tions used, since no increase in activity was observed in the presence of oxygen alone. A representative experiment which shows the reduced pyridine nucleotide specificity of cotton leaf microsomal *N*-demethylase is shown in Table 3. Both NADPH and NADH (the reduced form of nicotinamide adenine dinucleotide) served as cofactors for the *N*-demethylation of 3-(4′-chlorophenyl)-1,1-dimethylurea, but NADPH was approximately twice as effective as NADH. The combination of NADPH and NADH was only as effective as NADPH alone.

The observed requirements for molecular oxygen and reduced pyridine nucleotides, together with the localization of enzyme activity in the microsomal fraction, indicate that the *N*-demethylation of 3-(4′-chlorophenyl)-1,1-dimethylurea to 3-(4′-chlorophenyl)-1-methylurea is catalyzed by a mixed-function oxidase system in cotton leaves. Further studies are needed to purify and characterize this and other in vitro plant microsomal systems as possible sites for the metabolism of pesticides in plants.

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Estimating the Number of Animals: A Rapid Method for Unidentified Individuals

Abstract. *A proposed model yields the density of a mobile population from quick, cursory surveys in which the observer identifies none of the animals. When the spaces on which animals were seen are successively removed, the decline in the counts permits estimation of the average probability of seeing a given animal. The method showed promise in initial trials.*

Estimations of the density of populations (*1*), depend either on total counts on sample plots, on marking and later reobservation, or on physical removal of some animals (*2*), or on a "flushing count" (*3*). The model I now propose requires none of these methods and may lessen some of the difficulties in one of the central tasks of animal ecology.

A study area subdivided into equal sample spaces is inspected during a series of n cursory, randomized surveys, but the observer limits each survey to just the L_i spaces on which he has not previously seen animals in that given series of counts ($i = 1, 2, \ldots$ unless otherwise noted). During each i^{th} count the investigator notes the total number of observed animals, x_i, and the total number of spaces on which they occurred, l_i; following the survey he eliminates the l_i plots from further study in that given experiment (series of counts or surveys). The worker continues the surveys under uniform conditions, preferably until all plots have been removed or the remaining ones have no observed animals. The present "plot-removal method" is distantly related to the earlier removal procedures which Zippin and Tanaka (*4*) reviewed.

I consider the average probability, P, of seeing any one given animal, during any one survey that included its plot, under two cases: (i) the unusual one where the average value of P remains constant from one l_i group of plots to another l_i group (although not necessarily from plot to plot within each group); and (ii) the common case where it does not. The observer must make the surveys near each other in time, to prevent change in the size of the total population K but far enough apart to permit successive observations on any specific plot to be independent. Movements of animals on and off each l_i group of plots do not occur or the movements must tend to cancel out.

In the special case where P is constant and the investigator makes only two of the rapid, cursory counts on one study area (divided into numerous spaces), the following postulates and definitions can be made. If the assumptions are fulfilled, the number of animals seen on the first survey is given by

$$x_1 = PK \tag{1}$$

and the number seen on the second survey is

$$x_2 = P\,(K - x_1 - y_1) \tag{2}$$

where y_1 is the number of unobserved animals that occurred on the same l_1 spaces as the x_1 seen animals. The total number of unobserved animals on the entire area studied in the first survey, including those living on the l_1 plots as well as elsewhere, Y_1, is further defined by

$$Y_1 = K - x_1 \tag{3}$$

The spatial distribution of the Y_1 animals is now a major concern, for one must form some idea about what fraction of them constituted y_1. Although the situation undoubtedly differs somewhat in different populations, the strongest assumption that one can make in the present circumstance probably is that the number of unobserved animals, per plot, is proportional to the number of observed ones. If this is true, one can write that

$$y_1 = Y_1\,(x_1/K) \tag{4}$$

The preceding four equations contain the four unknowns y_1, Y_1, P, and K, of which P and K are the population parameters. After the equations are solved simultaneously, and after writing $\hat{K}$ for K, one obtains an estimate of abundance on one study area based on a constant P, $n = 2$, and the factor of proportionality x_1/K, as follows:

$$\hat{K} = x_1/[1 - (x_2/x_1)^{1/2}] \tag{5}$$

In this equation it is obvious that

$$\hat{q} = (x_2/x_1)^{1/2} \tag{6}$$

where q is defined as $1 - P$, and where the circumflex (∧) means estimated.

A different assumption about the distribution of the Y_1 unseen animals can be made, namely that their density throughout the study area is essentially uniform. If this is so, $(y_1/Y_1) = (l_1\,/\,L_1)$, and therefore

$$\hat{y}_1 = Y_1(l_1/L_1) \tag{7}$$

When one uses Eq. 7, instead of Eq. 4, as the factor of proportionality in developing the previous type of derivation, based on $n = 2$, one study area, and a constant P, the estimated density is

$$\hat{K} = \frac{x_1{}^2 (L_1 - l_1)}{[x_1 (L_1 - l_1)] - x_2 L_1} \qquad (8)$$

Although a few populations living in a relatively homogeneous environment, such as some insects in a grain field, may have a more or less uniform distribution, yet the great majority will not; and therefore Eq. 5 should generally be superior to Eq. 8.

Where the assumptions hold, Eqs. 5 and 8 can be generalized to fit any two successive surveys on a given study area, such as the fourth count compared to the fifth one, by writing x_{i-1} in place of x_1 and x_i in place of x_2, and by changing l_1 and L_1 accordingly in Eq. 8.

The average probability (based on all l_i plots of a given survey) that the observer will see a specific animal when the survey included the plot where the animal lived will actually vary from survey to survey, usually tending to fall lower and lower as the surveys continue. Therefore, the ecologist should (i) extend the work to include a number of study areas in one experiment to lessen random error, and he should (ii) average the results of a number of surveys conducted on the numerous study areas to lessen systematic error.

We now extend the previous special cases to the general one where the probability P_i varies from survey to survey, and where results obtained from m study areas, each containing L_i plots, and n surveys are considered simultaneously as one "experiment." Then let the number of animals observed on the i^{th} survey and the j^{th} study area be called x_{ij}. Since the worker will inspect all of the m study areas during each survey, he will accumulate a series of observations, each one being a sum of one survey, namely

$$\sum_{j=1}^{m} x_{1j}, \quad \sum_{j=1}^{m} x_{2j}, \quad \cdots \quad \sum_{j=1}^{m} x_{nj}$$

To simplify the notation, let the sum of observations made over all the study areas on the i^{th} survey now be called just X_i. Therefore, for one experiment the grand sum of all surveys conducted on all study areas and plots within areas is given by

$$X_1 + X_2 + \cdots X_n = \sum_{i=1}^{n} X_i$$

Then $\bar{q}^2$ for use in an expression such as Eq. 5 is approximated by the following averaging process (where E signifies expectation):

$$\begin{aligned} E(\bar{q}^2) &= E(\bar{X}_i)/[E(\bar{X}_{i-1})] \\ &= (X_2 + X_3 + \cdots X_n)/ \\ &\quad (X_1 + X_2 + \cdots X_{n-1}) \\ &= \sum_{i=2}^{n} X_i / \sum_{i=1}^{n-1} X_i \end{aligned} \qquad (9)$$

After Eq. 5 is accordingly modified to use these sums, the result is

$$\hat{K} = X_1/[1 - (\sum_{i=2}^{n} X_i / \sum_{i=1}^{n-1} X_i)^{1/2}] \qquad (10)$$

A few small experiments were made on adult mealworms (*Tenebrio molitor*) confined in pans containing bran flakes that served as cover. In one experiment, two surveys were made on six pans, each containing 300 mealworms, but the estimate of K was only about 45 percent of its true value. In another experiment, three surveys were made on two pans, each containing 300 of the beetles, and this time the estimate of K was about 60 percent of what it should have been. Four surveys were made on one pan in another experiment, giving $\hat{K} = 258$ when $K = 300$; a final experiment of the same type (four surveys on one pan) gave $\hat{K} = 510$ when $K = 550$. Since the mealworms usually gathered in a few huge clumps, mostly in the corners of the holding pans, and since each clump tended to be nearly entirely visible or else had few or no visible animals, these animals provided a severe test of Eq. 10. Nevertheless, from these few preliminary observations, I suggest that, when other conditions are equal, $\hat{K}$ should tend to converge toward K as the number of surveys are increased.

The densities of three very small, more or less isolated, natural populations were estimated from Eq. 10; the species were deer mice (*Peromyscus maniculatus*), canyon mice (*Peromyscus crinitus*), and cottontail rabbits (*Sylvilagus auduboni*). For the mice, the workers used a grid of live traps, and I treated the data as though each trap represented the entire space in which it was centered. Although the investigators operated each trap during each survey, after a trap caught a mouse I considered the trap and its surrounding space as having been removed from subsequent trappings, and problems of "trap-proneness" were thereby reduced. In effect, the investigators were making repeated counts of traps that had not yet caught a mouse. Based on $n = 3$ and one study area, the plot (trap) removal method gave $\hat{K} = 24$ for deer mice and $\hat{K} = 28$ for the canyon mice, but the mark and recapture technique gave only 17 and 18, respectively, for the two populations. Results by plot removal seemed slightly low but resembled densities found by various methods in similar nearby habitat, whereas results from marking seemed much too low, especially in view of the fact 15 mice were caught in the first trapping on each area. For the rabbits, where $n = 2$ on one study area, $\hat{K} = 21$, approximately, by plot removal compared to about 20 by repeated "total" counts on sample plots. Both estimates seemed very close to what K should have been.

Equation 10 describes a hyperbola in which $\hat{K}$ tends to infinity or to X_1 as P_i tends, respectively to 0 or to 1. When X_1 is kept constant, then as P_i declines below .3, $\hat{K}$ accelerates noticeably, and when P_i falls below .2, $\hat{K}$ rapidly approaches an asymptote. One should usually design the sampling so that $P_i \geqslant .3$, which requires that

$$(\sum_{i=2}^{n} X_i / \sum_{i=1}^{n-1} X_i) \leqq (.7)^{1/2} \leqq (.84)$$

Moreover, one should make at least four surveys per experiment, although seven or eight would be better; the smaller the plots are made, the longer the surveys can continue in one experiment.

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