# Studies on the population estimation for 

## insects of medical importance．

# II．A method of estimating the population size of larvae of Aedes togoi in the tide－water rock pool．${ }^{1)},{ }^{2)}$ 

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## 衡生害虫の個体数の推定に関する研究．2．海岸のロックプールのトゥゴウヤブカ幼虫数の推定方法．和田羲人，長崎大学医学部医助物学教室（主任：大森南三郎教授）

## Introduction

The larva of Aedes togoi is usually found in large numbers in tide－water rock pools of the seaside in Kyushu，Japan．To determine the total number of larvae within a given area is of fundamental importance in the ecology of this species．For this purpose，it may be necessary to know the number of pools within the area and further to estimate the number of larvae in each pool．The number of larvae collected during a unit time may be con－ sidered as an index of the larval density in a pool． But，even if the same number of larvae is collected in different pools，the total number of larvae will vary with pools according to the size of the pool， the distribution pattern of larvae and so on．

The writer reported in the previous paper（Wada， 1962）that the total number of mosquito larvae in a fertilizer pit can be estimated by the removal method．It seemed fruitful to apply the same principle to the larva of Aedes togoi in the tide－water
rock pool．In the present paper，the writer wishes to inform that the number of larvae of Aedes togoi can safely be estimated by the removal method， and also to discuss on another type of removal method obtaining the estimate for the population size through the relation between the collecting rate of larvae，and the size of the pool and the larval abundance，which was revealed to be equally useful and easier than the above method．

Before going further，the writer wishes to express his hearty thanks to Prof．N．Omori of Depart－ ment of Medical Zoology，Nagasaki University School of Medicine for constant guidance and en－ couragement in the course of this study．The writer is much indebted to Prof．S．Utida and Assistant Prof．T．Kono of Entomological Laboratory，Kyoto University for their criticisms and also to Mr．T． Oda，Dr．S．Ito and Dr．O．Suenaga of Depart－ ment of Medical Zoology，Nagasaki University School of Medicine for much help in the field work．

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## The place and method of collecting immature individuals of Aedes togoi

Collections of the larvae and pupae of Aedes togoi were carried out in tide-water rock pools along the seaside in Nagate-cho and Sakiyama-cho, Fukue City, Nagasaki Prefecture in 1961. Unless otherwise stated, "larva and pupa" is simply written as "larva" in the following. In each pool, the larvae were collected by a pipette which was made by attaching a rubber ball-teat to glass tubing of 4 mm in inside diameter and 15 cm in length, and the
number of larvae collected was recorded every one minute, except for a few cases in which the number was recorded every five minutes. In this paper one minute catch will be called a unit catch and five successive unit catches a super-unit catch. Five minute catch is nothing but a supper-unit catch.
A few collections were also made by a dipper of 15 cm in diameter and 3 cm in depth for comparison with the pipette collection. The pipette collection was usually found satisfactory for the larva of Aedes togoi in the rock pool, while the dipper was.

Table 1 The total number of larvae collected in pools of various sizes by different catching methods.

| (Group) <br> Catching method | Collec- <br> tion <br> Number | No. of unit catches | Total No. of larvae collected | Size of the pool |  | Date <br> of collection |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  | Surface area ( $\mathrm{cm}^{2}$ ) | Depth (cm) |  |
| (A) <br> One-minute catches by pipette were made 11 or more times | 1 | $1 \times 20$ | 88 | 1246 | 4 | Aug. 9 |
|  | 2 | $1 \times 30$ | (723) | 4557 | 10 | " |
|  | 3 | $1 \times 40$ | 722 | 1805 | 10 | Aug. 20 |
|  | 4 | $1 \times 11$ | 383 | 330 | 5 | Sept. 6 |
|  | 5 | $1 \times 55$ | 1656 | 500 | 8 | Sept. 7 |
|  | 6 | $1 \times 11$ | 159 | 523 | 2 | Sept. 8 |
|  | 7 | $1 \times 18$ | 286 | 1035 | 6 | " |
| (B) <br> One-minute catches by pipette were made 10 or less times | 8 | $1 \times 6$ | 87 | 378 | 6 | Aug. 19 |
|  | 9 | $1 \times 3$ | 49 | 154 | 2 | Sept. 6 |
|  | 10 | $1 \times 7$ | 8 | 1456 | 5 | Sept. 7 |
|  | 11 | $1 \times 9$ | 63 | 1170 | 8 | " |
|  | 12 | $1 \times 6$ | 160 | 746 | 5 | " |
|  | 13 | $1 \times 5$ | 17 | 9450 | 4 | Sept. 8 |
| (C) | 14 | $5 \times 10$ | (2361) | 1458 | 10 | Mar. 21 |
| Five-minute catches by pipette were made 3 to 10 times | 15 | $5 \times 3$ | 54 | 687 | 5 | Aug. 9 |
|  | 16 | $5 \times 7$ | 180 | 567 | 7 | Aug. 11 |
|  | 17 | $5 \times 5$ | 207 | 4200 | 4 | Oct. 29 |
|  | 18 | $5 \times 8$ | 170 | 930 | 3 | Dec. 26 |
| (D) <br> Catches by dipper were made 43 to 96 times |  |  |  |  |  |  |
|  | 19 | $1 \times 43$ | 317 | 4800 | 18 | Aug. 9 |
|  | 20 | $1 \times 96$ | 534 | 4540 | 10 | Aug. 19 |

Remarks: (1) The number of collected larvae is nearly equal to, or the approximate value of, the population size, while that in parentheses represents only a part of the whole larvae found in a pool (see text for further explanation).
(2) Collection No. 20 was made in the pool No. 2 on the other date.
rather suitable when the water of the pool is deeper than about 15 cm . Here, one dipping will also be called a unit catch and five successive dippings a super-unit catch.

In collecting larvae by the pipette, care was taken to catch as many larvae as possible within a given time. In dipper collections, the site of larval abundance was selected.

With each of eighteen pipette collections and two dipper ones, the number of unit catches, the total number of collected larvae, the size of the pool, and the date of the collection are given in Table 1. The eighteen pipette collections are divided into three groups, A, B, and C. In Group A, the unit catches (one minute catches) were made eleven or more times, in $B$ the unit catches were made ten or less times, and in C the super-unit catches (five minute catches) were made. In all collections excepting Nos. 2 and 14, catches were continued till almost all the larvae in a pool were collected, and therefore the total number of collected larvae shown in Table 1 is nearly equal to the population size of larvae in each pool. In collection Nos. 2 and 14, the total number of collected larvae is quite different from respective population size, because catches were suspended in spite of the existence of many larvae.

## Brief account for the removal method

Theoretical considerations on the removal method have been given in the writer's previous paper (Wada, 1962) and therefore only an account necessary for understanding about the following writings will be referred to.

The removal method is one of the methods for obtaining the estimate for the total number of animals in a given space. Assuming that the number of larvae of Aedes togoi caught in a unit catch in a pool is proportional to the number of larvae yet to be collected, the following equation may be derived,

$$
\begin{equation*}
A_{n}=a\left(S-Y_{n \cdot 1}\right) \tag{1}
\end{equation*}
$$

where $A_{\mathrm{n}}$ is the number of larvae caught in the $n$th unit catch, $S$ the population size of larvae in the pool, $Y_{n \cdot 1}$ the accumulated number of larvae caught
till the ( $n-1$ )th unit catch, $a$ a constant which shows the rate of the larvae caught in a unit catch. Next, the equation for the super-unit catch which consists of five successive unit catches is given as follows,

$$
A(5)_{n}=\left\{1-(1-a)^{5}\right\}\left(S-Y(5)_{n \cdot 1}\right) \cdots \cdots \cdot(2)
$$

where $A(5)_{\mathrm{n}}$ is the number of larvae caught in the $n$th super-unit catch, $Y(5)_{n \cdot 1}$ the accumulated number of larvae caught till the $(n-1)$ th super-unit catch.

The relation between $A_{\mathrm{n}}$ and $\boldsymbol{Y}_{\mathrm{n} \cdot 1}$ or between $A(5)_{n}$ and $Y(5)_{n-1}$

In Fig. 1, the relation between the number of larvae caught in the $n$th unit or super-unit catch ( $A_{\mathrm{n}}$ or $A(5)^{n}$ ) and the accumulated number of larvae obtained till the $(n-1)$ th unit or superunit catch $\left(Y_{n \cdot 1}\right.$ or $\left.Y(5)_{n-1}\right)$ is illustrated with each of the twenty collections shown in Table 1. In Groups $\mathrm{A}, \mathrm{B}$, and D the points of $A_{\mathrm{n}}$ to $Y_{\mathrm{n} \cdot 1}$ are plotted, while in A and D the points of $A(5)_{\mathrm{n}}$ to $Y(5)_{\mathrm{n} \cdot 1}$ are also plotted. In Goup C, naturally only the points of $A(5)_{\mathrm{n}}$ to $Y(5)_{\mathrm{n} \cdot 1}$ are plotted.

In Fig. 1, a linear relation between $A_{\mathrm{n}}$ and $Y_{\mathrm{n} \cdot 1}$ or between $A(5)_{n}$ and $Y(5)_{n \cdot 1}$ is clearly recognized in each collection. This may justify the assumption that the number of larvae captured in a unit catch or in a super-unit catch is proportional to the number of larvae yet to be collected.

Then, regression lines were drawn by the method of weighted least squares after Zippin, 1956. Here, the weight of each point is inversely proportional to $\left(S^{\prime}-Y_{\mathrm{n} \cdot 1}\right)$ or $\left(S^{\prime}-Y(5)_{\mathrm{n} \cdot 1}\right)$, where $S^{\prime}$ is the estimate for the population size as a first approximation. Although the total number of collected larvae in Table 1 was regarded as nearly equal to the population size, about one per cent of the total number remained in most cases. So, the total number of collected larvae plus its one per cent value was taken as $S^{\prime}$ in all collections, except for Nos. 2 and 14 in which the $Y(5)_{n \cdot 1}$ intercept of the line drawn by eye was taken as $S^{\prime}$, because the total number of collected larvae was quite different from the population size as stated above.

In cases when the larvae are concentrated in distribution, the number of larvae to be collected is larger; while they are scattered by disturbance

Fig. 1 The relation between the number of larvae obtained in the $n$th unit or super-unit catch ( $A_{\mathrm{n}}$ or $A(5)_{\mathrm{n}}$ ) and the number of larvae obtained till the ( $n-1$ )th unit or super-unit catch $\left(Y_{n-1}\right.$ or $\left.Y(5)_{n-1}\right)$ is shown by broken line or solid line. (Group A)


Fig. 1 Continued. (Groups B and C)





$$
Y_{n-1} \text { or } Y(5)_{n-1}
$$

Fig. 1 Continued. (Group D)


Remarks: (1) Solid and open circles represent unit and super-unit catches, respectively.
(2) As to the regression lines drawn, see text.
in catching larvae, the numbers to be collected thereafter become abruptly smaller. This may be the reason why the first unit catch, $A_{1}$, in collection Nos. 6 and 7 and the first two unit catchcs, $\Lambda_{1}$ and $A_{2}$, in No. 5 were greater than the numbers expected from the respective regression line.

It takes about two or three seconds in time for collecting larvae by one pipetting. The maximum number of the pipettings of larvae within one minute is, therefore, limited usually to about 25 , even in the case of plenty of larvae being found to be easily collected. So, if the larvae are very abundant and are scattered in distribution, the numbers to be collected may be smaller than those expected from the line. Examples for this may be seen in Fig. 1 in Collection Nos. 3, 5, and 6, in
the middle part, in which a series of points for the unit catches runs below off the line and in parallel to $Y_{n \cdot 1}$ axis.

## Estimation of the population size by regression method

$Y_{n \cdot 1}$ or $Y(5)_{n \cdot 1}$ intercept of the regression line in Fig. 1 by the method of weighted least squares will give the estimate for the population size of larvae in the pool. These will be called ESa and $E S(5) a$, respectively.

The above regression lines are obtained from all catches made in each pool. A regression line, however, can be drawn from at least three points of $A_{\mathrm{n}}$ to $Y_{\mathrm{n} \cdot 1}$. Then, the population size was tried to estimate by $\mathrm{Y}_{\mathrm{n}-1}$ intercept of the regression

Table 2 Notation of estimate for the population size.

| Abbreviation <br> of <br> estimate | Way of obtaining estimate |  |
| :---: | :---: | :---: |
| $\mathrm{ES}_{3}$ | Each estimate is <br> given by $Y_{\mathrm{n}-1}$ <br> intercept of the <br> regression line <br> obtained from | the first three unit catches. |
| $\mathrm{ES}_{4}$ | the first four unit catches. |  |
| $\mathrm{ES}_{5}$ | the first five unit catches. |  |
| ESa | Each estimate is <br> given by Y(5) <br> intercept of the <br> regression line <br> obtained from | the first three super-unit catches. |
| $\mathrm{ES}(5)_{3}$ | all the super-unit catches. |  |
| $\mathrm{ES}(5) \mathrm{a}$ | all thit catches. |  |

Table 3 Kind of estimates for the population size in each pool of the indicated collection number, obtained by the method given in Table 2.

| Collection No. | Kind of estimates |
| :---: | :---: |
| 1 | $\mathrm{ES}_{3}, \mathrm{ES}_{4}, \mathrm{ES}_{5}, \mathrm{ESa}, \mathrm{ES}(5)_{3}, \mathrm{ES}(5) \mathrm{a}$ |
| 2 | $\mathrm{ES}_{3}, \mathrm{ES}_{4}, \mathrm{ES}_{5}, \mathrm{ESa}, \mathrm{ES}(5)_{3}, \quad \mathrm{ES}(5) \mathrm{a}$ |
| 3 | $\mathrm{ES}_{8}, \mathrm{ES}_{4}, \mathrm{ES}_{5}, \mathrm{ESa}, \mathrm{ES}(5)_{3}, \mathrm{ES}(5) \mathrm{a}$ |
| 4 | $\mathrm{ES}_{3}, \mathrm{ES}_{4}, \mathrm{ES}_{5}, \mathrm{ESa}, \mathrm{ES}(5)_{3}, \mathrm{ES}(5) \mathrm{a}$ |
| 5 | $\mathrm{ES}_{3}, \mathrm{ES}_{4}, \mathrm{ES}_{5}, \mathrm{ESa}, \mathrm{ES}(5)_{3}, \mathrm{ES}(5) \mathrm{a}$ |
| 6 | $\mathrm{ES}_{3}, \mathrm{ES}_{4}, \mathrm{ES}_{5}, \mathrm{ESa}, \mathrm{ES}(5)_{3}(=\mathrm{ES}(5) \mathrm{a})$ |
| 7 | $\mathrm{ES}_{3}, \mathrm{ES}_{4}, \mathrm{ES}_{5}, \mathrm{ESa}, \mathrm{ES}(5)_{3}, \quad \mathrm{ES}(5) \mathrm{a}$ |
| 8 | $\mathrm{ES}_{3}, \mathrm{ES}_{4}, \mathrm{ES}_{5}, \mathrm{ESa}$, |
| 9 | $\mathrm{ES}_{3}(=\mathrm{ESa})$ |
| 10 | $\mathrm{ES}_{3}, \mathrm{ES}_{4}, \mathrm{ES}_{5}, \mathrm{ESa}$ |
| 11 | $\mathrm{ES}_{3}, \mathrm{ES}_{4}, \mathrm{ES}_{5}, \mathrm{ESa}$ |
| 12 | $\mathrm{ES}_{3}, \mathrm{ES}_{4}, \mathrm{ES}_{5}, \mathrm{ESa}$ |
| 13 | $\mathrm{ES}_{3}, \mathrm{ES}_{4}, \mathrm{ES}_{5}$ (=ESa) |
| 14 | $\operatorname{ES}(5)_{3}, \quad \operatorname{ES}(5) \mathrm{a}$ |
| 15 | $\operatorname{ES}(5)_{3}(=\operatorname{ES}(5) \mathrm{a})$ |
| 16 | $\mathrm{ES}(5)_{3}, \quad \mathrm{ES}(5) \mathrm{a}$ |
| 17 | $\mathrm{ES}(5)_{\mathbf{3}}, \quad \mathrm{ES}(5) \mathrm{a}$ |
| 18 | ES(5) ${ }_{\mathbf{3}}, \quad \mathrm{ES}(5) \mathrm{a}$ |
| 19 | $\mathrm{ES}_{3}, \mathrm{ES}_{4}, \mathrm{ES}_{5}, \mathrm{ESa}, \mathrm{ES}(5)_{3}, \mathrm{ES}(5) \mathrm{a}$ |
| 20 | $\mathrm{ES}_{3}, \mathrm{ES}_{4}, \mathrm{ES}_{5}, \mathrm{ESa}, \mathrm{ES}(5)_{3}, \mathrm{ES}(5) \mathrm{a}$ |

line drawn from the first three, four, or five unit catches ; these will be called $E S_{3}, E S_{4}$, and $E S_{5}$, respectively. Similarly, the estimate of the population size was obtained by $Y(5)_{n \cdot 1}$ intercept of the line from the first three super-unit catches, being called $E S(5)_{3}$. Notation of estimates for the
population size is summarized in Table 2. The kinds of estimates made for each collection are shown in Table 3.

In Group A, two regression lines from all catches in a pool are drawn in Fig. 1, one for the unit catch and another for the super-unit catch, and
accordingly two estimates for the population size (ESa and $E S(5) a$ ) are obtained. The relation between $E S a$ and $E S(5) a$ is illustrated in Fig. 2, showing an almost complete coincidence with each other.

Fig. 2 The relation between two estimates for the population size given by $Y_{n-1}$ and $Y(5)_{n-1}$ intercepts of the regression lines from the all unit and super-unit catches ( $E S a$ and $E S(5) a$ ) in the collections of Group A.


Remarks : Solid and open circles represent the pipette and the dipper collection, respectively.
The relation between the total number of collected larvae shown in Table 1 and the estimate for the population size given by $E S a$ or $E S(5) a$ is shown in Fig. 3 (except for Nos. 2 and 14), where in Group A $E S(5) a$ is conveniently taken as a representative of the estimate. The figure shows that the latter is extremely near to the former. This may be to justify the estimation method for the population size. It is the next problem to determine that the estimate to be obtained from the first 3 or how many unit catches could be sufficiently useful as a representative of the population size.

Then, the relation between the total number of collected larvae and each of the estimates given by $E S_{3}, E S_{4}, E S_{5}$, and $E S(5)_{3}$ is illustrated in Figs. 4, 5, 6, and 7 respectively. Here, in col-

Fig. 3 The relation between the total number of collected larvae and the estimate for the population size given by $Y_{n 1}$ or $Y(5)_{n-1}$ intercept of the regression line ( $E S a$ or $E S(5) a$ ).


Remarks : (1) As to solid and open circles, see Remarks of Fig. 2.
(2) In Group A, B, and DES(5)a and in C ESa are taken, respectively.
lection Nos. 2 and 14, ES(5)a is taken instead of the total number (This is to be repeated in the following). These figures show that the estimate obtained from a greater number of unit catches approaches more closely to the total number of collected larvae. However, when the total number of collected larvae is considerably large, the number is found, in most cases, to exceed the estimate, by the probable reason that the first one or two catches are often larger than those expected from the regression line as stated above.
Next in Table 4, the number of unit catches (time in minutes or number of dippings) necessary for obtaining the estimate of population size represented by the total number of collected larvae within 10,20 or 40 per cent error is tabulated.

Table 4 shows varying results with different pools. In collection Nos. 8, 9, and 11, three unit catches or three minutes are enough to estimate the

Fig. 4 The relation between the total number of collected larvae and the estimate for the population size from the first three unit catches ( $E S_{3}$ ).


Remarks : As to solid and open circles, see Remarks of Fig. 2.

Fig. 5 The relation between the total number of collected larvae and the estimate for the population size from the first four unit catches ( $E S_{4}$ ).


Remarks: As to solid and open circles, see Remarks of Fig. 2.

Fig. 6 The relation between the total number of collected larvae and the estimate for the population size from the first five unit catches ( $E S_{5}$ ).


Remarks : As to solid and open circles, see Remarks of Fig. 2.

Fig. 7 The relation between the total number of collected larvae and the estimate for the population size from the first three super-unit catches $\left(E S(5)_{3}\right)$.


Remarks : As to solid and open circles, see Remarks of Fig. 2.

Taßle 4 Time in minutes (No. of unit catches) needed for estimating the population size (approximate value) within the estimation error of the indicated percentage, by the regression method.

| Collection <br> Number | Approximate value of population size | Time in minutes necessary for estimation within the error of |  |  |
| :---: | :---: | :---: | :---: | :---: |
|  |  | 10\% | $20 \%$ | 40\% |
| 1 | 88 | 6 | 3 |  |
| 2 | 1922* | 30 | 30 | 5 |
| 3 | 722 | 40 | 35 | 35 |
| 4 | 383 | 6 | 3 |  |
| 5 | 1656 | 20 | 20 | 15 |
| 6 | 159 | 5 | 5 | 3 |
| 7 | 286 | 8 | 7 | 4 |
| 8 | 87 | 3 |  |  |
| 9 | 49 | 3 |  |  |
| 10 | 8 | 6 | 6 | 4 |
| 11 | 63 | 3 |  |  |
| 12 | 160 | 5 | 5 | 3 |
| 13 | 17 | 5 | 4 | 3 |
| 14 | 3619* | 45 | 35 | 25 |
| 15 | 54 | $<15$ |  |  |
| 16 | 180 | $<15$ |  |  |
| 17 | 207 | $<15$ |  |  |
| 18 | 170 | $<15$ |  |  |
| 19 | 317 | 35 | 35 | 9 |
| 20 | 534 | 50 | 25 | 20 |

Remarks :(1) * represents the estimate for population size, given by $E S(5)$ a shown in Table 2.
(2) Time in minutes represents the time required for the estimation of the approximate value of population size within the error of the indicated percentage from the regression line from the first 3 to 10 points of unit catches or from the first 3 or more points of super unit catches.
(3) In collection Nos. 19 and 20, for "time in minutes" read "the number of dippings".
(4) Time in minutes indicated by " $<15$ " shows that the estimate obtained from the first 3 super-unit catches approaches already to the approximate value of the population size within the $10 \%$ error.
population size within the error of 10 per cent, while 50 dippings in collection No. 20, and 40 minutes in No. 3 are required for obtaining the estimate within the same percentage error. More minutes or dippings are generally needed for the estimation within a given error in pools having a greater number of larvae. In pipette collection, fifteen minutes (three super-unit catches) may
usually be enough to estimate the population size in pools having a moderate number of larvae within the error of 10 per cent, but in pools of larger population size, estimation precision is not so good as seen in Fig. 7. With dipper collection, further studies will be necessary.

## The relation of the collecting efficiency to the size of the pool and the larval abundance

In equation (1), the absolute value of the inclination of the regression line for the unit catch in Fig. 1 represents the estimate of constant $a$ which is the rate of larvae to be captured in a unit catch. Similarly, in equation (2), the absolute value of the inclination of the line for the super-unit catch represents the estimate of constant $1-(1-a)^{5}$, and therefore the estimate for $a$ can also be obtained by letting the absolute value of the inclination be equal to $1-(1-a)^{5}$.

In each of collections in Groups A and D, two estimates for $a$ are obtained from two regression lines in Fig. 1, as in the case of the estimation of the population size. The relation between the two estimates of $a$ thus obtained is shown in Fig. 8, showing a noticeable similarity with each other. Accordingly, in the following description, as the estimate

Fig. 8 The relation between two estimates for the constant $a$ in equation (1) or (2) obtained from two regression lines in each collection of Group A.


Remarks : (1) As to solid and open circles, see Remarks of Fig. 2.
(2) For further explanation for the two estimates, see text.
of $a$ in Groups A and D, that from super-unit catch will be conveniently used. The estimates for $a$ in the collection No. 2 by pipette and No. 20 by dipper made in the same pool but on different date are 0.0156 and 0.0323 , respectively. The condition of the pool was not so much different on the days when the both collections were made. Therefore, it could be considered that the unit catch by pipette is roughly equal to that by dipper.

It is questionable whether or not the collecting efficiency represented by the estimate of $a$ varies with the size of the pool or the number of larvae in it. So, in the first the relation between the collecting efficiency and the surface area of the pool is examined in Fig. 9. The figure shows that the relation is roughly represented by a rectangular

Fig. 9 The relation between the collecting efficiency represented by the estimate for the constant $a$ and the surface area ( $\mathrm{cm}^{2}$ ) of the pool.


Remarks: As to solid and open circles, see Remarks of Fig. 2.
hyperbola, though a point on the right of the figure is far apart. Secondly, the relation of the efficiency to the product of the surface area by the depth of the pool is examined in Fig. 10, with the result that clearer hyperbolic relation is observed than in Fig. 9. Thirdly, Fig. 11 gives the relation of the efficiency to the number of larvae collected during the first five minutes $\left(A(5)_{1}\right)$ taken as an index for the larval abundance in each pool, between

Fig. 10 The relation between the collecting efficiency represented by the estimate for the constant $a$ and the product of the surface area $\left(\mathrm{cm}^{2}\right)$ by the depth $(\mathrm{cm})$ of the pool.


The product of the area by the depth
Remarks : As to solid and open circles, see Remarks of Fig. 2.

Fig. 11 The relation between the collecting efficiency represented by the estimate for the constant $a$ and the number of larvae collected during the first five minutes $\left(A(5)_{1}\right)$.

$A(5)_{1}$
Remarks : As to solid and open circles, see Remarks of Fig. 2.
which a hyperbolic relation is also observed. Finally, the relation of the efficiency to the product of three parameters, surface area, depth, and $A(5)_{1}$, is presented in Fig. 12, in which the hyperbolic relation is much more clearly shown than the above three cases. It may reasonably concluded from these figures that the larger and the deeper the pool is and the greater the number of larvae per pool is, the lower, in general, the collecting efficiency is.

Fig. 12 The relation between the collecting efficiency represented by the estimate for the constant $a$ and the product of three parameters, the surface area $\left(\mathrm{cm}^{2}\right)$, the depth (cm) of the pool, and $A(5)_{1}$.


The product of three parameters
Remarks : As to solid and open circles, see Remarks of Fig. 2.

## A more simplified method for obtaining the estimate for the total number of larvae

The hyperbolic relation is most clearly shown between the collecting efficiency represented by the estimate of $a$, and the product of the three parameters mentioned above. From the nature of the rectangular hyperbola, it is expected that the relation of the reciprocal of the estimate of $a$ to the product shows a linear regression passing the origin (Fig. 13). Here, however, a point derived from dipper collection is far apart from the regression. There may exist somewhat different relations in the dipper collection and the pipette one. Accordingly, in the following, further discussion will be made using only the points derived from catches by pipette, because in these experiments collections were made mainly by pipette.

Then, a regression line in the form of $Y=k X$ is drawn by the method of least squares, where $Y$ is the reciprocal of the estimate of $a, X$ the product of three parameters, and $k$ a constant. This is represented as

$$
\begin{equation*}
\mathrm{Y}=0.007956 \mathrm{X} \tag{3}
\end{equation*}
$$

In the above, the population size of larvae was

Fig. 13 The relation between the reciprocal of the estimate for the constant $a(Y)$ and the product of three parameters, the surface area $\left(\mathrm{cm}^{2}\right)$, the depth (cm) of the pool, and $A(5)_{1},(X)$.


Remarks : (1) As to solid and open circles, see Remarks of Fig. 2.
(2) As to the regression line passing the origin, see text.
estimated by regression method. However, it is troublesome to continue the collection of larvae till the clear-cut regression line of $A_{\mathrm{n}}$ to $Y_{\mathrm{n} \cdot 1}$ could be obtained. Moreover, the period in minutes necessary for this may be different with pools, as seen from Table 4. However, if a five minute catch is carried out and the value of $X$ is determined in a given pool, then the vaule of $Y$ could be obtained from equation (3). The reciprocal of the value of $Y$ is another estimate of $a$.

By letting $n$ be equal to 1 in equation (2), the following is derived,

$$
\begin{equation*}
S=\frac{A(5)_{1}}{1-(1-a)^{5}} \tag{4}
\end{equation*}
$$

By applying the above estimate of $a$ and the number of larvae obtained in the first five minutes $\left(A(5)_{1}\right)$ to equation (4), another estimate for the population size of larvae $(S)$ is given.

The relation of the total number of collected larvae shown in Table 1 to the estimate for $S$ thus obtained is illustrated in Fig. 14. The figure

Fig. 14 The relation between the total number of collected larvae and the estimate for the population size obtained by equation (4).


Estimate for the population size by equation(4)
indicates that the population size can be well expressed more accurately by the estimate obtained by equation (4) than by $E S(5)_{3}$ (cf. Fig. 7), except for collection No. 5. Pool No. 5 was $500 \mathrm{~cm}^{2}$ in surface area and 8 cm in depth, one third of which was shallow, 2 cm or less in depth, having few larvae. If two thirds of $500 \mathrm{~cm}^{2}$ are taken as surface area, the value of $X$ and accordingly the value of $Y$ are both reduced by one third. Using the estimate of $a$ from this reduced value, the population size is estimated by equation (4) at 2455 instead of the former value of 3526 . This may be a reason for the over-estimation of the population size. The first super-unit catch, $A(5)_{1}$, is considerably larger than the number expected from the regression line as seen in Fig. 1; this may be another reason for the over-estimation.

## Changes in the rate of the number of individuals of immature stages with progress of catches

In pipette collection, care was taken to catch as many larvae as possible within a given time,

Fig. 15 Changes in the rate of individuals of each stage to the whole larvae and pupae, obtained till a certain time of super-unit catch.


No. of super-unit catches
Remarks : I, II, III, IV, and P in the figure indicate the first, the second, the third, and the fourth instar larvae, and pupae respectively.
namely, larvae were pipetted from the site of higher larval concentration in a pool. It appears that the younger larvae may be less easy in collection owing to their small size than the older larvae and pupae. Then the rates of individuals of each stage to the whole larvae plus pupae captured till a certain time in collection of super-unit catch are illustrated in Fig. 15 with only the collections in which four or more super-unit catches were made.

The figure shows that in most cases the rate of older larvae is slightly higher and that of younger ones is slightly lower at the beginning of catches than in the later ones. However, in the case when younger and older larvae mingle completely in a pool, little change in the rate may happen, as seen in collection No. 14. In dipper collections this may be also the case, but more investigations will be necessary to obtain data enough to compare the trend of change in the two collection methods.

## Discussions

It was concluded in an earlier paragraph that the collecting efficiency generally became lower with the increase in surface area, depth of the water and also in the number of larvae. Additionally, there are some factors affecting the efficiency. For example, if the water of the pool is thick, or if the bed of it is dark in color or rugged in structure, the efficiency will be lowered owing to the difficulty of finding larvae. Further, if the pool is ill situated for collection, the efficiency will also be lower than in the well situated pool. Though such factors might have any influence on the estimation of the population size, the introduction of these factors may result in much more confusion. So, using only the above three factors, the estimation of the collecting efficiency and of the population size was made. Individual variation of the collector in capturing larvae is not considered here, as the collections were made only by the writer himself. Therefore, the error seen in Fig. 14 in the estimation of the population size by equation (4) may be attributable partly to the factors being not taken into consideration.

The estimate for the population size from equation
(4) by only one super-unit catch stands comparison in precision with that by regression method using the first three super-unit catches. Of course, using more catches, the estimate by regression method will become much better in precision than that by using equation(4). However, the estimation method by equation (4) is obviously more convenient by the reasons of being nearly sufficient in precision and being economical in time.

## Summary

Estimation for population size of immature individuals of Aedes togoi was tried with satisfactory result, in 1961 in Fukue City, Nagasaki Prefecture. The larva of this aedine mosquito breeds in tidewater rock pools of various sizes along rocky seaside. In the pool, the larvae were collected by a pipette, and the number was recorded every minute (one minute catch), or every five minutes (five minute catch). One minute catch is called a unit catch, and both five successive unit catches and five minute catch are called a super-unit catch.

Between the number of larvae obtained in the $n$th unit or super-unit catch and the number of larvae obtained till the $(n-1)$ th unit or super-unit catch, the linear regression was clearly recognized. This may indicate the justification of the assumption that in the removal method the number of larvae obtained in a unit or a super-unit catch is proportional to the number of larvae yet to be collected in the pool.
The estimates for the population size of larvae in the pool were obtained by using regression lines from the first three, four, and finally all unit catches and also super-unit catches. On comparing the results, it is concluded that fifteen minutes (three super-unit catches) may be enough to estimate the population size within the estimation error of ten per cent, though in the pool of a large population size estimation precision decreases to some extent.

The hyperbolic relation was revealed to exist between the collecting efficiency (the rate of the larvae collected in a unit catch) and the product
of surface area，depth of the pool and the first super－unit catch．This shows that the linear regression passing the origin may hold good between the reciprocal of the collecting effeciency and the product．If one super－unit catch is made in a given pool，an estimate for the collecting efficiency could be obtained from the above relation．By
substituting this estimate（a）and the first super－ unit catch $\left(A(5)_{1}\right)$ into $A(5)_{1} /(1-a)^{5}$ ，another estimate for the population size can be given． The estimate obtained by this method was proved to be rather convenient and satisfactory than that by the regression method from the first three super－ unit catches．

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総 括
1961年，福江市海岸の18のロックプールに抢いて，トウゴウヤブカ幼虫をスポイトで採集し，各プール毎に採集数を1分毎に，あるいは5分每に記録した。ある回の採集数とその前の回ま での採集数の合計との間には何れのプールにおいてあ直線回帰が認められ，採集数はそとに採集されずに残っている個体数に比例するという除去法の仮定が満足されることがわかった。そ てで回帰直線を求めて総個体数の推定を行なった結果，10\％以内の誤差で推定するためには，各プールに打さて 5 分間の採集を 3 回行竞ば概ね充分であると結論された。

次に回帰直線から求められた採集能率（1 分間に採集される幼虫の割合）とプールの表面積 $\left(\mathrm{cm}^{2}\right)$ ，深さ（cm）及び最初の5分間の採集数の3つの值の積との間の関係を調べた所，直角双曲線状の関係が得られたので，前者の逆数をとって原点を通る回帰直線を求めた。この回帰直線を利用して，各プールにつき，上記3つの値の積から採集能率の推定値（a）を求め，との値 と最初の 5 分間の採集数（ $\mathrm{A} ~(5) 1$ ）を $\frac{\mathrm{A}(5) 1}{1-(1-a)^{5}}$ 式に代入して総個体数の新しい推定値を得た。 この方法は，先に述べた3回の5分間採集から求めた総個体数の推定値より屯一般によい推定値を与えると考えられる。

Reccived for publication May 15， 1962


[^0]:    1）Contribution from the Research Institute of Endemics，Nagasaki University No． 403 and Contri－ bution No， 110 from the Department of Medical Zoology，Nagasaki University School of Medicine．

    2）This investigation was supported in part by a Grant in Aid for Fundamental Scientific Research from the Ministry of Education，and in part by a PHS research grant E－3328（ $\mathrm{R}_{1}$ ）from National Institute of Allergy and Infectious Diseases，NIH．，Public Health Service．

