WORLD HEALTH ORGANIZATION
TECHNICAL REPORT SERIES
No. 368

MOSQUITO ECOLOGY

Report of a WHO Scientific Group

WORLD HEALTH ORGANIZATION
GENEVA
1967

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PRINTED IN FRANCE

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Geneva, 31 October - 5 November 1966

Members:

Professor L. C. Birch, Challis Professor of Biology, School of Biological Sciences, University of Sydney, New South Wales, Australia

Mr J. A. Downes, Chief, Experimental Biology Section, Entomology Research Institute, Canada Department of Agriculture, Ottawa, Canada (*Rapporteur*)

Professor W. R. Horsfall, Department of Entomology, University of Illinois, Urbana, Ill., USA (*Chairman*)

Dr F. Kühlhorn, Principal Curator, Zoological Museum of the State of Bavaria, Munich, Federal Republic of Germany

Dr W. W. Macdonald, Sub-Department of Entomology, School of Tropical Medicine, Liverpool, England

Professor P. A. Petriščeva, Head of Department of Diseases with Natural Focality, Gamaleja Institute for Epidemiology and Microbiology, USSR Academy of Medical Sciences, Moscow, USSR (*Vice-Chairman*)

Professor J. A. Rioux, Faculty of Medicine, University of Montpellier, France

Dr T. R. E. Southwood, Reader in Insect Ecology, Department of Zoology and Applied Entomology, Imperial College of Science and Technology, University of London, England

Secretariat:

Dr N. G. Gratz, Vector Control, WHO (*Co-Secretary*)

Dr M. Laird, Chief, Environmental Biology, Division of Environmental Health, WHO (*Co-Secretary*)

Dr R. C. Muirhead-Thomson, London School of Hygiene and Tropical Medicine, London, England (*Temporary Adviser*)

Mr J. W. Wright, Chief, Vector Control, WHO

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MOSQUITO ECOLOGY

Report of a WHO Scientific Group

A Scientific Group on Mosquito Ecology met in Geneva from 31 October to 5 November 1966. Dr A. M.-M. Payne, Assistant Director-General, opened the meeting. Professor W. R. Horsfall was elected Chairman and Professor P. A. Petriščeva Vice-Chairman. Mr J. A. Downes was appointed Rapporteur.

1. INTRODUCTION

Despite the eradication of malaria and urban yellow fever from many countries, and the success of widespread control programmes based on insecticides in reducing the intensity and spread of other mosquito-borne infections, many new and challenging problems have arisen in connexion with these diseases.

Foremost among the problems attributable to the extensive use of insecticides are the development of resistance in many species of mosquito and environmental contamination with nonselective chemical pesticides. In addition, there are areas in several countries where insecticides are only partly effective against mosquitos, either because the local vector species occur in places where insecticides cannot be effectively applied, or because they avoid resting on insecticide-treated surfaces.

In many countries there has been a marked increase in the number and size of towns and cities. Often there has been no corresponding increase in such services as sewage disposal and piped water supplies, and mosquitos have been provided with numerous new larval habitats. As a result, there has been, for example, an increase in the extent of urban filariasis transmitted by *Culex pipiens fatigans* and the appearance in South-East Asia of haemorrhagic fever transmitted by *Aedes* (*Stegomyia*) spp.

The increase in areas of the world brought under irrigation, notably for rice growing, and the construction of major dams and man-made lakes, have created vast new areas that are highly favourable for the development of large populations of mosquitos. Extensive developments of this type in tropical Africa and in Asia have brought about the existence of large populations of mosquitos where none existed before. Further problems are likely to arise when additional areas are flooded in connexion with future irrigation schemes. Furthermore, agriculture has been extended into regions where it had not been developed before (e.g., parts of tropical Africa and the USSR). Mosquito populations that had been dependent entirely upon native animals in these regions have found in man an eminently suitable new host, leading to the hazard of mosquito-borne disease from natural foci.

In considering the extent to which ecological studies could contribute to a solution of the problems posed by these serious new developments, it is immediately apparent that there is a serious lack of quantitative information on many aspects of mosquito ecology. The information that is required must provide a picture of changes in density and dispersal of the vectors in relation to changes in the environment. Understanding of the epidemiology of the diseases and assessment of control programmes depend on accurate measurements of population, and knowledge of the causes of the growth and decline of mosquito populations is essential for the integration of different approaches to vector control.

2. SYNOPTIC AND QUANTITATIVE APPROACH TO MOSQUITO ECOLOGY : LIFE-TABLE ANALYSIS

Many previous studies of mosquito ecology have had a piecemeal approach. As a result, there was often a lack of the quantitative information that is essential for field control programmes. The study of the numbers of animals in a population, and of their survival and reproductive capacity in relation to their environment, is central to ecology. It is, therefore, axiomatic that methods for the measurement of the actual numbers of individuals in a population (i.e., the absolute size of the population) are a prior requirement for ecological research. Techniques that can provide such information are necessary. Many of the methods recently developed for the study of populations of other animals could profitably be adapted for use in mosquito studies.

Better methods for the measurement of numbers of mosquitos will provide more than an understanding of population dynamics. Such methods are essential for assessing the efficacy of control measures and for relating the density of the vector and its various hosts to the epidemiology of diseases. The potentials of stochastic theory (i.e., theory providing for an element of randomness, as opposed to deterministic theory, which involves no random elements) and computers for studying and forecasting changes in vector populations and patterns of disease frequency and transmission will remain unrealized without more accurate measures of population size and understanding of population dynamics.

Rational control programmes, involving the integration of biological, chemical, genetic, and other approaches (including environmental manipulation), should be based on models of vector populations. Ideally, such models for mosquitos would make it possible to recognize the most vulnerable point in the life cycle and to forecast outbreaks under known conditions, thus providing a sound basis for the planning of control measures.

In the past decade, various ecologists, particularly those concerned with forest entomology, have developed methods for the construction and analysis of a life-table (or life-budget), and this new approach should be applied to mosquitos. The life-table describes, in terms of absolute population size or density, the numbers of individuals of a generation or cohort passing through each stage, and the contribution of these individuals to the next generation. If the numbers of each stage (e.g., eggs laid, eggs hatching, larvae, pupae, and emerging adults) in a given population are counted, and the logarithm of each number subtracted from that for the preceding stage, the results (the " mortalities " over the different age intervals) are called k values. The sum of the k values gives the total " mortality " for the generation.[1] When a series of life-tables are available for successive generations of a population, the quantitative relationships of such k values to population fluctuations, to population density, and to various environmental factors may be determined. This involves the quantification of significant environmental factors as they change throughout the period of study.

Such a life-table analysis makes possible the recognition of (1) environmental factors that are important for the prediction of population fluctuations; and (2) the relationship of mortality (and dispersal) at each stage of the life cycle, and of natality, to population density. (Knowledge of this relationship is essential for building a model of the way in which numbers are determined.)

Thus, information derived from this type of analysis provides the basis for the study of population dynamics. Studies of the effects of environmental factors on the mosquito throughout its life-cycle are also important.

3. APPRAISAL OF KNOWLEDGE OF MOSQUITO ECOLOGY

This section indicates the nature of present knowledge of mosquito ecology, and the gaps in such knowledge that are important for studies of the mosquito as a vector of disease. Some 3000 species of mosquito

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[1] For further information, see Southwood, T. R. E. (1966) *Ecological methods*, London, Methuen.

are distributed over the world in a great variety of environments. There is a corresponding variety of patterns of life cycle, survival, reproduction, and dispersal. Despite this diversity, however, there are many common problems that can be approached in a similar way.

In order to understand the factors that determine mosquito numbers, knowledge of the effects of environment upon survival, fecundity, and dispersal is necessary. The data required are the total numbers of eggs, larvae, pupae, and adults in the area under study at different times, together with an analysis of environmental factors. Changes in numbers can then be related to changes in environment. Existing studies on mosquitos are deficient on two counts : (1) there are few adequate studies of total numbers of eggs, larvae, pupae, and adults; and (2) the many effects of the environment on survival and reproduction have not been fully explored, still less adequately quantified.

3.1 Ecology of immature stages

3.1.1 *Eggs*

In many species, the selection of particular oviposition sites is determined by certain qualities of the aqueous, organic, and mineral content of such sites that attract adult mosquitos. The zone of attraction may be at the surface of a body of water or it may simply be a film of water covering a substrate such as soil (certain species of *Aedes* and *Psorophora*) or some vegetable growth (*Mansonia*). Egg deposition is influenced by the physico-chemical qualities of the film and, in some instances, by those of the substrate.

Eggs may hatch within a few days following deposition (e.g., *Culex* and *Anopheles*), or several months or even several years later (e.g., the subgenus *Ochleroatus* of *Aedes*). In the former case, they lose their viability unless they remain in contact with water; in the latter, they may survive desiccation. Eggs on the surface of water are seldom subjected to great temperature variations, but those able to withstand long periods of drought may be subjected to extremes of heat or cold. Indeed, they may actually require exposure to freezing or near-freezing temperatures for the reactivation of the embryo.

Hatching follows the completion of embryonic development. Eggs not resistant to desiccation may hatch without further external stimuli. Eggs of tropical and most subtropical species that resist drying also hatch immediately following embryogeny while in a water film; they do so, however, only after being exposed to a decrease in the oxygen content of the water. Most northern species require conditioning by a sequence of stimuli before hatching can occur. Prolonged exposure to freezing or near-freezing temperatures, followed by exposure to rising temperatures, brings embryos to a state in which hatching is triggered by a decrease in the dissolved oxygen content of the water. Such a decrease in oxygen content is caused by the increased microbial activity that takes place when the temperature rises.

In the past there has been a preoccupation with the control of mosquito larvae; very little thought has been given to the possibility of developing methods for the control of eggs and pupae. There is a real possibility of exploiting the ecology of the egg for the purpose of controlling mosquitos. Since the eggs of certain aedine species have been shown to have long survival periods, additional information on this point and its epidemiological and control significance is desirable.

3.1.2 *Larvae and pupae*

3.1.2.1 *Physical factors*

Although many species tolerate relatively wide temperature fluctuations, extremes of heat or cold restrict the times and places where larvae can survive and thrive. Other physical and chemical factors that affect larvae and pupae include salinity, pH, hardness, aeration, pollution, and movement of water and intensity of light. The specific limits within which certain species tolerate such factors merit further investigation, as an aid to the better understanding of the occurrence and distribution of the species concerned. Further work in this area would assist the development of a new branch of limnology—“ microlimnology ”—concerned with the small bodies of water that are so extensively utilized by mosquitos but that have been largely neglected by hydrobiologists.

3.1.2.2. *Food*

Although a great deal has been recorded about the matter that is ingested by larvae (e.g., particles of organic matter, diatoms, and other algae), much less is known about the nutrients they utilize, except for some carnivorous species such as members of the subgenus *Lutzia* of *Culex*). Food is also important in influencing the rate of growth, the development of imaginal organs (e.g., ovaries in autogenic species), and the process of infection by pathogens. Greater knowledge of the nutritional requirements of larvae might lead to ways of making the food less accessible.

3.1.2.3 *Vegetation*

In addition to providing food, vegetation—as part of the environment of immature stages—acts in a number of other ways. It directly affects the temperature, evaporation, surface characteristics, and chemical composition of the water; it influences the amount of light reaching the water surface; it provides harbourage (e.g., tree holes and leaf axils) for immature stages; and it provides sites of attachment and a source of oxygen for larvae and pupae of *Mansonia.* For these reasons it is of great importance to maintain a close watch on changes in the vegetation (e.g., *Eichhornia* and *Pistia*) in areas subject to man-made alterations in water level. Such changes (as have recently occurred in the Tennessee Valley Authority area through the introduction of *Myriophyllum spicatum*) have caused great increases in local mosquito populations.

Vegetation, both macroscopic and microscopic, is a convenient and dependable indicator of total ecological conditions. In this connexion it is noted with concern that field investigators often experience great difficulty in identifying the species of plants. Also, while total floras of given localities may be well known, local plant associations may not. Greater use might be made of vegetation maps, which are sometimes already available as a result of work in other scientific fields. In recent years, too, considerable advances have been made in the use of phytoplankton for biological monitoring of the extent of water pollution. There is a great deal of valuable information on this subject in the sanitary engineering literature.

3.1.2.4 *Associated fauna*

WHO is conducting studies of the natural enemies and diseases of mosquitos and other arthropods of public health importance. In recording its approval of this activity, the Scientific Group wishes to emphasize the necessity of detailed knowledge in this area as a prerequisite not only to the selection of practical biological control agents, but also to the most effective field application of such agents and the properly selective use of chemical larvicides in integrated control. This information is also necessary for an understanding of the way in which predators and disease determine host numbers.

There is already a considerable body of literature relevant to the pathogens, parasites, and predators of mosquitos. Much of this information, however, comprises chance observations made during studies of a broader nature, and little of it is based upon really sound experimentation. There is a decided need for competent reviews of the data that are now scattered through a multitude of journals and reports—many of which are difficult to obtain—and for a widespread census of predators of mosquitos. Also, as most of the pertinent experiments that have been conducted involved only single variables, it is not yet possible to evaluate the significance of disease and natural enemies in relation to the density of wild populations of even one mosquito species.

The available evidence makes it clear that, from the moment of oviposition, the mosquito egg is subject to attack by pathogens, including fungi, and to destruction by predators such as certain members of the orders Coleoptera and Hemiptera. It is also clear that the aquatic stages are continuously subjected to such hazards, with intermittent phases of extreme vulnerability (e.g., at hatching, successive moults, pupation, and emergence of the adult). The extent of the hazards varies greatly, due to factors such as locality, season, and type and nature of the larval habitat. Ecology should also aim at studying those intrinsic factors that promote or inhibit excessive multiplication of pathogens.

Knowledge of the ways in which the various factors discussed in this section affect the immature stages of mosquitos could certainly be utilized to design appropriate alterations of the environment. Such changes, effected by drainage, filling, removal of vegetation and shade, water-level fluctuation, or related measures, have been used successfully for reducing mosquito numbers. Because of their extremely important control implications, they merit the most careful consideration from the earliest planning stage in any water management project.

3.2 Adult mosquitos and their environment

Survival and fecundity are two of the basic factors that determine the numbers of mosquitos. In order to understand how numbers are determined, knowledge of the ways in which survival and fecundity are affected by environment is essential.

From the time when it commences to emerge from the pupal skin, a mosquito is subject to the direct influence of non-aquatic environmental factors. In the earliest phase of its adult life, and again at oviposition, it may of course also be subjected to aquatic influences, a fact that may be useful in the design of control measures.

Non-aquatic environmental factors include temperature, humidity, rainfall, wind, and less obvious ones such as changes in air pressure and the electrical fields of a mosquito's surroundings. Also of importance are predators, diseases, and factors that are dependent upon population density and that influence a mosquito's contact with its own and other species. Furthermore, food, shelter and resting sites, a mate, and oviposition sites are all required for survival and reproduction.

An energy-yielding food, usually nectar, may be required by both sexes; and the female, in addition, typically requires a blood meal for maturation of the eggs.

Probably none of these influences and requirements has a simple incidence. Their effects vary with age and in relation to two strongly marked periodicities that run throughout the life-span. The first hours and days are characterized by maturation phenomena, perhaps mating, and sometimes special dispersal. Then follows a period of maturity, when (in several species that have been investigated) the death-rate remains constant and apparently subject only to steady external influences. Finally, there is a period of senescence, with changes in feeding, reproduction, and mortality. The two periodicities that also influence the effects of environmental factors are (1) a daily periodicity of activity (controlled by light, temperature, and an internal rhythm) that may follow different patterns in the two sexes; and (2) a gonotrophic periodicity, shown by the female only, so far as is known, that is related to the repeated cycle of blood sucking, egg maturation, and egg laying, correlated with which are great changes in activity, behaviour, and physiology. Repetition of the gonotrophic cycle may hasten senescence, and hibernation and aestivation pose special hazards, such as unusual vulnerability to certain types of fungal attack.

All these influences and requirements probably affect the two sexes differently. It is commonly found that all females more than one or two days old are already mated, and it has been assumed that survival of the male is of minimal significance. However, it is now known that there is a marked tendency for the females of several species to mate only once; it also seems possible that, as with *Drosophila*, the quantity of sperm transferred is not always in great excess but rather of the same order as the number of eggs produced. This being so, the role of the male in determining the number of progeny becomes more nearly comparable to that of the female.

In genetic control techniques (including the use of sterile or incompatible males) all aspects of male dispersion and survival are obviously of great significance.

Certain co-ordinating processes of mosquito biology have begun to attract attention. First, there is the grouping of processes in the first days of adult life, among which male maturation, mating, dispersal, and the first meals find a place, perhaps in a definite sequence. The endocrine system is now believed to control nutritional metabolism and the onset and progress of ovarian development, and also perhaps the general level of locomotor activity. Thus, dispersal—a most important element in epidemiology—may be related to the age and reproductive condition of the insect. Flight is essential, in typical species at least, for dispersal, food-finding, mate-finding, and the discovery of resting and hibernation sites. It is known to be controlled by various sensory (mainly visual) processes, and its speed and extent are related to the feeding and to the energy expenditure of the insect. Again, the periodicity of flight has a great effect upon the times of feeding, mating, resting, and ovipositing of many mosquitos; and it thereby influences other ecological factors and leads to such situations as the special vulnerability of male swarms to predators.

Systematic and intensive study of all these co-ordinating processes, which can evidently have far-reaching effects upon mosquito ecology, is urgently required.

There is not only wide variation in many aspects of behaviour of mosquitos generally, but there are sometimes very striking contrasts between different phases of life or between closely related species. Certain anopheline females do not need a sugar meal, and can obtain all the energy they require from the blood meal. The females of a number of species, representing several genera, can dispense with a blood meal in the first cycle and mature their eggs on materials stored at the time of larval feeding. This autogeny may yield a full or a very incomplete batch of eggs; it may be genetically or environmentally determined; and it may affect the whole or only a part of the population, perhaps with geographical variation. Mating may take place in quasi-stationary flying swarms formed by the assembly of individuals from wide-ranging dispersive flight; it may take place, again in flight, but with scarcely perceptible aggregation and in the neighbourhood of the larval habitat; or it may take place on the substrate and not in flight. Dispersal may occur by a relatively large-scale migration, usually at the beginning of adult life, or by smaller-scale movements related to daily activities throughout adulthood. Moreover, when the insect is flying low enough to be able to perceive the ground pattern, movement is frequently up-wind; however, when it is too high to perceive the ground pattern its orientation is uncontrolled and its over-all movement is down-wind at wind speed. Many other analogous examples could be given.

The measurement of dispersal is important for understanding the role of the adult in epidemiology, in infesting new regions, or in re-infesting areas where control measures have been carried out—and also, as noted above, in the design of sterile-male and related techniques. Understanding of the distinctions between the various modes of dispersal is obviously essential for meaningful quantification.

The aggregation of males in a swarm, characteristically positioned over a definable landmark, is in many species a necessary preliminary to mating. The concentration of insects in the swarm renders them vulnerable to predators such as bats and dragonflies. A female entering the swarm is recognized by its flight (wing-beat) tone, and a male responds by flying towards the female. This stimulus and response can possibly be exploited as a means of trapping large numbers of males for destruction, or for dosing with chemosterilants.

Observation of swarms, and earlier behavioural studies in the laboratory, have given some information on the way in which the pattern and speed of flight are controlled by the observation of landmarks from the air.

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[1] It is often possible to induce the formation of a swarm over an artificial landmark; this suggests a possible control method that merits investigation.

It might now be possible, with relatively little developmental work, to establish the conditions for sustained flight and mating in reasonably small cages, and thus to make possible the breeding of additional species in the laboratory.

There is growing evidence that the females of various species of mosquito are less selective in their choice of hosts for the blood meal than was formerly believed. This has been noticed particularly by workers in the USSR in studies of species in natural habitats that have not been disturbed by human settlement. The natural hosts of certain species of *Anopheles*, *Culex*, *Culiseta*, and *Aedes* include mammals, birds, reptiles, and amphibians. Elsewhere, it is apparent that the favoured host varies with region and even microhabitat, and with the season of the year and even the time of day; precipitin tests have shown that an individual mosquito may take successive blood meals from different species (even three or four different species) of host. In general, experimental work supports these observations; the chemical and visual stimuli that lead a mosquito to find and alight on a host are mainly of a kind produced by many, if not all, species of land vertebrates. Such selectivity as there is, at least for the species that have been investigated, is based principally on ecological factors (e.g., the size, number, availability, and time and place of activity of the hosts) rather than on specific stimuli, and is thus inherently modifiable. This reduced host selectivity is one of the properties that give to mosquitos their great importance in the transmission of zoonoses and the spread of zoonotic agents.

Little is known of the sugar meal in nature, and studies of its importance, frequency, timing, and specificity are necessary. It has already proved unexpectedly specific in males of certain *Aedes* species in Canada, and of *Anopheles sergenti* at Siwa Oasis, United Arab Republic, emphasizing the great desirability of additional observations under natural conditions elsewhere. In considering these questions it must always be kept in mind that experimental trophic behaviour may differ from field trophic behaviour. It is possible that studies in this area might lead to new or improved control techniques—for example, trials might be conducted to assess the effect of removing the preferred host plants (or certain parts of them, since the only relevant observations have been of flowers and the stomata on the under surfaces of leaves).

Oviposition, which follows the digestion and utilization of the blood meal, has not been widely studied. Most species lay their eggs on a moist substrate or open water—individually, or clustered, or as rafts. It seems probable, therefore, that the periods devoted by different mosquitos to searching for oviposition sites and to egg-laying vary considerably. In general, little is known about the sensory testing of the site before the eggs are laid, but this must be an important process since it usually determines, once and for all, the habitat available to the larvae.

4. POPULATION-STUDY METHODS

4.1 Relative and absolute population estimates

There are two principal types of estimate of animal populations : absolute estimates of the actual numbers per unit area; and relative estimates of the numbers in terms of some trapping period or other unit whose relation to the size of the total population is unknown. The central requirement of life-table work is absolute population estimates. As the fiducial limits of such estimates may be wide, it is always of value, whenever possible, to make estimation by more than one method; this also helps to ensure that part of the population is not totally overlooked. The internal consistency of the life-table provides yet another check on the reliability of the estimates.

Much information on the ecology of adult mosquitos is based on catches in various types of trap, such as light traps, shelter traps and bait traps. Catches of this kind are affected by both the density of the population and the behaviour of its constituent individuals. They give a measure of the population in relation to a particular stimulus; when this stimulus is the natural one of a host (e.g., man) such a measure is meaningful, and is also important for the assessment of the epidemiological significance of the vector. However, relative estimates are affected by so many factors that they are of limited use for the construction of a life-table.

Since much of the previous work on mosquitos has emphasized relative methods, studies aimed at the construction of a life-table will initially have to be based largely on techniques developed for other animals. The aims of such studies will be to provide estimates of absolute population and to assess the density of the vector in relation to transmission.

4.2 Estimation of absolute numbers

4.2.1 *Eggs*

Satisfactory methods have been devised for sampling the eggs of flood-water species, but improved methods are necessary for species that use more permanent water and for container breeders. Techniques might be based on the use of unit-area sampling frames for eggs on free water (e.g., ponds), and on the use of experimental containers for species that lay eggs in natural containers (e.g., coconut husks) and man-made ones (e.g., tin cans).

4.2.2 *Larvae*

Traps based on the habit of larvae of ascending to the surface have generally not given satisfactory samples of larval populations. A better assessment would be obtained by unit-area samples. Some traps have been designed to enclose a volume of water having a standard surface area; the contents of such traps can be removed and sieved. Alternatively, a base plate can be inserted into the submerged trap and the water allowed to drain away as the trap is removed. The available traps are not ideal, and new methods of collecting larvae are required.

Two other techniques are available : the mark-release-recapture method, and removal trapping. The mark-release-recapture method has not been widely used, and although techniques for the mass marking of large numbers of larvae are available, the problems associated with the marking of individual larvae are still to be overcome. Methods of analysis of recapture data are well developed but have been insufficiently utilized in studies of larval populations. The removal method is based on the theory that the number of animals captured per unit time is proportional to the number available, and the total population is estimated from the results of a series of catches. The method has been applied to mosquitos but, since catching efficiency often varies during successive catches, it is, perhaps, more appropriate as a supplementary method to check estimates obtained by other means.

4.2.3 *Pupae*

Pupal populations might be measured by methods similar to those mentioned above. Quantitative data would have the additional value of verifying the assumption that estimates of pupal density bear a close relationship to estimates of adult populations.

4.2.4 *Adults*

Direct estimates of the numbers of adults produced per unit area, or per container, might best be obtained by the use of emergence traps, which have hitherto been little used in mosquito studies. Floating traps of transparent materials could be valuable in open-water habitats, and it would be easy to cover entire containers with a simple emergence trap. In both types of habitat, a standard 24-hour trapping period would seem appropriate, the trap then being sited elsewhere. The measurement of adult emergence patterns and of adult production per unit area provides a better assessment of the relative importance of different kinds of habitat than do estimates of larval population in the habitats.

Mark-release-recapture techniques have been shown to be practical for some species of mosquito, and modern modifications of the Lincoln Index[1] allow estimates to be made of additions to and losses from a population, as well as of population size. At present, there is no other satis-

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[1] The index derived by dividing the total number of marked units released by the proportion of marked units recaptured.

factory technique for measuring the absolute density of a population (see Annex 1).

Few attempts have been made to measure the total number of airborne adults at a given time. Estimates can be obtained using suction traps or sticky traps, allowance being made for the effect of wind speed at different heights. Studies of the time taken by a given population to fly a certain distance, similar to those performed successfully with other insects, would be valuable, and might include exploration of ways to obtain sustained flight conditions in the laboratory.

4.3 Natality and mortality

At the same time as the actual populations of each stage are being estimated, it is valuable to determine the role of the different factors that affect the three ways in which populations change : natality, mortality, and dispersal. Such measurements may be based on two different approaches : direct measurement in the field (e.g., a count of the number of non-embryonated eggs), and experimental assessment in the field (or in the laboratory, using material collected in the field).

The potential influence of physical environmental factors, such as temperature and salinity, can be determined by laboratory experiments. The role of parasites and pathogens may be quantified by analysing their frequency in samples drawn from the natural population and by determining their pathogenicity. It is difficult to determine the relevance to natural populations of laboratory findings on the numbers of a particular life-history stage of a mosquito that may be consumed by predators. However, Canadian studies have shown that the number of prey consumed in the field by different predators can be estimated by employing radioisotope markers. This approach should be extended, and consideration should also be given to the possibility of using serological techniques to identify the meals eaten by predators, as has been done in work on some nonflying insects. A great deal could also be learned about the significance of aquatic predators in the natural regulation of mosquito populations by using an appropriate pesticide to kill all predators in selected habitats and then restocking with mosquitos alone.

4.4 Dispersal

Techniques for monitoring insect dispersal leave much to be desired. Some studies of mosquito dispersal have been made by releasing large numbers of marked individuals at a central point. Such experiments certainly yield information on the potentiality for dispersal; however, the contrived conditions of the release mean that it is not necessarily a reliable guide to movements that take place in nature.

A satisfactory method has yet to be developed for marking all the adults of a natural population at emergence. It is true that dyes and other materials have been applied to larval habitats at the time of emergence; however, the results have been of relatively limited value because of failure to mark all emerging adults, and the technique has not lent itself to the systematic marking of individuals during the larval stages. The use of genetic markers also offers possibilities, but it must be borne in mind that any behavioural changes associated with this or indeed any method could invalidate its use.

The Scientific Group discussed these questions at length and was informed of a promising mark-release-capture experiment based on the fact that if newly-emerged adults collected in an emergence trap are individually marked and carefully released, the relation of the rate of movement to the age of individuals can be studied, and any migratory phase recognized (see Annex 1).

Recent experiments involving the release of numbers of radioactively marked newly-emerged individuals under more or less natural conditions have provided evidence on the spread of the population concerned. Quantitative information on dispersal could also be obtained by placing traps at various distances from a study area in which at least some of the population have been marked. The rate of decrease in numbers with distance, and the normality or skewness of the curve plotted from this rate, give information on the degree of dispersal and the extent to which the tendencey to disperse is normally distributed within the population.

Long-distance migration may (particularly if the insects are windborne) be studied through the use of suitable traps placed in appropriate locations at different heights above the ground. Continuous records of the wind speed at each height are also necessary, so that the results may be corrected for the effects of wind. Data obtained in this way with insects other than mosquitos have been used for the calculation of total airborne populations and the mean distance travelled by individuals. Mobile traps (e.g., those attached to land, air, or sea transport vehicles) can also give useful information on the movement of individuals.

4.5 Environmental factors

The other biotic and physical components of the mosquito's environment must also be measured, although this assessment should be limited to organisms and factors likely to have some direct effect on the insect.

Where estimates of the absolute population of associated fauna are required, the general principles outlined above with regard to mosquito studies are applicable.

Various methods may be employed for the quantitative assessment of the vegetation associated with larval habitats. With macroscopic plants, for example, a square or a linear analysis may be applicable; under other conditions, mapping on a more or less extensive scale may be preferable.

Microscopic flora and fauna (e.g., protozoan and other plankton) may be studied by means of modifications of the collecting and laboratory techniques already widely used by limnologists.

ACKNOWLEDGEMENTS

The Scientific Group acknowledges the contributions made by the following staff members of WHO : Mr R. F. Fritz, Vector Control; Dr J. W. Kliewer, Consultant (University of California mosquito control research group, Fresno, Cal., USA); Mr M. Nicole, Terminology, Division of Editorial and Reference Services; and Dr R. Pal, Vector Control.

Annex 1

THE MARK-RELEASE-RECAPTURE EXPERIMENT AT THE WHO *AEDES* RESEARCH UNIT, BANGKOK

WHO has established an *Aedes* Research Unit in Bangkok, Thailand. This Unit is carrying out research on the biology, population dynamics, and insecticide susceptibility of *Aedes aegypti* and closely related *Stegomyia* species. Through a better understanding of these factors it is hoped to develop effective and economical methods of reducing *Aedes* vector populations to a level at which the transmission of dengue and dengue-like fevers will cease. A mark-release-recapture experiment is being conducted with *A. aegypti*. The purposes of the experiment, and the methods used, are as follows.

The objectives of the experiment are (1) to obtain estimates of the adult population in a given area, (2) to estimate the daily increases in numbers and the survival rates of males and females, (3) to measure the short-range movements of the mosquitos, and (4) to follow seasonal fluctuations.

The study area is a group of houses that forms part of one of Bangkok's temples, Wat Samphaya. The area measures approximately 94 × 56 m. There are 31 houses with 94 numbered bedrooms, and at each visit collections of resting *A. aegypti* are made in 30 of the bedrooms, the 30 rooms being selected by means of random-number tables. On average, three or four mosquitos can be found in a room during a search of 5-10 minutes.

The mosquitos are collected with an aspirator, transferred individually to glass tubes, and taken to the marking station in the temple. They are then anaesthetized with ether, and each is given an individual series of marks. A quick-drying paint is employed for marking, the paint being applied with a fine nylon bristle from a test-tube brush. One to eight spots can be painted on a mosquito at three sites on the costa of each wing and at two on the thorax. A ninth spot, posteriorly on the thorax, can be used as a series mark. Each spot represents a number, and, using a binary system, 255 mosquitos of each sex can be marked with one colour of paint. Red, yellow, and white have proved the most satisfactory colours. When these three colours have been used to mark 765 mosquitos of each sex, the ninth, or series, mark can be introduced. Three more series, each of 765 mosquitos, can then be marked, using red, yellow, and white series marks in turn. Additional colours can be introduced if necessary.

Each mosquito, therefore, has a unique number. The room where it was first collected is recorded, and a record is also made of the condition of females (unfed, fully-fed, semi-gravid, or gravid). After being marked, each mosquito is transferred to a clear plastic box, 6 cm in diameter, on which are written the number of the mosquito and the number of the room where it was found. Later in the day the mosquitos are returned to these rooms; those that do not fly or that were injured during handling are killed and the fact is recorded.

When marked mosquitos are subsequently recaptured, a record is made of their number, the room where they were found, and, in the case of females, their condition.

Three visits a week are usually made to the study area. The recapture rate has been 7-8% for females and 4-5% for males. The population size and survival rates are being estimated by the methods of Fisher & Ford [1] and Jolly.[2] (These two methods and others are reviewed by Parr [3] and Southwood. [4]) Day-to-day estimates can be made with the use of a calculating machine. However, the recording forms for the Bangkok experiment have been designed so that the data can be transferred with ease to punch cards. These are to be fed into a computer programmed to make the necessary calculations of population size, rates of addition to the population and of survival, and the variances in these factors, together with estimates of the characteristics of short-range flight.

Annex 2

DESIGN OF A POPULATION STUDY OF ADULT *CULEX PIPIENS FATIGANS*

The WHO Filariasis Research Unit in Rangoon, Burma, has carried out extensive investigations of the biology, ecology, and control of the main urban vector of filariasis, *Culex pipiens fatigans*. The Unit is developing insecticidal and other control methods intended to reduce *C. p. fatigans* to a point at which transmission of urban filariasis will be interrupted.

The most efficient available method of estimating the absolute density of a population of adult *C. p. fatigans* in a given area is the mark-

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[1] Fisher, R. A. & Ford, E. B. (1947) *Heredity*, **1**, 143-174.

[2] Jolly, G. M. (1965) *Biometrika*, **52**, 225-247.

[3] Parr, M. J. (1965) *Field Studies*, **2**, 237-282.

[4] Southwood, T. R. E. (1966) *Ecological Methods*, London, Methuen.

release-recapture technique. Depending upon the amount of information required about the population, this technique is performed in one of three different ways.

If only the population density is required, an estimate can be obtained by two days' marking. On the first day, as many individuals as possible are collected in the study area and given a standard mark representing the date of collection. The mosquitos are then released. On the second marking day, which should preferably follow the first as soon as possible, the procedure is repeated but the mosquitos are given a different mark; recaptures of mosquitos marked on the first day are recorded and the mosquitos are killed. On subsequent collecting days (which need not be at regular intervals) only marked individuals need be recorded, and after recording they are killed. Collecting of marked individuals should continue until the recovery rate is nil. Applying the Jolly method of analysis, the population size during the second day of collecting and marking can be estimated.

If estimates of both the population size and survival rate are required, mosquitos must be collected and date-marked on at least three occasions. Recaptured mosquitos are given the mark of the day of recapture, in addition to the previous mark(s), and released once more. After the last day of marking, collecting should be continued until no further recaptures are made (all recaptured individuals are killed and the fact is recorded).

In both these approaches to population study, some information on flight range can be gathered if the mosquitos are released at a central station, and subsequent collections made at some distance from this station. However, it is more important that the mosquitos be returned to the habitats from which they were collected, at the expense of information on short-range movements. If data on dispersal are required, a unique mark for each individual is necessary. A method of giving individuals unique marks is described in Annex 1.