長崎大学風土病紀要 第4巻 第1号：22～30頁 1962年3月

Studies on the Population Estimation for Insects of Medical Importance.

1. A method of estimating the population size of mosquito larvae in a fertilizer pit.*

Yoshito WADA

Department of Medical Zoology, Nagasaki University School of Medicine

(Director : Prof. N. OMORI)

衛生害虫の個体数の推定に関する研究. 1. 水肥溜の蚊幼虫数の推定方法. 和田義人, 長崎大学医学部医動物学教室（主任：大森南三郎教授）

Introduction

Determination of the total number of an animal in a given space is of basic importance for the analysis of various problems such as the population dynamics of the animal, the effectiveness of chemicals applied to animals in the field, the injury caused by the animal, and so on. It is, however, very difficult or practically impossible in most cases to count up the complete number of animals. So, the method for obtaining the estimate of population size is required and many literatures on the subject have been reported. Mark-and-release method is the widely known one for mobile animals (see e. g. Bailey, 1952), but this can not be applied to the animal which can not stand such a procedure owing to its weekness in structure, rapidity in development, or other reasons. In such cases, the following method, which has been developed chiefly in the field of the population estimation of small mammals, will be suitable (Moran, 1951 ; Zippin, 1956). This is the method that when caught animals are removed from the population the initial number of animals will be estimated from the results of a series of catches on the basis of the assumption that the number of animals captured during unit time is proportional to the number present, and it was called "the removal method" by Zippin (1956). Wada (1958) applied this method to the spider mite feeding on the Japanese cedar. Kono (1953) and Webster et al. (1954) presented independently a similar method of estimating the number of insects and ticks, which is based on the assumption like the removal method, and it will be called "the time unit collecting method".

The writer attempted to estimate the number of the larva of *Culex pipiens pallens* in a fertilizer pit. In this paper, theoretical considerations necessary for the estimation of the total number, especially when several successive catches are grouped, are given, and the relation between the removal method and the time unit collecting method is discussed, and finally, through the results of the present investigation, it will be reported that the removal method can be applied to the estimation of the total number of mosquito larvae in fertilizer pits.

The writer wishes to express his sincere appreci-

*Contribution from the Research Institute of Endemics, Nagasaki University No. 388 and Contribution No. 96 from the Department of Medical Zoology, Nagasaki University School of Medicine.

ation to Prof. N. Omori of Department of Medical Zoology, Nagasaki University School of Medicine for constant guidance and encouragement in the course of the study. The writer is much indebted to Prof. R. Tanaka of Zoological Laboratory, Kochi Women's University and Prof. S. Utida and Assistant Prof. T. Kono of Entomological Laboratory, Kyoto University for their helpful suggestions. Thanks are also due to Messrs. Y. Beppu, K. Harada, S. Honda, M. Matsui, Y. Matsuo, T. Saji and H. Tagawa, students of Nagasaki University School of Medicine, for their practical assistance in the field.

Theoretical considerations

Let us take a case in which the larvae of *Culex pipiens pallens* in a fertilizer pit are collected by a dipper. Now, assuming that the number of larvae collected by one dipping is proportional to the number of those yet to be dipped, the following equation may be derived,

$$A_n = a(S - Y_{n-1}) \quad \cdots\cdots (1)$$

where A_n is the number of larvae collected by the nth dipping, Y_{n-1} the accumulated number of larvae collected till the $(n-1)$th dipping, S the initial number of larvae in the pit at the start of dipping, a a proportional constant. This is the equation used for the estimation of the number of small mammals, removing the trapped animals from the population (Zippin, 1956 ; Tanaka, 1958).

From equation (1),

$$\frac{A_n}{A_{n-1}} = \frac{a(S-Y_{n-1})}{a(S-Y_{n-2})} = \frac{S-Y_{n-2}-A_{n-1}}{S-Y_{n-2}} = \frac{S-Y_{n-2}-a(S-Y_{n-2})}{S-Y_{n-2}} = 1-a. \quad \cdots\cdots (2)$$

It is, therefore, expected that the number of collected larvae decreases in geometrical progression in which the first term, A_n, is aS and the common ratio is $1-a$. The sum of n terms of the progression is given by the following equation,

$$Y_n = \frac{aS\{1-(1-a)^n\}}{1-(1-a)} = S\{1-(1-a)^n\}. \quad \cdots\cdots (3)$$

Wada (1958) had already presented the above conception, which was used for the estimation of the total number of a spider mite, *Paratetranychus hondoensis*, on a twig of the Japanese cedar.

Regarding one dipping used in the above equations as a unit catch and t successive unit catches as a super-unit catch, then the number of larvae to be obtained in the nth super-unit catch, $A(t)_n$, will be

$$A(t)_n = \sum_{n=t(n-1)+1}^{tn} A_n = Y_{tn} - Y_{t(n-1)} = S\{1-(1-a)^{tn}\} - S\{1-(1-a)^{t(n-1)}\} = \{1-(1-a)^t\}S(1-a)^{t(n-1)} = \{1-(1-a)^t\}(S-Y_{t(n-1)}).$$

Let the accumulated number of larvae obtained till the $(n-1)$th super-unit catch be $Y(t)_{n-1}$, then

$$Y(t)_{n-1} = Y_{t(n-1)} \quad \cdots\cdots (4)$$

and accordingly,

$$A(t)_n = \{1-(1-a)^t\}(S-Y(t)_{n-1}), \quad \cdots\cdots (5)$$

or in another form,

$$A(t)_n = \{1-(1-a)^t\}S - \{1-(1-a)^t\}Y(t)_{n-1}. \quad \cdots\cdots (6)$$

Equations (1) and (5) are isomorphic. The assumption that the number of collected larvae is proportional to the number of those yet to be collected is applicable also in the case of super-unit catch, where proportional constant is, however, not a but $1-(1-a)^t$.

From a comparison between equations (1) and (5), it is easily obtained that

$$\frac{A(t)_n}{A(t)_{n-1}} = (1-a)^t. \quad \cdots\cdots (7)$$

The same result may be arrived at in another way as follows,

$$\frac{A(t)_n}{A(t)_{n-1}} = \frac{\{1-(1-a)^t\}(S-Y(t)_{n-1})}{\{1-(1-a)^t\}(S-Y(t)_{n-2})} = \frac{S-Y_{t(n-1)}}{S-Y_{t(n-2)}} = \frac{S-S\{1-(1-a)^{t(n-1)}\}}{S-S\{1-(1-a)^{t(n-2)}\}} = \frac{(1-a)^{t(n-1)}}{(1-a)^{t(n-2)}} = (1-a)^t.$$

This indicates that the number of larvae obtained in a super-unit catch decreases in geometrical progression in which the first term, $A(t)_1$, is

$$A(t)_1 = \sum_{n=1}^{t} A_n$$
$$= Y_t$$
$$= S\{1-(1-a)^t\},$$

and the common ratio is $(1-a)_t$. Therefore, the sum of n terms of the progression is

$$Y(t)_n = \frac{S\{1-(1-a)^t\}\{1-(1-a)^{tn}\}}{1-(1-a)^t}$$
$$= S\{1-(1-a)^{tn}\}. \quad \cdots\cdots\cdots(8)$$

If a unit catch can be divided into u successive sub-unit catches, namely the number of animals obtained in the $(n+1)$th unit catch, A_{n+1}, can be shown as $\sum_{n=un+1}^{u(n+1)} A\left(\frac{1}{u}\right)_n$, where $A\left(\frac{1}{u}\right)_n$ is that obtained in the nth sub-unit one, then $A\left(\frac{1}{u}\right)_{un+1}$ may be represented as

$$A\left(\frac{1}{u}\right)_{un+1} = \{1-(1-a)^{\frac{1}{u}}\}(S-Y_n). \quad \cdots\cdots(9)$$

When u=1, equation (9) is

$$A(1)_{n+1} = a(S-Y_n).$$

This is identical with equation (1). $A\left(\frac{1}{u}\right)_{un+1} / \frac{1}{u}$ is apparently the value which shows the collecting efficiency at the $(un+1)$th sub-unit catch. When u is infinitely large, let the limiting value of this efficiency be $\frac{dY_n}{dn}$ then

$$\frac{dY_n}{dn} = \lim_{u\to\infty} \frac{A\left(\frac{1}{u}\right)_{un+1}}{\frac{1}{u}}$$
$$= \lim_{u\to\infty} \frac{\{1-(1-a)^{\frac{1}{u}}\}(S-Y_n)}{\frac{1}{u}}$$
$$= \lim_{v\to 0} \frac{\{1-(1-a)^v\}(S-Y_n)}{v}$$
$$= \lim_{v\to 0} \{-(1-a)^v \log(1-a)\}(S-Y_n)$$
$$= -\log(1-a)(S-Y_n). \quad \cdots\cdots\cdots(10)$$

Thus the same equation as Kono (1953) and Webster et al. (1954) proposed for the time unit collecting method is obtained. Equation (10) shows that the collecting efficiency, at the time n from the start of collection, is proportional to the number yet to be collected. Now, let the proportional constant, $-log(1-a)$, be p, then

$$\frac{dY_n}{dn} = p(S-Y_n), \quad \cdots\cdots\cdots(11)$$

where

$$p = -\log(1-a),$$

or

$$a = 1-e^{-p}.$$

From equation (11), the following may be derived,

$$Y_n = S(1-e^{-pn}). \quad \cdots\cdots\cdots(12)$$

This is equivalent to equation (3).

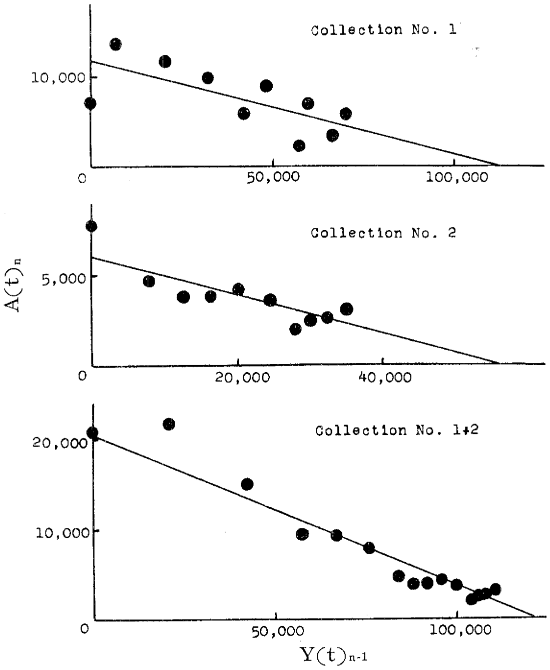
The proportional constants, a for the removal method and p for the time unit collecting one, should not be confused with each other. While p is the constant showing the instantaneous collecting efficiency, a is by itself the rate of the larvae collected in the unit catch to those yet to be collected. Equations (1) to (8), which are set up for the removal method, will be used in the following description, on account of the reason that the constant for the removal method has a concrete meaning.

Application to the larva of *Culex pipiens pallens*

Collections of mosquito larvae were made by a dipper of 15 cm in diameter and 3 cm in depth in a fertilizer pit of 2. 2 m in diameter in the suburbs of Nagasaki City in mid-July, 1960. Dippings were carried on at a site of the highest larval density in the pit. The dominant species in it was *Culex pipiens pallens* and the fourth instar larvae were found in about 85 per cent. *Culex vorax* and *Armigeres subalbatus* were concurrently found but only in less than 0. 05 per cent. On the first and the second days ten super-unit catches (a super-unit catch consists, on respective day, of five and ten successive unit catches) were consecutively made in the same pit, where a unit catch meant one dipping. These two collections and the sum of the two will be named Collection No. 1, No. 2 and No. 1+2 respectively.

From equation (5) or (6) a linear regression will be recognized between the number of larvae obtained in the nth super-unit catch, $A(t)_n$, and the accumulated number of larvae till the $(n-1)$th super-unit catch, $Y(t)_{n-1}$, and if the above is really

Fig. 1 Relation between the number of larvae obtained in the nth super-unit catch ($A(t)_n$) and the accumulated number of larvae till the (n-1)th super-unit catch ($Y(t)_{n-1}$).



Remarks : (1)A super-unit catch consists of t successive dippings, where t is 5, 10 and 10 for collection No. 1, 2 and 1+2 respectively.
(2)As to the regression lines drawn in the figure, see text and also Table 1.

done, then it will be expected that the estimate for the collecting rate per super-unit catch will be given by the absolute value of the inclination of the line and the estimate for the initial number of larvae will be given by $Y(t)_{n-1}$ intercept. The relation between $A(t)_n$ and $Y(t)_{n-1}$ is shown in Fig. 1 for Collection No. 1, 2, and 1+2, where t in No. 1 is five and in No. 2 ten, therefore in No. 1+2 ten are taken as t. Considerable deviations in each point from the regression line may be responsible to the alternation of workers during the experiment. The linear regression is clearly seen in all of the collections ; this may indicate that the above stated assumption that the number of larvae obtained in a super-unit catch is proportional to the number of those yet to be collected is satisfied. To fit the regression line of $A(t)_n$ on $Y(t)_{n-1}$, Zippin(1956)'s weighted least squares method was applied. Here the weight of each point is inversely proportional to (S'-$Y(t)_{n-1}$), where S' is the $Y(t)_{n-1}$ intercept of the line drawn by eye as a first approximation. From the regression equations thus obtained, the estimates for the initial number of larvae at the start of dipping, S, and also the collecting rate in a unit catch, namely one dipping, will be easily obtained from equation (6). These results are shown in Table 1.

Table 1 Regression equations of the number of larvae obtained by the nth super-unit catch ($A(t)_n$) on the accumulated number of those till the (n-1)th super-unit one ($Y(t)_{n-1}$) with estimates for the total number of larvae (S) and the collecting rate per unit catch (one dipping) (a)

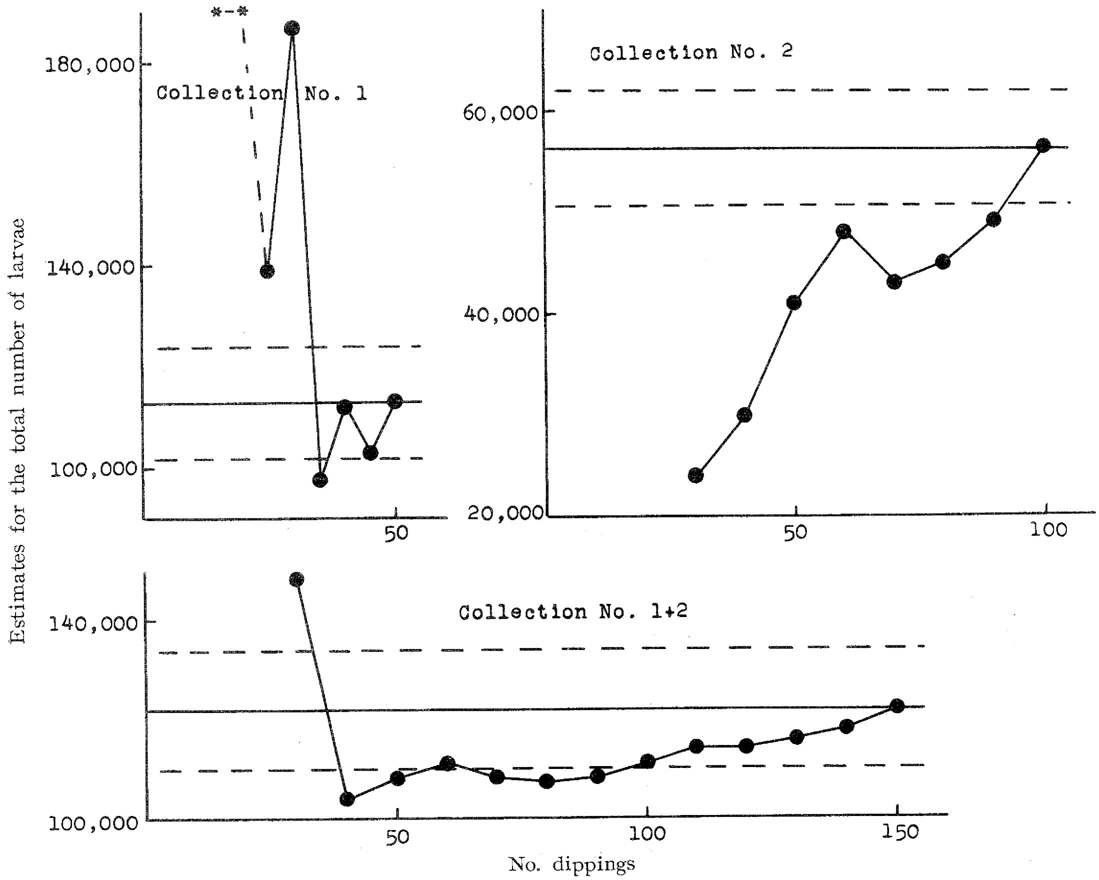
Collection No.	t	Regression equation	Estimate for	
			S	a
1	5	$A(5)_n = 11,792 - 0.1043Y(5)_{n-1}$	113, 058	0. 02180
2	10	$A(10)_n = 6,026 - 0.1070Y(10)_{n-1}$	56, 318	0. 01124
1+2	10	$A(10)_n = 20,443 - 0.1675Y(10)_{n-1}$	122, 048	0. 01816

Remarks : (1) Regression equation is determined from the data shown in Fig . 1 by weighted least squares method.
(2) The estimate for S is given as the value of $Y(t)_{n-1}$ when $A(t)_n$ is zero and that for a is obtained by letting the regression coefficient be $-\{1-(1-a)^t\}$.
(3) A super-unit catch consists of t successive unit catches.

In Collection No. 1 ten super-unit catches were made and a total of 75, 730 larvae was captured and in No. 2 56, 318 larvae were given as the estimate for the total number on the next day in the same fertilizer pit. Accordingly the initial number of larvae in the pit may be estimated at the sum total of the above two figures; that is 132, 048. Other estimates for the initial number are calculated at 113, 058 and 122, 048 from the results of Collections No. 1 and 1+2 respectively as shown in Table 1. These three estimates are similar with each other; this may justify that the removal method can be applied for the estimation of the number of mosquito larvae in the fertilizer pit.

The method of weighted least squares can be applied so long as there are three points of which co-ordinates are $Y(t)_{n-1}$ and $A(t)_n$. Thus, estimates for the total number were obtained from the result of super-unit catches of the first three, four, and so on, and finally ten in Collection No. 1 and No. 2 and fifteen in No. 1+2, and shown in Fig. 2.

Fig. 2 Series of estimates for the total number of larvae (S). An estimate is given as $Y(t)_{n-1}$ intercept of the regression line by the method of weighted least squares for each of the first three, four, ………… , ten super-unit catches in Collection No. 1 and No. 2, and ………… , fifteen super-unit catches in Collection No. 1+2.



Remarks : (1) Solid and broken straight lines represent the estimate for S in Table 1 and its ± 10 per cent values respectively.

(2) * means the impossibility in estimation owing to the positive inclination of the regression line obtained.

From the figure, it is indicated that the estimation precision is not so good if the number of dippings is small, though relatively better in Collection No. 1+2. This may be due to the fact that deviation in each point from the regression line is considerable owing to such a reason as stated ealier. Repeated experiments are required to determine how many dippings are demanded for the estimation for the total number within a given limit of the error.

If the collecting rate in a nuit catch, *a*, can be given, the total number, *S*, will be estimated without determining the regression equation. From equation (5),

$$S = \frac{A(t)_n}{1-(1-a)^t} - Y(t)_{n-1}, \quad \cdots\cdots(14)$$

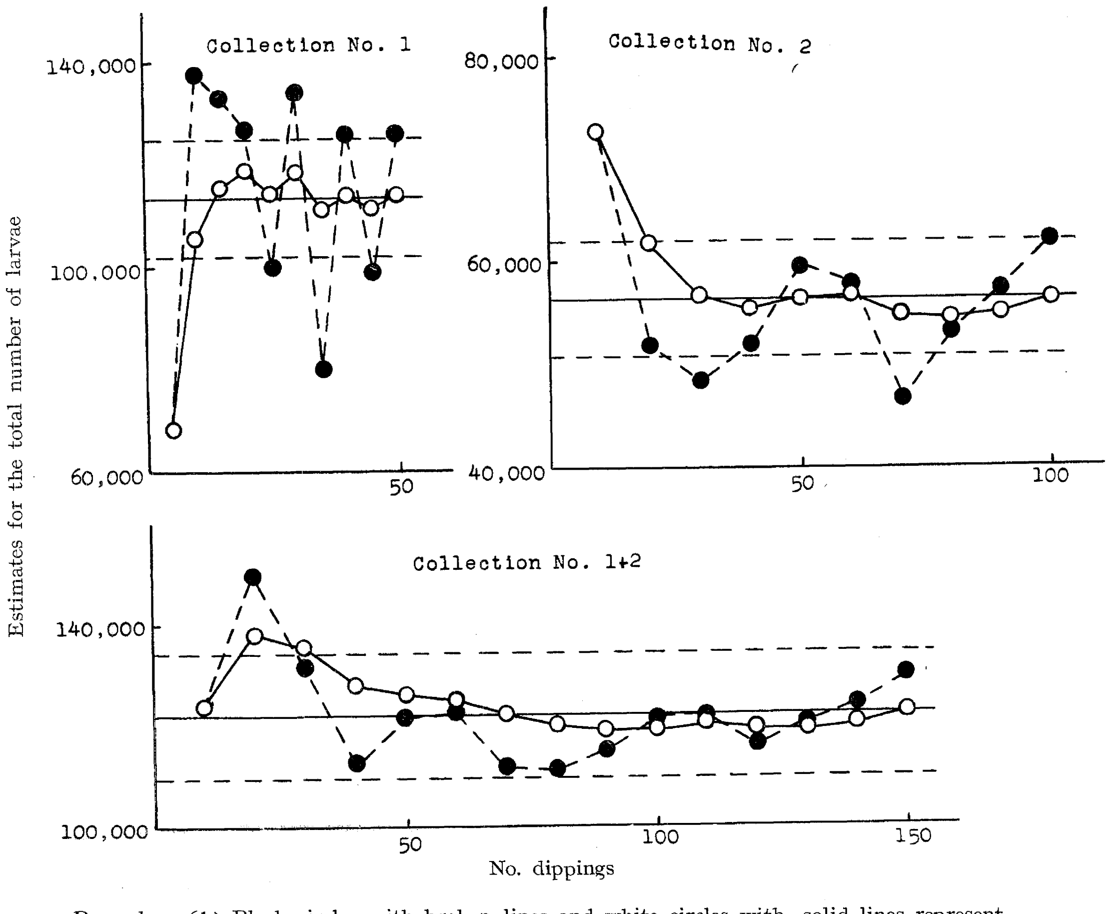
and from equation (7),

$$S = \frac{Y(t)_n}{1-(1-a)^{tn}}. \quad \cdots\cdots(15)$$

Both equations (14) and (15) will give the estimate for *S*.

Taking the estimate for *a* in Table 1 as the collecting rate in a unit catch, the variation was examined in the estimates for *S* obtained by

Fig. 3 Series of estimates for the total number of larvae (*S*) based on the data till *n* dippings from the start by applying the estimate for *a* in Table 1 to equation (14) and (15).



Remarks : (1) Black circles with broken lines and white circles with solid lines represent the estimates for *S* by equation (14) and (15) respectively.

(2) Solid and broken straight lines represent the estimate for *S* in Table 1 and its ± 10 per cent values respectively.

the above two equations with the progress in dipping and represented in Fig. 3. From the figure, it is evident that the results obtained by using equation (15) are higher in precision than by equation (14). This may be a matter of course, since equation (15) involves only the accumulated number of larvae till the definite dipping time, while equation (14) does, in addition to the above number, the number of larvae obtained in an individual super-unit catch. The figure also shows that the initial population size of larvae in the pit can safely be estimated from ten or twenty dippings by using equation (15), if the collecting rate in a unit catch is given. But the collecting rate is usually unknown in advance, and as far as the situation is so, the above procedure may have no value. An approach to this subject will be the clarification of the relation between the rate and the size of the fertilizer pit, and this will be discussed later.

Discussions

Zippin (1956) stated in his paper "The removal method assumes a stationary populating during the trapping program and also that the probability of capture during a given trapping is the same for each animal and does not change from trapping to trapping." The assumption he set up in the trapping of small mammals is considered to hold in the case of dipping the mosquito larvae.

It can be safely said that the population of mosquito larvae in the pit is quite stationary, and the probability of capture is naturally the same for individuals as far as the dipping of larvae is confined to 50 or 100 times, owing to the reason that the larvae are passively dipped.

However, it is a question whether the collecting rate holds constant during the collection period. The rate will be affected by the distribution pattern of the larvae in the pit. The collecting rate in one dipping will probably be higher in clumped case than in less so. The larval density may also be an affecting factor, though it is closely related to the distribution pattern. The distribution of larvae of *Culex pipiens pallens* is generally not uniform but rather clumped, and with the progress in dipping the larval density will usually become lower and the distribution less clumped. It is therefore conceivable that the collecting rate will become lower if the dipping is on progress. Whether or not the fact that a in Collection No. 1 is larger than in No. 2 as shown in Table 1 can be attributable to the above reason is unknown now, but it is the subject to be studied.

In addition to such factors as the distribution pattern and the density of larvae, another factor which may affect the collecting rate is the size of the fertilizer pit. This factor was not considered in this paper, since all collections were made in the same fertilizer pit. But it can easily be considered that the collecting rate in a given fertilizer pit will be higher than in a larger one if there exists the same number of larvae in the two pits of different size. The relation between the collecting rate and the size of the pit is also remained to be made clear.

Summary

"The removal method" has been known as a method for determination of the total number of an animal in a given space. This is based on the assumption that the number of animals captured during unit time is proportional to the number of those yet to be captured. Assuming the above and taking that a unit catch represents one dipping and a super-unit catch consists of a certain number of unit catches, the following equations will be given,

$$A(t)_n=\{1-(1-a)^t\}(S-Y(t)_{n-1}),$$

where $A(t)_n$ is the number of the larvae captured in the nth super-unit catch, $Y(t)_{n-1}$ the accumulated number of those captured till the $(n-1)$th one, S the initial number of larvae in a given space at the start of collection, a a proportional constant; a super-unit catch consists of t successive unit catches.

The writer attempted to estimate the total number of larvae of *Culex pipiens pallens* in a fertilizer pit by the removal method. Collections of mosquito larvae were made by a dipper of 15 cm in diameter and 3 cm in depth in a pit of 2.2 m in diameter in the suburbs of Nagasaki City in

mid-July, 1960. Dippings were carried out at sites of the highest larval density in the pit. On the consecutive two days ten super-unit catches consisting each of five and ten successive unit catches were made. These two collections and the sum of the two will be named Collection No. 1, No. 2 and No. 1+2 respectively.

The results of experiments showed that a linear regression holds clearly between $A(t)_n$ and $Y(t)_{n-1}$ as expected from the form of the equation. The regression equation obtained by Zippin (1956)'s weighted least squares method for each of three collections are as shown in Table 1.

The initial number of larvae in the fertilizer pit is estimated at 113,058 and 122,048 in Collection No. 1 and No. 1+2 respectively. Another estimate for the initial number is obtained by adding up the total number of larvae captured in Collection No. 1, 75,730, to the estimate for the total number in No. 2, 56,318; that is 132,048. These three estimates are nearly similar with each other ; this may justify that the removal method can be applied for the estimation of the total number of mosquito larvae in the fertilizer pit. Further studies are, however, necessary to determine how many dippings are required for estimating the total number within a given limit of the error.

Literatures

1) **Bailey, N. T. J.** : Improvements in the interrelation of recapture data. J. Anim. Ecol., **21** : 120-127, **1952**.

2) **Glasgow, J. P.** : The extermination of animal populations by artificial predation and the estimation of populations. J. Anim. Ecol., **22** : 32-46, **1953**.

3) **Kono, T.** : On the estimation of insect population by time unit collecting. Researches on population ecology, **2** : 85-94, **1953**. (In Japanese with English summary).

4) **Moran, P. A. P.** : A mathematical theory of animal trapping. Biometrika, **38** : 307-311, **1951**.

5) **Morris, R. F.** : Population studies on some small forest mammals in eastern Canada. J. Mammal., **36** : 21-35, **1955**.

6) **Tanaka, R.** : Theories and practical uses of a census method of small mammal populations. Hoppo-Ringyo, No. 115 : 1-5, **1958**. (In Japanese).

7) **Tanaka, R.** : A field study of effect of trap spacing upon estimates of ranges and populations in small mammals by means of a latin square arrangement of quadrats. Bull. Kochi Wom. Univ., **9** : 8-16, **1961**.

8) **Wada, Y.** : On a method of population estimation of a spider mite, *Paratetranychus hondoensis* EHARA, feeding on the Japanese cedar by the aid of "beating". J. Jap. For. Soc., **40** : 288-292, **1958**. (In Japanese with English summary).

9) **Webster, A. P. & DeCoursey, J. D.** : The catch curve of insects. Ann. Ent Soc. Amer., **47** : 178-189, **1954**.

10) **Zippin, C.** : An evaluation of the removal method of estimating animal populations. Biometrics, **12** : 163-189, **1956**.

総　　括

ある空間内の動物数を推定する方法の１つに〃除去法〃がある．それは単位採集により得られる動物数がその空間に残っている動物数に比例するという仮定に立つもので，次式で表現される．

$$A(t)_n = \{1-(1-a)^t\}(S-Y(t)_{n-1})$$

こゝで、$A(t)_n$は単位採集を t 回続けたものをまとめて１回とした場合の第n回目の採集数，$Y(t)_{n-1}$ は同様にした場合の第(n-1)回目までの採集数の計，aは単位採集によって採集される動

物の割合，Sは総個体数である．

1960年7月中旬，長崎市郊外の直径2.2mの水肥溜において，直径15cm，深さ3cmの柄杓で，最も多く幼虫がいると思われる所からすくうという方法で採集を続けた．1回の柄杓採集を単位採集として，先ず最初の日に5回ずつまとめて10回，合計50回の採集を行ない（採集No.1），翌日同じ水肥溜で10回ずつまとめて10回，合計100回の採集を行なった（採集No.2）．またNo.1とNo.2を一諸にしたものについても検討した（採集No.1+2）．なお、この水肥溜の蚊幼虫は殆んどすべてアカイエカであった．

$A(t)_n$及び$Y(t)_{n-1}$を両軸にとって，得られた資料をプロットすれば，採集No.1，2及び1+2の何れにおいても，前式から期待されるように直線回帰が認められ，先の仮定が満足されていることがわかった．そこで重みつきの最小二乗法により求めた回帰直線から総個体数の推定を行なった．採集No.1及びNo.1+2からの推定総個体数はそれぞれ113,058及び122,048であった．また採集No.1の採集総数とNo.2からの推定総個体数の合計は132,048であった．これら3つの値が何れもよく近似していることは，この方法によって水肥溜の蚊幼虫の総個体数を推定することが可能であることを示すものと考えられる．しかし乍ら，ある与えられた精度で総個体数を推定するには何回のすくい取りが必要であるかという点については今後の研究に待ちたい．

Received for publication February, 1962