{2}+s_{A}^{2}=0.0116$
$+0.0152=0.0268$.
Therefore we have

$$
\begin{equation*}
\frac{0.0268}{n_{b}}=d^{2} \tag{10}
\end{equation*}
$$

or

$$
\begin{equation*}
n_{b}=\frac{0.0268}{d^{2}} \tag{11}
\end{equation*}
$$

Thus we can calculate the values of $n_{b}$ with various $d$, as shown in Fig. 1. It is apparent that the number of paddy= fields to be examined, $n_{b}$, decreased with the increasing value of $d$. If we wish to estimate the mean of $y$ with the accuracy that the standard error, $s_{\vec{y}}$, taken as $d$ is about 0.005 , then the number of paddy-fields to be examined is as many as 1,000 , but if $s_{\bar{y}}$ is about 0.02 , then 70 paddy-fields will be enough.


Fig. 2. The relation between $\bar{y}$ and $D$ for various $n_{b}$, obtaind from the expression $n_{b}=0.0268 / D^{2} \bar{y}^{2}$.

Usually $d$ is expressed as $d=\mathrm{D}_{\bar{y}}$, where $\bar{y}$ is the mean of $y$, and $D$ is a decimal which shows the rate of $s_{\bar{y}}$ to $\bar{y}$. So, the expression (11) can be written as

$$
\begin{equation*}
n_{b}=\frac{0.0268}{D^{2} \bar{y}^{2}} \tag{12}
\end{equation*}
$$

From the expression (12) we have the relation between $\bar{y}$ and $D$ for various $n_{b}$, as shown in Fig. 2, and the relation between $\bar{y}$ and $n_{b}$ for various $D$, as shown in Fig. 3. Fig. 2 shows that when the same number of paddy-fields $\left(n_{b}\right)$ are examined, the value of $D$ decreases with increasing $\bar{y}$, and Fig. 3 shows that
when the value of $D$ is the same, the number of paddy-fields to be examined ( $n_{b}$ ) decreases with increasing $\bar{y}$. In other words, when $\bar{y}$ is small, $D$ is large (the level of accuracy is low), and vice versa. For example, in order to estimate the mean of $y(\bar{y})$ in the accuracy within $10 \%$ of $s_{\bar{y}}$ to $\bar{y}$, about 10 paddy-fields are enough when $\bar{y}$ is 0.5 , but more than 1,000 paddy-fields are required when $\bar{y}$ is 0.05 .

As Fig. 2 and Fig. 3 were based on the values of $y$ which had been transformed from the number of larvae in a dip ( $x$ ) by $y=\log (x+1)$, it may be convenient


Fig. 3. The relation $\bar{y}$ and $n_{b}$ for various $D$, obtained from the expression $n_{b}=0.0268 / D^{2} \bar{y}^{2}$.
to convert back the values of $\bar{y}$ to the original figures of $\bar{x}$ by using the relation $\bar{y}=\log (\bar{x}+1)$ and the relation between $\bar{x}$
and $D$ for various $n_{b}$ and the relation between $\bar{x}$ and $n_{b}$ for various $D$ were


Fig. 4. The relation between $\bar{x}$ and $D$ for various $n_{b}$, obtained by converting $\bar{y}$ into $\bar{x}$ in Fig. 2.


Fig. 5. The relation between $\bar{x}$ and $n_{b}$ for various $D$, obtained by converting $\bar{y}$ into $\bar{x}$ in Fig. 3.
given in Fig. 4 and Fig.5, As $\bar{x}$ increases when $\bar{y}$ increases, the similar relations found in Figs. 2 and 3 are seen in Figs. 4 and 5 , namely when $\bar{x}$ (the mean number of larvae per dip) is small the level of accuracy is low. Figs. 4 and 5 may be used to know the approximate number of paddy-fields which need to be examined in a given accuracy or to know the level of accuracy when a given number of paddy-fields are examined, in estimating the mean number of larvae per dip in a particular area. How many paddy-fields must be chosen in planning the larval survey of $C . t$. summorosus in paddyfields in a particular area depends on the value of $D$ which is determined by the purpose of the survey and the abundance of larvae. If we take $10 \%$ as $D$, as usual, then the approximate number of paddy-fields to be examined is 400 when the mean number of larvae per dip is around 0.2 on an average, 90 when the mean is around 0.5 and 30 when the mean is around 1.0. In usual breeding situation of C. $t$. summorosus larvae in Nagasaki area, 100 paddy-fields may be sufficient for most purposes in relation to Japanese encephalitis epidemiology.

As mentioned above, the relative density of larvae in water-filled paddy-fields can be estimated with a given precision, however the total number of $C, t$. summorosus larvae breeding in a particular area as a whole is often required for the seasonal changes. The total number depends on the density of larvae in the water-lodged paddy-fields which are the main breeding place for this mosquito larvae, and also on the surface area of the whole paddy-fields in the area.

Accordingly, for the index of the total number of larvae, it is advisable to multiply the mean number of larvae per dip by the rate of water-lodged surface area in the paddy-fields in the area.

In the present paper, the number of larvae per dip ( $x$ ) was transformed into $y=\log (x+1)$ so that the variance of the transformed variate was independent of the level of mean, and then the estimation method of relative density of the larvae was discussed. Here, the relation between the variances before and after transformation will be mentioned.

If a variate $x$ is transformed into $y$ by $y=f(x)$, then

$$
y=f(m)+f^{\prime}(m)(x-m)+\cdots \cdots \cdots, \quad \text { (13) }
$$

where $m$ is the expectation of $x$. Therefore, approximately

$$
\begin{equation*}
(y-M)=f^{\prime}(m)(x-m) \tag{14}
\end{equation*}
$$

where $M$ is the expectation of $y$. Letting the variance of $y$ be $\sigma^{2}$ and that of $x$ be $\sigma^{2}$, we have

$$
\begin{equation*}
o^{\prime 2}=\left\{f^{\prime}(m)\right\}^{2} \sigma^{2} \tag{15}
\end{equation*}
$$

If $\sigma^{2}$ is related to $m$ as $\sigma^{2}=\lambda^{2} m^{2}$ ( $\lambda$ is a constant), $\sigma^{2}$ becomes nearly constant by adopting the transformation $y=$ $\log (x+1)$, and in negative binomial distribution this transformation seems likely to prove good enough in many cases (Beall, 1942). Since $f^{\prime}(x)=\log e /(x+1), f^{\prime}(m)$ $=0.4343 /(m+1)$, and accordingly from expression ( 1 ( 5 ),

$$
\begin{equation*}
\sigma^{\prime 2}=0.189 \sigma^{2} /(m+1)^{2} . \tag{16}
\end{equation*}
$$

Thus, we have the approximate relation between the variances before and after the transformation, $\sigma^{2}$ and $o^{2}$. If $m$ is fairly large, then $\sigma^{2}$ comes near to 0.189 $\lambda^{2}$ since $\sigma^{2}=\lambda^{2} m^{2}$.

## References

1）Bartlett，M．S．：The use of transforma－ tions．Biometrics，3：39－50， 1947.

2）Beall，G．：Transformation of data from entomological field experiments so that the analysis of variance becomes applicable．Biome－ trika， $32: 243-262,1942$.
3）Southwood，T．R．E．：Ecological methods with particular reference to the study of insect
populations．London， $391 \mathrm{pp.,1966}$.
4）Wada，Y．，Mogi，M．and Nishigaki，J．： Studies on the population estimation for insects of medical importance．III．Sequential sampling technique for Culex tritaeniorhynchus summorosus larvae in the paddy－field．Trop．Med．，13（1）： 16－25， 1971.

## 衛生害虫の個体数の推定に関する研究

IV．ある地域の水田全体のコガタアカイエカ幼虫の
相対密度を推定する方法

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柄杓当りのコガタアカイエカ幼虫数 $x$ を $y=\log (x+1)$ 式で変換した後に，水田内及び水田間の幼虫数の分散を求め，これを基として，ある地域全体に棲息する幼虫の相対密度を推定する方法を研究した。その結果，最も能率よく推定するには，1枚の水田でのすくいとり回数を1回とし，その代 りに調査する水田数を増すのがよいことがわかった。その場合，調査水田数が多い程推定の精度は勿論高く，水田数が同じ場合には平均幼虫密度が高い程精度は高い。


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