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AN EASY FIELD METHOD FOR ESTIMATING THE ABUNDANCE OF CULICID LARVAL INSTARS

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ABSTRACT. A new method is proposed that avoids manual counting of mosquito larvae in order to estimate larval abundance in the field. This method is based on the visual comparison between abundance, in a standardized sampling tray (called an abacus), with 5 (abacus 5) or 10 (abacus 10) diagrammatically prepared abundance classes. Accuracy under laboratory and field conditions and individual bias have been evaluated and both abaci provide a reliable estimation of abundance in both conditions. There is no individual bias, whether people are familiar or not with its use. They could also be used for a quick estimation of larval treatment effectiveness, for the study of population dynamics and spatial distribution.

KEY WORDS Culicidae, abacus, abundance, visual tool

INTRODUCTION

Information about animal populations is sought for a variety of purposes. Studies are frequently used to provide information on distribution and abundance for conservation or management programs. For pests or parasites, this knowledge of distribution and abundance provides assessments of incidence or damage and may guide the application of control measures (Southwood and Henderson 2000).

Population estimates can be classified into 2 different types: absolute and relative estimates (Morris 1955). The absolute population is defined as the number of animals per unit area. The relative estimates, in which the number caught cannot be expressed as a density per area unit, only allow comparisons in space or time. They are especially useful in extensive work on species distributions, monitoring changes in species abundance and richness and recording patterns of animal activity (Southwood and Henderson 2000). The abundance is defined as the number of individuals and density as the ratio between number of individuals and a surface or a volume unit.

In the case of culicid species, determination of the total number of larvae in a given place is essential for the analysis of various problems such as population dynamics, effectiveness of chemical treatment, and biological agents applied against larvae in the field (Wada 1962a). Numerous methods have been used to estimate the abundance and the density of mosquito larvae: capture–recapture (Hassett and Jenkins 1949; Bailey 1952; Croset et al. 1976), dipping (Andis et al. 1983; Linthicum et al. 1984; Reisen et al. 1989), removal method (Wada 1962a, 1962b; Service 1971; Croset et al. 1976), and sequential sampling (Waters 1955 in Knight 1964; Wada 1965). However, all these methods demand that the operator make a manual count to estimate larval abundance and appraise the larval density when the sampling surface is known. In most cases, it is very difficult or even impossible to count the total number of larvae. Moreover, mosquito larvae, like many other aquatic invertebrates, have a notorious nonuniform distribution (Hocking 1953; Nielsen and Nielsen 1953; Papierok 1972; Stewart and Schaefer 1983; Service 1993), and numerous samples are required for a reliable estimation of abundance when the population is randomly dispersed (Zippin 1956; Resh 1979; Morin 1985).

Up to now, manual counting was the sole method used to estimate mosquito larvae abundance and density. Agronomic methods (Chiarappa 1971) and a black fly study (Palmer 1994) were the first to use and test a visual tool called an abacus as a substitute for manual counting. We developed this type of tool for mosquitoes in southern France. This relative method is based on a visual comparison of larvae present in a standardized sampling tray with diagrammatically prepared abundance classes. The accuracy of the abacus as well as individual bias were tested in the laboratory and in the field. The abacus was also used to estimate the efficiency of a larvicide treatment.

MATERIALS AND METHODS

The method

A series of schematic representations of pictures with a given known number of larvae made up the abacus. The range of the abacus goes from 1 larva to the maximum larval abundance, which is likely to occur in the field (Babinot, unpublished data). When the range of the abacus is established, it is divided into classes based on a semilog scale. Each class is visualized by the maximum number of larvae of this class. For each maximum number, the corresponding number of larvae is counted and put in a 13.5- × 13.5- × 6-cm white standardized tray and is photographed. The schematic representation of each photo, which represents an abundance class, was obtained with Microsoft PowerPoint® software. All schematic representations were printed on a single A4-size sheet of paper so that it

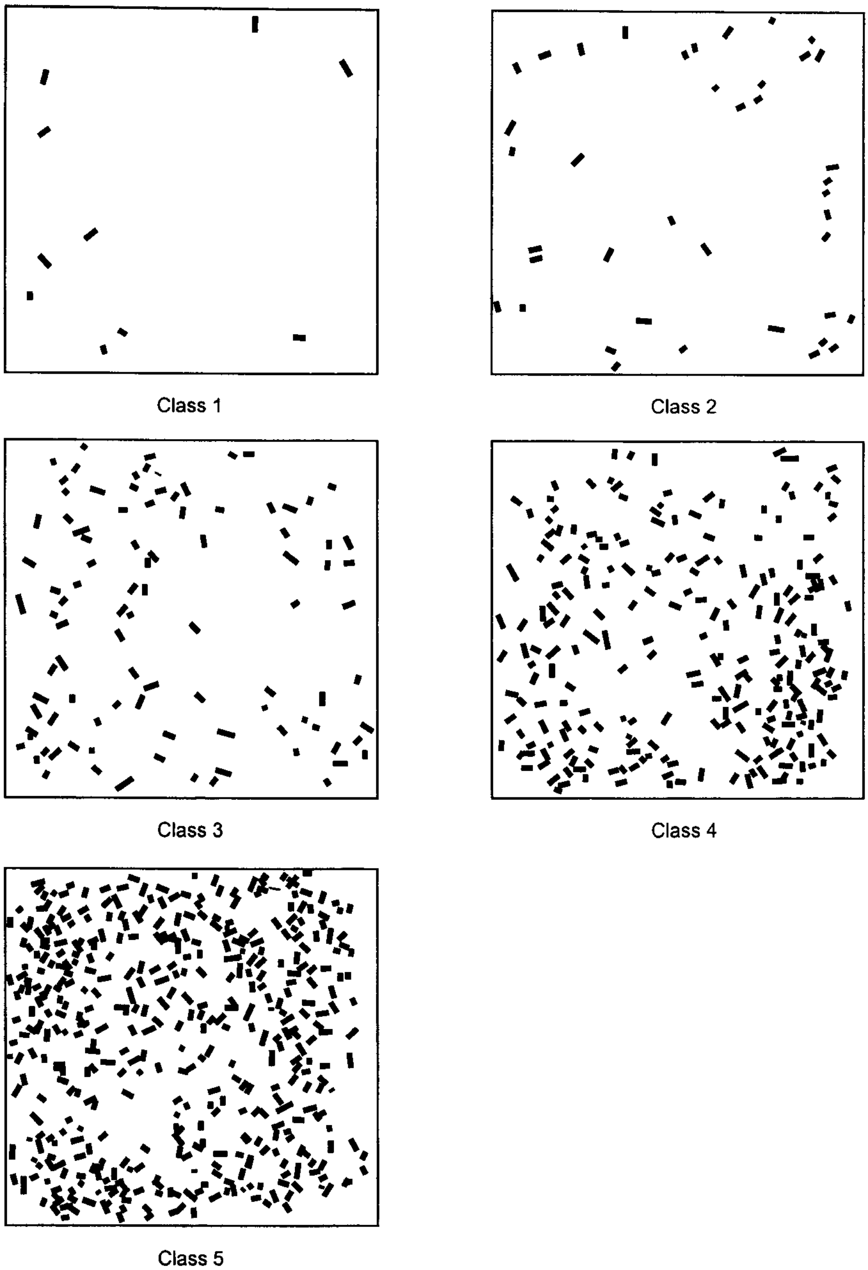

Fig. 1. Abacus 5, diagrammatic representation of semilogarithmically defined abundance scale to estimate population abundance of mosquito larvae. Classes correspond to the numbers shown in Table 1.

could be held with 1 hand, and be conveniently used in the field.

The sample is collected with the standardized tray. This sample is compared with the abacus from the first to the last class. When a doubt remains between 2 classes, the abundance is considered to belong to the upper class.

Two abacuses are proposed: an abacus with 5 abundance classes on a single A4 sheet (abacus 5, Fig. 1) and a more accurate one with 10 abundance classes on 2 sheets (abacus 10, Fig. 2). Classes range from 1 larva (class 1) to an excess of 250 (class 5, Table 1) for abacus 5 and 1 larva (class 1) to an excess of 500 (class 10, Table 2) for abacus 10.

Validation of the method: laboratory accuracy and individual biases

This experiment was designed to test the accuracy of estimates and the possible effect of bias of

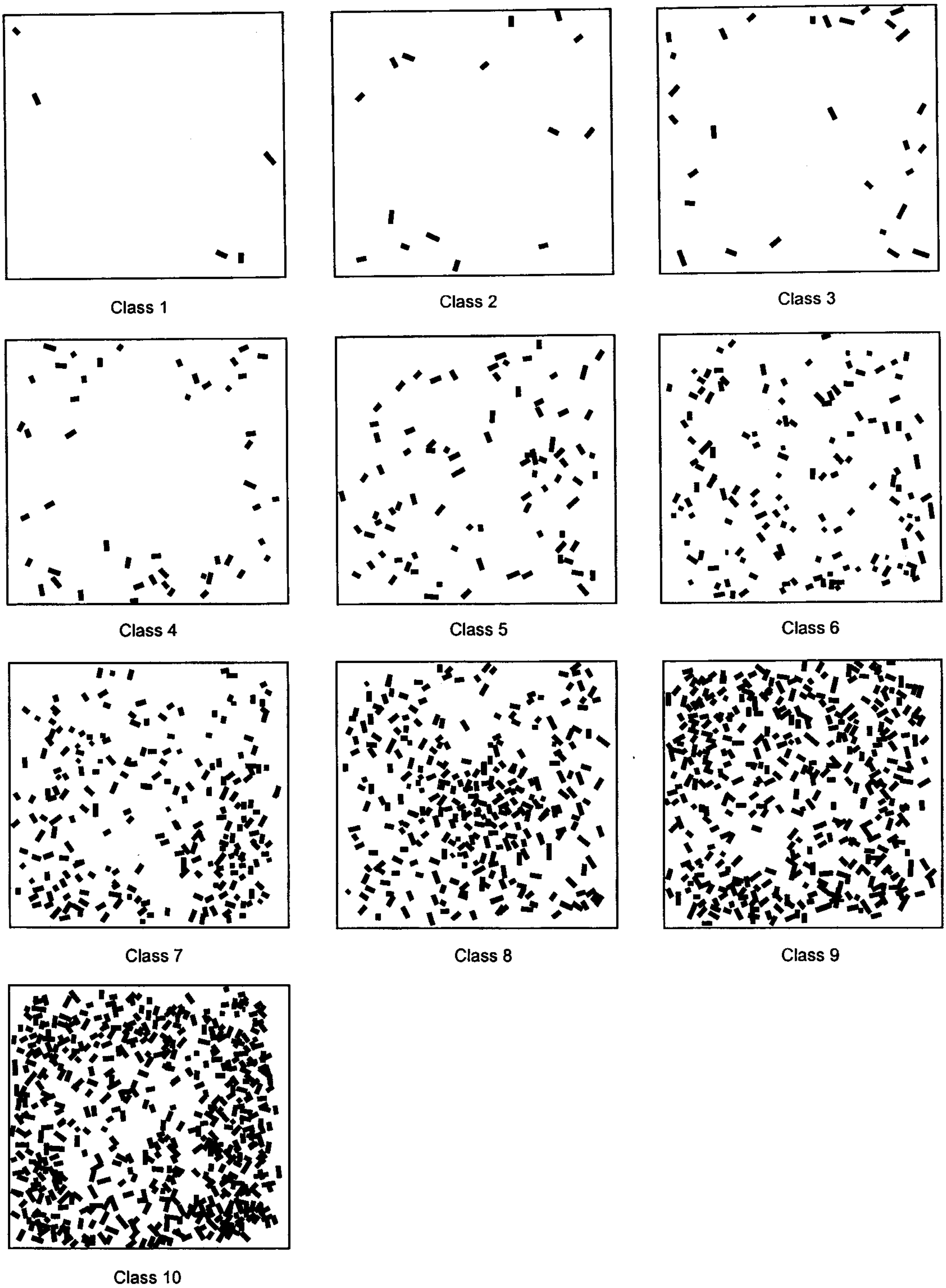

Fig. 2. Abacus 10, diagrammatic representation of semilogarithmically defined abundance scale to estimate population abundance of mosquito larvae. Classes correspond to the numbers shown in Table 2.

Table 1. Larval numbers in each of the 5 abundance classes of abacus 5 used for estimating the abundance of mosquito larvae. Ranges for the classes are given in brackets.

Class	Larvae
1	10 (1–10)
2	40 (11–40)
3	100 (41–100)
4	250 (101–250)
5	600 (>250)

Table 2. Larval numbers in each of the 10 abundance classes of abacus 10 used for estimating the abundance of mosquito larvae. Ranges for the classes are given in brackets.

Class	Larvae
1	5 (1–5)
2	15 (6–15)
3	30 (16–30)
4	50 (31–50)
5	90 (51–90)
6	150 (91–150)
7	250 (151–250)
8	350 (251–350)
9	500 (351–500)
10	600 (>500)

field and laboratory operators and to compare the 2 abacuses. A laboratory experiment was conducted at room temperature and under natural light and photoperiod. Three aquariums of 92.5 × 35 × 47 cm, with 10 cm of water, were used. An unknown number of 3rd- and 4th-stage larvae of *Culex pipiens* L. was put in each aquarium. Aquariums 1, 2, and 3 received respectively 1, 3, and 5 field dippings in order to obtain increasing abundances.

An operator collected larvae in an aquarium with the standardized tray. The abundance was estimated with abacus 5, then with abacus 10; then the larvae were manually counted. This larval number gave the true value of abundance, translated into true abundance class with Table 1 for abacus 5 and Table 2 for abacus 10. The larvae were put back into the aquarium. In order to test the laboratory accuracy of the abacus, comparisons were made between abundance classes, appraised with abacus 5 and abacus 10, and the true abundance classes obtained with manual counting.

Three operators carried out this experiment for each aquarium: operator A, familiar with the use of abacuses and unfamiliar with sampling, made 10 replicates; operator B, unfamiliar with the use of abacuses and familiar with sampling; and operator C, familiar neither with the use of abacuses nor with sampling, performed 5 replicates so that 20 replicates were made in each aquarium. Comparing the estimations of abundance classes of the 3 operators allowed displaying individual biases and manual counting allowed for testing the differences in larval sampling between operators.

Comparison between the two abacuses

Twenty replicates for each aquarium were used and the "residues" between estimated abundance and the true value of abundance were used to compare the accuracy. These residues were calculated by subtracting the estimated value from the true value of abundance given by manual counting and the absolute value of these differences was calculated. The estimated value is the maximum number of the estimated abundance class. For abacus 5, the abundance class is translated into abundance value with Table 1. For abacus 10, Table 2 is used. Twenty residues for each abacus were calculated and compared for each aquarium. The null hypothesis is that the residue values of abacus 10 are inferior to the residues of abacus 5.

Validation of the method: field accuracy

To test the field accuracy of the 2 abacuses, an operator collected 12 samples with the standardized tray in a nonturbid water ditch (50 × 0.5 × 0.4 m) colonized with 3rd- and 4th-stage instars of *Cx. pipiens* larvae (Mas de Badet, southern France). To avoid sampling bias, the operator must have the sun in one's eyes to avoid alarm reaction (e.g., diving) of the larvae due to his or her shadow (Hocking 1953). Moreover, when the operator reached the point of sampling, water is disturbed and the larvae dive (Duhrkopf and Benny 1990), so the operator must wait at least 1 min before dipping. The abundance in each tray was estimated with abacus 5, then with abacus 10, and true values of abundance were determined by manual counting.

Efficiency of a larvicide treatment

The abacus can also be used to estimate the efficiency of larvicides used for mosquito control, mainly against *Ochlerotatus caspius* (Pallas, 1771) along the French Mediterranean coast. To estimate the percentage of mortality (Tables 3 and 4), differences between classes before and after treatment were used:

$$\% \text{ mortality} = (Y - X)/Y$$

where Y is the maximum number of larvae for the class before treatment and X is the maximum number of larvae for the class after treatment.

The larvicide used was *Bacillus thuringiensis* ser. *israelensis* (*Bti*, Vectobac® 12 AS, Valent Bioscience Inc., France, 1,200 UTI/mg). A dilution of 0.120 liter of *Bti* in 2.4 liters of water was applied with a knapsack sprayer during 3 min across a 1,200 m² marsh sheltering 3rd- and 4th-stage instars of *O. caspius* larvae. The discharge flow during the treatment was 800 ml/min. The estimation of the

Table 3. Changes in numbers between abundance classes for abacus 5 were used to estimate percentage mortality of mosquito larvae as a result of larvicide application.

	% mortality Class before treatment (*Y*)				
Class after treatment (*X*)	1	2	3	4	5
1	0	75	90	96	98
2		0	60	84	93
3			0	60	83
4				0	58
5					0

abundance at 15 points, localized by posts, was made immediately before and 24, 48, and 72 h after treatment with abacus 10.

Statistical analysis

All statistical tests were done with SYSTAT 9 software (SPSS Inc.). To test the laboratory and the field accuracies, a sign test (Zar 1999) was used to compare the abundance classes, appraised with the abacus, with the true values determined by manual counting translated into classes with Table 1 or Table 2. To evaluate individual bias, a Kruskall–Wallis test (Zar 1999) was used to compare the estimations of abundance classes appraised with both abacuses and to compare the expected classes given by the manual counting of the 3 operators. To test if abacus 10 was more accurate than abacus 5, a Kolmogorov–Smirnov test (Zar 1999) was used to check the normality law of the residues. A paired-sample t-test (Zar 1999) was then used to compare the residues calculated with abacus 5 and abacus 10. A Wilcoxon signed-rank test (Zar 1999) was used to study the differences in percentage of mortality induced by the treatment.

RESULTS

Laboratory accuracy and individual bias

There were no significant differences between the estimates with the 2 abacuses and manual counting (Table 5). For aquariums 1 and 2, no significant differences were found between the 3 operators with both abacuses and manual counting (Table 5). For aquarium 3, there were significant differences among the 3 operators in estimating the abundance with the 2 abacuses (Kruskall–Wallis one-way ANOVA, $KW = 6.815$, df = 2, $P = 0.033$ for abacus 5; $KW = 7.76$, df = 2, $P = 0.021$ for abacus 10) and for manual counting expected abundance classes (given by Table 2, Kruskall–Wallis one-way ANOVA, $KW = 7.039$, df = 2, $P = 0.03$).

Comparison between the 2 abacuses

All residues were adjusted to the normality (all Kolmogorov–Smirnov tests were not significant). There were highly significant differences between the residues calculated with abacus 5 and abacus 10 (paired-sample t-test, $t = 2.956$, df = 19, $P = 0.008$ for aquarium 1; $t = 3.715$, df = 19, $P = 0.001$ for aquarium 2; and $t = 3.183$, df = 19, $P = 0.005$ for aquarium 3). The residues calculated with abacus 5 were higher than those calculated with abacus 10.

Field accuracy and efficiency of a larvicide treatment

There were no significant differences between estimated abundances and manual counting (sign test, $P = 0.25$ for abacus 5; $P = 0.625$ for abacus 10, Table 6). The *Bti* treatment induced a reduction of the larval population (Fig. 3a, Table 7) and abacus 10 provided a quick estimation of this reduction (Fig. 3b). After 24, 48, and 72 h, a reduction of 51, 69, and 70%, respectively, of larval abundance was noted (Table 7). There was a significant difference

Table 4. Changes in numbers between abundance classes for abacus 10 were used to estimate percentage mortality of mosquito larvae as a result of larvicide application.

	% mortality Class before treatment (*Y*)									
Class after treatment (*X*)	1	2	3	4	5	6	7	8	9	10
1	0	67	83	90	94	97	98	98.6	99	99.2
2		0	50	70	83	90	94	95.7	97	97.5
3			0	40	67	80	88	91.4	94	95
4				0	44	67	80	85.7	90	91.7
5					0	40	64	74.3	82	85
6						0	40	57.1	70	75
7							0	28.6	50	58.3
8								0	30	42
9									0	16.7
10										0

Table 5. Results of the laboratory experiment. The *P*-values were calculated for abacus and manual counting estimates and aquarium and operator differences with a Kruskall–Wallis test.[1]

	Aquarium 1				Aquarium 2				Aquarium 3			
Operator	Abc5	Mc1	Abc10	Mc2	Abc5	Mc1	Abc10	Mc2	Abc5	Mc1	Abc10	Mc2
A	3	3	5	5	3	4	6	6	4	4	7	9
A	3	3	4	5	4	4	7	7	5	5	9	9
A	2	3	4	4	4	3	6	6	5	4	8	7
A	3	3	5	5	4	4	6	6	4	4	7	7
A	3	3	5	6	4	4	7	7	4	4	7	7
A	3	3	5	5	4	4	7	7	5	5	8	9
A	4	3	6	6	4	4	7	7	5	5	8	8
A	4	4	6	6	4	4	6	6	5	5	8	8
A	4	4	7	7	4	4	6	6	5	5	9	9
A	4	4	6	7	4	4	6	6	4	4	7	7
B	4	4	6	6	4	4	7	7	5	5	9	9
B	4	4	7	7	4	4	7	7	5	5	9	9
B	3	3	5	5	4	4	7	7	5	4	8	7
B	3	3	5	5	5	5	8	8	5	5	9	9
B	5	5	8	8	5	5	8	8	5	5	9	8
C	3	3	6	6	5	5	8	8	4	4	7	7
C	3	3	5	6	3	3	5	5	4	4	6	6
C	4	4	6	6	3	3	5	5	3	3	5	5
C	3	3	5	5	4	4	7	7	5	5	9	8
C	3	3	5	5	4	4	6	7	4	4	6	6
P-value	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	*	*

[1] NS, $P > 0.05$; *, $P < 0.05$. Abc5, abacus 5; Mc1, manual counting translated into classes with table 1; Abc10, abacus 10; Mc2, manual counting translated into classes with table 2.

in the percentage of mortality between 24 h and 48 h after treatment (Wilcoxon signed-rank test, count of differences = 6, $P = 0.046$) but no significant difference between 48 h and 72 h after treatment (Wilcoxon signed-rank test, count of differences = 9, $P = 0.476$).

DISCUSSION

In the laboratory, there were significant differences among the 3 operators when larval abundance was high (aquarium 3). In this aquarium, the significant differences noted between operators in estimating the abundance with abacus 10 were due to significant differences in sampling (Table 5). These differences were probably due to the fact that a more skilled operator collected significantly larger numbers of organisms (Clifford and Casey 1992). Operators A, B, and C collected respectively 275.9 ± 59.9, 342.8 ± 52.3, and 170.2 ± 92.5 larvae, which explained the significant differences found with abacus 5 in aquarium 3. No test was made with an operator familiar both with the abacus and with sampling because there is no such operator in the staff of the EID Mediterranee.

All the experiments were done with 3rd- and 4th-stage instars in nonturbid water. However, this visual method might give unreliable results when high densities of 1st- and 2nd-stage instars occur, as small larvae can be easily overlooked (Palmer 1994). Moreover, when the water is turbid, larvae are not easily seen and estimation of abundance is biased. Turbid water could be diluted, but operators do not always have clean water near at hand. When the water contains large organic fragments, they should be carefully removed.

Although abacus 5 is easier to use, there are significant differences among operators when the abundance is high. Moreover, the residues calculated with abacus 10 are narrower than those obtained with abacus 5. Therefore, abacus 10 provides more accurate estimations. Either abacus can be used according to the accuracy wanted.

The abacus gives an estimation of abundance and not an estimation of density. However, estimation of larval density is possible: the larval numbers caught with a dipper related to the estimated water

Table 6. Results of the field experiment.[1]

Replicate	Abc5	Mc1	Abc10	Mc2
1	4	4	6	6
2	3	3	5	5
3	3	3	5	5
4	4	4	7	6
5	4	4	7	7
6	4	4	6	7
7	3	4	5	6
8	3	3	5	5
9	4	5	7	8
10	4	4	6	6
11	4	4	7	7
12	3	4	6	6

[1] Abc5, abacus 5; Mc1, manual counting translated into classes with table 1; Ab10, abacus 10, Mc2, manual counting translated into classes with table 2.

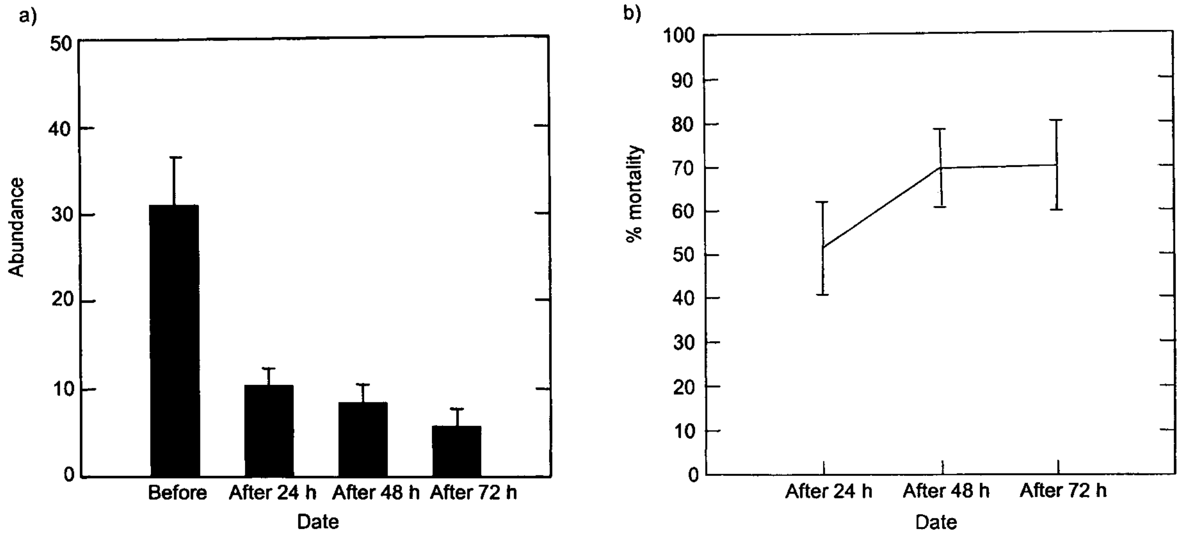

Fig. 3. (a) Changes in larval abundance induced by a larvicide treatment and (b) percentage of larval mortality induced by this larvicide treatment. The larval abundance considered is the upper number of the abundance class estimated with abacus 10. The mean and the standard error are represented for (a) the abundance estimated with abacus 10 and (b) the percentage of mortality.

volume allows calculating larval population estimates (Dixon and Brust 1972). In our experiments, the dipper used is a standardized tray. This method could be used with other dippers or other sampling techniques, but to use the abacus, the larvae collected must be transferred in the standardized sampling tray.

Manual counting is the most accurate existing method but it is time consuming and tiresome. Relative methods such as abacus or dipping are satisfactory in extensive research for nuisance-control programs (e.g., measurement of larvicidal efficiency; Papierok 1972) and may provide a reliable population estimate (Papierok et al. 1975; Stewart and Schaefer 1983) if a minimum number of samples is collected. Such is the case with mosquito larvae due to their nonuniform distribution (Resh 1979; Service 1993). The abacus can help to collect large numbers of samples as the operator spends less time to count larvae.

For life-table analysis (Service 1971; Lakhani and Service 1974; Reisen et al. 1989) or when different culicid species are present (Linthicum et al. 1983, 1984), instars and/or species must be separated. In such a case, the abacus cannot be used and the operator must proceed with manual count-

Table 7. Abundance classes before and after a larvicide treatment and percentage mortality, using Table 4.

Point	Class before treatment	Class 24 h		Class 48 h		Class 72 h	
		After treatment	% mortality	After treatment	% mortality	After treatment	% mortality
1	2	2	0	1	67	1	67
2	3	2	50	2	50	1	83
3	5	1	94	2	83	1	94
4	4	1	90	1	90	0	100
5	3	1	83	0	100	0	100
6	3	2	50	1	83	0	100
7	3	1	83	1	83	1	83
8	3	0	100	0	100	0	100
9	3	1	83	1	83	0	100
10	4	2	70	2	70	1	90
11	2	2	0	2	0	1	67
12	2	2	0	1	67	1	67
13	2	1	67	1	67	2	0
14	1	1	0	0	100	1	0
15	3	3	0	3	0	3	0
$\bar{x}$ percentage of mortality			51.33		69.53		70.07

ing. The abacus is a reliable tool to estimate the efficiency of larvicide treatments when the minimum sample number is known. In southern France, it was advisable to take 1 sample every 2 m to have an accurate estimation of larvae abundance (Croset et al. 1976). The abacus gives a relative estimation of abundance that could be used to study the influence of methodological and environmental factors on the efficiency of larvicides.

This visual method is effective with minimum equipment (1 or 2 sheets of paper) and minimum training. Whatever the sampling method used, the abacus allows the estimation of larval abundance without any manual counting. Despite its limitations, the visual method is practical, simple, and rapid, and is convenient for use in mosquito control programs whenever assessment of larval abundance is required.

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