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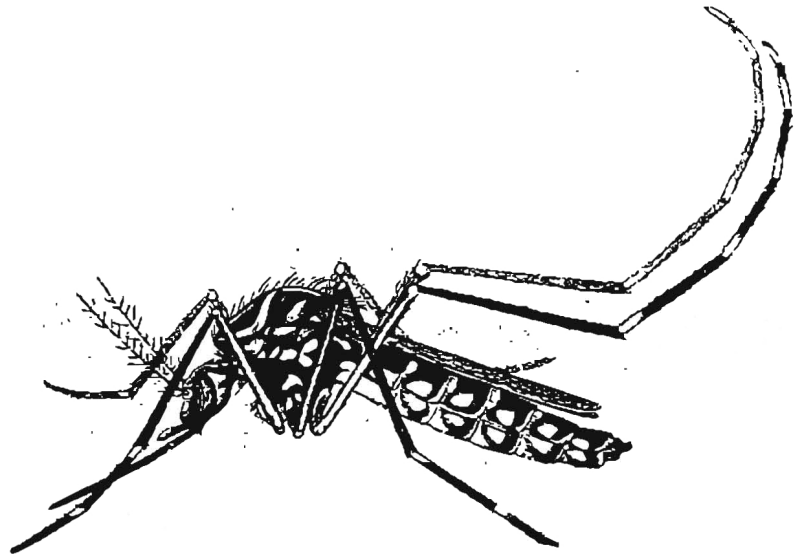
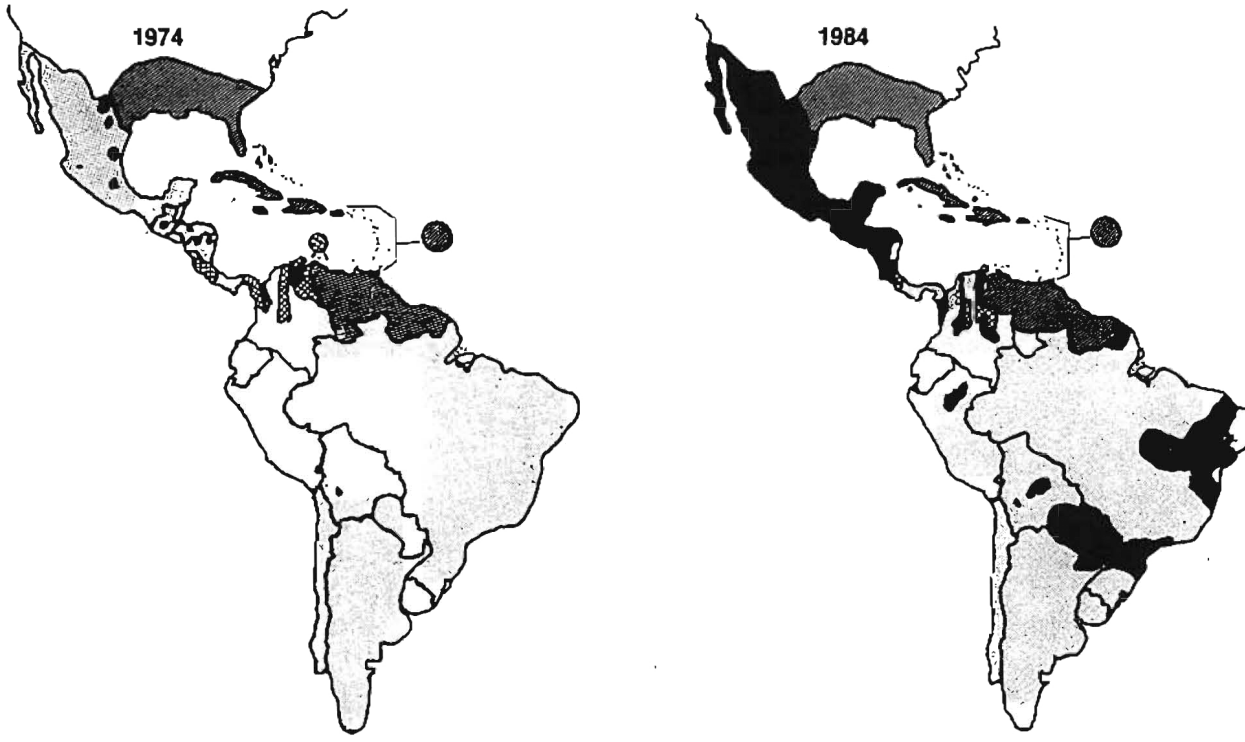


AEDES AEGYPTI: BIOLOGY AND ECOLOGY

Michael J. Nelson



PAN AMERICAN HEALTH ORGANIZATION
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AEDES AEGYPTI: BIOLOGY AND ECOLOGY

Michael J. Nelson
Regional Advisor in Entomology
Communicable Diseases Program

PAN AMERICAN HEALTH ORGANIZATION
Pan American Sanitary Bureau, Regional Office of the
WORLD HEALTH ORGANIZATION
525 Twenty-Third Street, N.W.
Washington, D.C. 20037, U.S.A.
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PREFACE

The mosquito Aedes aegyti and the diseases it transmits, dengue and yellow fever, are still present in the Americas. The emergency situation created by the epidemics of classical dengue that affected most of the countries of the Caribbean, Central America, northern South America, and Mexico during 1977-1979, and the first epidemic of dengue hemorrhagic fever reported in Cuba during 1981, have renewed interest in strengthening programs to control or eradicate Aedes aegypti in the countries of the Region.

Prevention and control of diseases produced by flavivirus depend basically on the levels of infestation and control of the vector. Use of preventive measures such as control and vigilance, combined with a multisectorial focus and effective community participation, represents the recommended approach. This strategy, when adapted in a flexible way to local transmittal conditions and resources, will permit stimulation of progress in public health programs.

This strategic approach must take into account ecological, social, cultural, economic, and political conditions related to migratory patterns, agricultural practices, environmental characteristics, vector resistance to insecticides, and the ability of health services personnel to achieve the proposed goals and objectives. National programs play an important role in the development and execution of this control strategy. The Pan American Sanitary Bureau, taking into account the limitations in human and financial resources, seeks to incorporate the most effective, efficient, and safe methods of control and to promote personnel training activities and

operational research as basic elements in the development of programs of prevention, surveillance, and control of these diseases.

It is our hope that this manual will contribute to the training of health care personnel in the methodology for study, surveillance, and control of Aedes aegypti.

Dr. Francisco J. López Antuñano
Coordinator, Communicable
Diseases Program

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INTRODUCTION

Aedes aegypti has traditionally been known as the "yellow fever mosquito." For centuries, yellow fever was a serious disease in the tropical Americas and in Africa, and it spread during the summers to temperate areas in violent epidemics with high mortality rates, especially in seaports and river cities. In 1881, the Cuban physician Carlos Finlay propounded the theory that the yellow fever pathogen was carried by the Aedes aegypti mosquito. In 1900, the Yellow Fever Commission of the United States Army, composed of Walter Reed, James Carroll, Jesee W. Lazear, and A. Agramonte, proved conclusively Finlay's hypothesis. In Havana and Panama, W.C. Gorgas demonstrated the feasibility of eradicating yellow fever by reducing the Ae. aegypti population. Subsequently, a sylvatic cycle of yellow fever was discovered in the Americas between mammals (especially some species of monkeys) and mosquitoes of the strains Haemagogus and Sabethes. In Africa a similar mammal-mosquito cycle exists, with the species Aedes africanus and Ae. luteocephalus figuring most prominently in the sylvatic cycle and Ae. simpsoni in the peridomestic cycle. Humans usually become infected when they enter the forest and are bitten by infected mosquitoes.

Outbreaks of sylvatic yellow fever continue in the Americas in Bolivia, Brazil, Peru, Ecuador, Colombia, Venezuela, and Trinidad and Tobago. Several outbreaks have occurred in rural populations in Colombia with migration of viremic patients to nearby cities and towns that were heavily infested with Ae. aegypti. However, for some unknown reason, no urban transmission has been documented in the Americas for the past four decades.

Aedes aegypti is currently more important as a vector of dengue. This disease, commonly called breakbone fever, has also been with us for

many centuries. In the New World dengue is principally an urban disease with only one cycle -- man-Ae. aegypti-man -- and with no indication of a feral cycle. In urban areas of Asia, the disease is transmitted mostly by Ae. aegypti, but in some peri-urban areas other Aedes of the subgenus Stegomyia, Aedes albopictus and of the Aedes scutellaris group are vectors. In Malaysia there is also some evidence of sylvatic transmission by the Ae. (Finlaya) niveus subgroup.

Although dengue is seldom fatal, there have been pandemics causing very high morbidity rates. During 1977 and 1978, dengue serotype 1 spread through most of the countries of the Caribbean, Central America, northern South America, and Mexico, and even into Texas in the United States.

In 1954, a more serious form of dengue was reported in the Phillipines: dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). This form of the disease was often fatal, especially infected children from ages 2 to 13 years. It quickly spread to the other countries of Southeast Asia and the South Pacific, causing over 350,000 hospitalizations and nearly 12,000 deaths from 1956 until 1981. This syndrome did not spread to the Americas until June 1981, when an outbreak of dengue hemorrhagic fever occurred in Cuba, causing 159 deaths. Of the 344,203 cases, 116,143 required hospitalization.

The etiology of the DHF syndrome is not clearly understood. The most commonly accepted hypothesis suggests that it is an extreme immunological response to sequential infections by two of the four different dengue serotypes, with dengue 2 usually being the second type in the sequence.

DISTRIBUTION

Aedes aegypti probably originated in Africa where there are three forms of this species: Ae. aegypti aegypti (the typical form), Ae. aegypti queenslandensis, and Ae. aegypti formosus (a smaller, darker, forest mosquito). Only the first two forms are found in the Americas. They were probably first transported from the Old to the New World in water barrels on ships during the early European explorations and colonizations.

Aedes aegypti is a tropical and sub-tropical species found around the globe, usually within the limits of 35° north and 35° south latitudes, corresponding to a summer isotherm of 10° C (see Figure 1). Although it has been found as far as 45° north latitude, these invasions occur during the warm season and the mosquitoes do not survive the winter.

At the beginning of this century, Ae. aegypti was found in all territories of the Americas from southern United States to Buenos Aires, Argentina. During the 1920's, advances were made in the attack on this vector, and in 1947, the member nations of the Pan American Health Organization resolved to eradicate Ae. aegypti from the Western Hemisphere. By 1965, eradication was confirmed in 17 of 49 countries and political units of the Americas. However, due to financial, political, technical, and administrative problems, most of the countries have become reinfested. In 1980, Bolivia was reinfested after being free of this vector for 37 years; in 1981, 25 years after eradication, Paraguay became reinfested; and in 1984 the Amazon River region of Peru was reinfested. In 1985, only 6 countries were still free of this species (see Figure 2 and Table 1). Panama and Ecuador experience frequent reintroductions of Ae. aegypti but, to date, have been able to eliminate each new infestation.

Distribution of Ae. aegypti is also limited by altitude. Urban Ae. aegypti has been reported at 2121 meters above sea level in India and at 2200 meters in Colombia where the mean annual temperature is 17°C. Farther from the equator, this species is rarely found above an altitude of 1000 meters.

In the New World, Ae. aegypti is primarily a domestic species, infesting man-made or natural containers found in or near human dwellings. The female Ae. aegypti feeds on human blood or on that of domestic animals. The mosquito is rarely found more than 100 meters from houses, although exceptions have been reported in the West Indies and the southern United States. In Cayman Brac, Ae. aegypti larvae were found in roof catchment cattle cisterns over 400 meters from human dwellings, and on the island of Anguilla, infestation occurred in fossilized coral rock holes (Karst solution holes) sometimes more than one kilometer from dwellings. Both populations apparently had the coloration of the "domestic" form of Ae. aegypti. In southeast Texas, eggs of Ae. aegypti were found in oviposition traps over 8 kilometers from the nearest human habitation, and larvae were collected from a tree hole 3.2 kilometers from human dwellings.

Because of its close association with man, Ae. aegypti, is essentially an urban mosquito. However, Brazil, Mexico, and Colombia have reported significant rural invasions sometimes many kilometers from the nearest urban center and the nearest vehicular road. Rural infestation is probably common in other countries as well. Ae. aegypti apparently invades rural areas as eggs or larvae found in domestic containers that are transported from urban areas to rural houses for water storage.

BIOLOGY

The life cycle of Ae. aegypti progresses from the egg stage through four larval instars and a pupal stage to the adult stage (see Figure 3).

The Egg

Ae. aegypti eggs are approximately 1 millimeter long, cigar shaped, and smoother than the eggs of most other container breeders (see Figure 4). The eggs, which are fertilized at the moment of oviposition, are deposited singly on the container wall just above the water level. When laid, the eggs are white but very rapidly turn to shiny black. Embryonic development is usually completed within 48 hours if the environment is humid and warm, but it may take up to 5 days at lower temperatures. Once embryonic development is complete, the eggs can withstand long periods of desiccation, sometimes more than a year. When the eggs are eventually flooded, the bacterial action on the organic matter in the water lowers the oxygen tension and provides an egg-hatching stimulus. Some eggs hatch within 15 minutes of flooding, but others may not respond until they have been inundated several times.

The property of the eggs to withstand drying is one of the most important obstacles to the control of Ae. aegypti. Eggs can be transported over great distances in dry containers. Elimination of adults and larvae from a locality during many months does not prevent reinfestation from eggs that have been hidden in dry containers.

The Larva

The larvae and pupae of Ae. aegypti are entirely aquatic. As with most holometabolic insects (i.e., those undergoing complete metamorphosis), the larval stage is a period of feeding and growth in which larvae spend most of the time using fan-like mouth brushes to feed on submerged objects and on any organic material found on the sides and bottoms of containers.

Larval Morphology and Characteristics. Ae. aegypti larvae are similar to other mosquito larvae in that they have ovoid heads and thoraxes and 9-segmented abdomens. The posterior and anal segment of the abdomen has four lobed gills for osmotic regulation and a siphon or air tube for respiration at the water surface (see Figure 5). Aedes larvae can be distinguished from most other genera with the naked eye (see Figure 6). The siphon is shorter than that of most other culicines and, in anophelines, there is no siphon. The resting position at the water surface is also different among the various species: Anopheles larvae lie parallel to the water surface; Culex larvae rest at an angle; and Aedes larvae hang almost vertically. Ae. aegypti larvae swim with a distinctive, serpentine movement, unlike Culex larvae which are characterized by jerky side-to-side movements of the abdomen. Ae. aegypti larvae are also very sensitive to sudden changes in light intensity and will swim to the bottom of the container when disturbed.

A stereoscopic dissecting microscope is necessary to distinguish Ae. aegypti from many other species of the Aedes genus. As Figure 5 shows, the most pronounced distinguishing characteristics are the prominent lateral spines, two on each side of the thorax, and the straight row of 7 to 12 comb scales on the eighth abdominal segment each of which has a median spine and lateral teeth.

Larval Development and Survival. The first instar is that which emerges from the egg. After a day or two of feeding and growth, moulting occurs and the second instar emerges. Immediately after moulting, the head capsule and siphon are soft and transparent, but after expanding to allow for further growth, they harden and darken. After the second stage, the head capsule and siphon will not change in size, but the thorax and abdomen will grow considerably during the subsequent stages.

Duration of larval development depends on temperature, food availability, and larval density in the receptable. Under optimal conditions, the time from hatching to pupation can be as short as 5 days but more commonly lasts 7 to 14 days. The first three instars develop quickly, while the fourth instar takes longer and increases more in size and weight. Under harsh conditions of low temperatures or scarcity of food, the fourth stage of larval development may last several weeks before pupation occurs. The male larvae and pupae develop more rapidly than the female.

In a stable environment, the highest mortality among the immature larval forms usually occurs during the first two larval stages. However, the majority of larval habitats are not stable. Most of the waste containers that serve as breeding sites are small (e.g. tires, tin cans, and bottles) and are found outdoors. Moreover, they are vulnerable to drying from the sun and to flooding and overflowing from rain. Frequently many of the containers used for domestic water storage are emptied and washed, or variable amounts of water are removed from them. These disturbances probably account for most of larval and pupal mortality.

The Pupa

Pupae do not feed. Their function is metamorphosis from the larval to the adult stage. Mosquito pupae are different from those of other holometabolous insects in that they readily react to external stimuli such as vibrations, and they actively swim about the container. When inactive they float at the water surface because of their buoyancy, a property that facilitates emergence of the adult. The pupal stage usually lasts two or three days.

Pupal Morphology and Characteristics. At the base of the thorax of the pupa is a pair of breathing tubes or "trumpets" that pierce the water surface to allow breathing (see Figure 7). At the tip of the abdomen there is a pair of oars or paddles used for swimming. Aedes pupae can be distinguished from those of other genera by the short non-flared trumpets and the single hair at the tip of each swimming paddle. Pupae of Aedes aegypti are different from those of other Aedes species in that they have stout, well-developed setae on the underside of the corners of the second to sixth abdominal segments (see Figure 8).

The Adult

The adult stage of Ae. aegypti is the reproductive stage. In most flying insects, including other mosquito species, the adult stage is also the important stage for dispersion. However, in the case of Ae. aegypti, there is probably more passive dispersion resulting from the transport of eggs and larvae in containers than active dispersion by adult flight.

Adult Morphology and Characteristics. Adult Aedes and other Culicinae may be distinguished from Anopheles by their shorter palps and their resting position which is more horizontal or parallel to the resting surface (See Figure 9). Aedes can be differentiated from most of the other Culicinae by their pointed abdomen and the absence of spiracular bristles. Ae. aegypti is a dark mosquito with white bands at the bases of the tarsal leg segments and a distinctive "lyre"-shaped design on the mesonotum (see Figure 10). With age the lyre marking may disappear, but the distinctive white scales on the clypeus and palpi usually remain.

The male, as in other culicine species, is distinguished from the female by its feathered antennae and by its longer, more developed palps.

Emergence. After emergence from the pupal case, the adult rests on the container wall for a few hours to allow the exoskeleton and wings to harden and, in the case of the males, to rotate the male terminalia 180°.

Mating. Within 24 hours after emergence, both sexes can mate, and females can take a blood meal. These two activities often occur simultaneously because the males are attracted to the same vertebrate host as the females, which facilitates mating. Mating usually takes place during flight, although sometimes it occurs on a vertical or, occasionally, a horizontal surface. The male clasps the tip of the female abdomen with his terminalia and inserts his aedeagus into the genital chamber. The female bursa copulatrix becomes filled with sperm that pass within one or two minutes to the spermathecae where they are stored prior to fertilization of the eggs. One insemination is sufficient to fertilize all the eggs that a female will develop during her lifetime.

Flight Range. Usually the female Ae. aegypti does not fly more than 50 meters in the course of a lifetime. The female will often remain in the same house where it emerged, provided that adequate hosts, resting places, and oviposition sites are available. However, if suitable containers are not present, a gravid female can fly up to three kilometers in search of a place to lay eggs. Males disperse less than females.

Resting behavior. When mosquitoes are not mating, searching for a host, or migrating, they seek a dark, quiet place to rest. Most commonly they rest indoors in bedrooms, bathrooms, and kitchens and only occasionally outdoors in garden vegetation. The preferred resting surfaces are walls, furniture, and hanging articles such as clothing, towels, curtains, and mosquito netting. Most resting occurs on vertical surfaces, although sometimes mosquitoes rest on ceilings and the underside of furniture, such as beds.

Longevity. Ae. aegypti adults can stay alive for months in the laboratory but they usually live only a few weeks in nature. Many adults die at the time of emergence or soon after, but daily survival is fairly constant thereafter. With a typical daily mortality of 10%, half of the mosquitoes would die during the first week and 95% during the first month. In spite of the great reduction in numbers, if the original emerging population is large, the resulting older population will be sufficiently large to transmit disease and support an epidemic.

HABITAT OF IMMATURE FORMS

Aedes aegypti is a container breeding mosquito. This is not to say that the larvae would not thrive in ground pools if given the chance. However, gravid females prefer to deposit eggs on the hard walls of containers immediately above the water level. These receptacles are of all imaginable kinds (see Figure 11) and may be classified as either artificial containers or natural containers.

Artificial Containers

Water Storage Containers. There are several kinds of water storage containers that serve as breeding sites for immature forms of Ae. aegypti.

- o Tanks are large, immovable, cuboidal or cylindrical containers, usually made of concrete, which may hold up to several thousand liters of water. These containers may be:
  - -- Elevated tanks, which are generally used for long-term storage of water that comes to the house by gravity flow, and which are usually covered, although mosquitoes frequently have access to them via poorly sealed covers on overflow pipes; and
  - -- Ground-level tanks, which are sometimes large depositories for water (cisterns) or sometimes smaller wash tanks (albercas) used daily for cleaning clothes and dishes.

- o Drums are usually 55-gallon (i.e., approximately 200-liter) metal containers, painted to prevent oxidation, and generally found outdoors or on covered patios. They frequently have lids, which are usually ill-fitting or left ajar, and are not mosquito-proof. In the Caribbean, drums are the containers most frequently found positive for Ae. aegypti.

- o Water jars are earthenware (clay, ceramic) containers of 20 to 200 liters in capacity, frequently found inside houses. Seepage of water through the porous container walls provides evaporative cooling of the contents.

Discarded Containers. The types of discarded, man-made containers that become breeding sites for the Ae. aegypti are even more numerous and include those described below.

- o Automobile tires are probably the single most important breeding source of Ae. aegypti, for several reasons:

  - -- They are one of the most common containers found in the urban environment.
  - -- They are usually the preferred habitat of Ae. aegypti.
  - -- They are almost never truly "discarded" in developing countries even though they are found lying about gardens and vacant lots. They are transferred from new cars to older cars then to mule carts, and they may eventually be used for the manufacture of shoe soles and flower containers or for fuel in sugar mills. Therefore, there may be objection by the populace to the removal of tires during cleanup campaigns.
  - -- Because of their commercial value, there is considerable movement of used automobile tires, both nationally and internationally. They are probably the most common means of dispersal of Ae. aegypti larvae and eggs.

  The percentage of positive receptacles (Container Index) is usually higher for tires than for any other container because they are black, with a dark, cool interior which is the preferred kind of oviposition site for Ae. aegypti; their configuration limits the amount of evaporation and makes it difficult to empty out the water; and they are difficult to destroy.

- o Tin cans are ubiquitous in gardens and garbage heaps in the developing world. Although their Container Index is low, their large numbers make them an important source of Ae. aegypti.

- o Bottles are also very common, but their positivity is very low. Glass jars, with wider mouths, are more frequently infested.

- o Vases, holding cut or live plants in water, are sometimes an important source inside houses or in cemeteries. The increasing practice of using artificial flowers has reduced this problem somewhat.

- o Roof gutters, when badly constructed or when not kept clean, frequently accumulate water and provide larval habitats. They are often missed by the Aedes inspectors. In Surinam, roof gutters are the most important source of Aedes aegypti.

- o Animal water dishes are sometimes important. In Puerto Rico they are the most common breeding source.

- o Water closets, especially unused toilets or sometimes toilets in use, can support larval breeding. When the toilet is flushed, some of the water always stays in the water closet, and most of the larvae, which have dived to the bottom because of the disturbance, also remain. This potential source is often not inspected by field crews.

Natural Containers

There are fewer types of natural receptacles used as Ae. aegypti breeding sites. Among the natural containers are the following:

- o Tree holes in gardens near houses, which are easily missed because they are often high above the ground and hard to detect, are frequently found with Ae. aegypti larvae.
- o Leaf axils are seldom found positive.

- o Rock holes are favored breeding places.

Ground pools with earth walls are seldom found with Ae. aegypti larvae; but wells, abandoned latrines, and other deep, dark pits in the ground that are lined with bricks or rock are attractive to ovipositing females.

Other Containers

Among the multitude of other containers which have been found harboring Ae. aegypti larvae are buckets, bowls, cups, milk cans, drum lids, cemetery urns, conduit pipes, boots, shoes, bamboo, coconut husks, fallen leaves, snail shells, broken light bulbs, boats, plastic tarps, and even bowls of Holy water in churches.

Relative Importance of Container Types

The importance of a container type is measured by the mosquitoes preference for it (Container Index) and the relative frequency of the container type in the community. Thus tires are important breeding sources because they are preferred by mosquitoes and very common. Bottles are not as important because, although very common, they are rarely positive.

Another important consideration is adult production per container type. For example, there may be many positive tin cans in the community, but daily production of adults per can may be very low. On the other hand, only a few heavily infested water storage tanks may contribute more to adult production than do tins. A simple, indirect estimate of daily adult production can be obtained by counting the number of pupae per container and dividing by two because pupal developmental takes approximately two days.

Ae. aegypti population densities usually fluctuate with rainfall, especially when a large percentage of the breeding sites are discarded containers that are found outdoors and exposed to the rain. Conversely, in areas with poor water supply where a great number of large, water-filled containers are kept year round, the Ae. aegypti population stays more constant.

SURVEILLANCE

In areas infested by Ae. aegypti it is necessary to determine the distribution, density, and effects of control measures. In uninfested areas a program of vigilance must be established to detect the influx of mosquitoes. Methods for sampling mosquito populations include larval, adult, or ovitrap surveys.

Larval Surveys

For larval surveys, the inspector needs a carrying case, vials, vial labels, dipper, medicine dropper (pipette), mirror, flashlight, forms, tea strainer, pencils, and chalk (to mark inspected houses and containers) (see Figure 12).

The field worker should explain the purpose of his visit to each household and then examine every water container inside the house, on the patio, and in the garden. A flashlight is used indoors to see into the containers. Outdoors a hand mirror is frequently used to reflect the sunlight into the containers. The inspector should note all those containers with water and those with Ae. aegypti larvae or pupae. (In some programs, the presence of larvae and pupae of other species also is noted in a separate column of the report form.) If detailed information on the relative importance of container types is required, the form should have separate columns for each type (e.g., drums, tanks, and tires). For quick surveys where only the House Index (see below) is required, the search continues only until the first breeding site is found.

If identification of the species from each positive container is required, one fourth-stage larva or, in its absence, one third-stage larva or one pupa, is placed in a small vial (e.g., in procaine vials such as those used in dental offices) in 70% ethanol. Next a label, written in pencil and containing the date, province, municipality, locality, house number, kind of receptacle, and name of worker, is inserted into the vial. Pasting labels to the outside of the vial or attaching them to the outside with a rubber band is not recommended because these labels tend to get lost.

At the end of each day the field worker must sum up the results of his findings at the bottom of the form and deliver the form and vials to the section head. The larvae are inspected in the laboratory under a stereoscopic microscope (at least 20x magnification) and when necessary corrections are made to the field form to rectify any errors in identification of species. On a second form the totals of all workers are added to give the overall total for the day. When the survey of the locality is finished, the daily totals are added to give the total for the locality.

Standard Larval Indices. The commonly used larval indices are defined below:

- o House Index or percentage of houses infested =

$$\frac{\text{number of infested houses}}{\text{number of inspected houses}} \times 100$$

- o Container Index or percentage of containers infested =

$$\frac{\text{number of infested containers}}{\text{number of inspected containers with water}} \times 100$$

- o Breteau Index or number of infested containers per 100 houses =

$$\frac{\text{number of infested containers}}{\text{number of inspected houses}} \times 100$$

In campaigns against Ae. aegypti, the larval House Index is the most frequently used and best understood index. The Container Index indicates the relative preference for larval breeding for each kind of receptacle. The Breteau Index combines the other two indices and gives a better measure of total larval production per house. The Breteau Index by container type measures the relative importance of each type in larval positivity.

Usually the House Index and Breteau Index are highly correlated, and one can be predicted from the other. If the Breteau Index is much higher than expected when compared to the House Index, intensive focalized breeding in only a small section of the locality is indicated. In some cases, the Breteau and House Indices are nearly the same as found in very crowded areas without gardens where almost every house has only one water container (e.g., a drum or water jar). Otherwise, similarity between the Breteau and the House Indices may indicate that the inspectors are examining the containers in each house only until they find the first positive one.

Data from such surveys have been used to establish criteria for interpreting the probability of the transmission of yellow fever by Aedes aegypti. If the Breteau Index is less than 5, the House Index less than 4, and the Container Index less than 3, urban transmission of yellow fever is considered unlikely; but where these figures are greater than 50, 35, and 20 respectively, there is a high risk of yellow fever transmission. Comparable criteria have not yet been established for dengue, but a similar interpretation of the indices may also be valid. In both diseases there is a relationship among the indices of Aedes aegypti, transmission of the virus, and the level of immunity in a population.

Other Indices. Other indices that may be used as part of the larval survey include the following:

- o Pupal Density Index, which gives the number per positive container and which is a useful method to estimate adult production; and
- o Larval Density Index, which shows the number of larvae, by stage, for each positive container, and which is usually used only for special studies.

Adult Surveys

Adult surveys show the various species present and their relative abundance. These data and information on reproductive habits can be used to conduct an effective search for larval breeding sites. Additional information obtained from adult surveys can be used to:

- o Determine the need for a control program including when and where control measures should be applied and by what method;
- o Determine if a disease potential may exist; and
- o Evaluate previously applied control measures.

The necessary equipment for adult collection includes a flashlight, an aspirator tube, a small net, cardboard containers with mesh tops, and forms attached to a clip board.

Human Bait Collections. In human bait collections, the field worker can collect mosquitoes either from his own body or from that of a second person, if collectors are working in pairs. Trials should be conducted before this type of survey is initiated, and the methods to be used should be standardized because individuals vary in their degree of attractiveness to mosquitoes. A twenty-minute collection period for each house is recommended between the hours of 0900 to 1100, the period of greatest mosquito activity. The inspector must sit quietly in a dimly lit room (usually a bedroom), with shoes and stockings removed and legs exposed to the knee. An aspirator is used to capture all mosquitoes, both male and female, that land on the inspector. If the mosquitoes are allowed to bite before being captured, results are expressed as a "biting rate." If they are captured immediately on landing, results are called a "landing rate." In some programs a small net is used to capture the mosquitoes before they land. Results of this approach could be expressed as an "attraction rate."

Resting Collections. Resting collections are conducted by searching for adult mosquitoes in bedrooms and in other rooms in houses, garages, and outbuildings. Mosquitoes may also be collected from yards, cemeteries, tires, and junkyards. The adults are captured with small vials, hand sweep nets, or aspirators. A small net may be used to flush the mosquitoes from under furniture and then capture them in flight. However, if information on type of resting surface is required, only an aspirator should be used to capture the mosquitoes directly from the surface.

Mosquitoes usually rest in shaded places and dark corners on mosquito nets and under tables, chairs, or beds. Aedes aegypti can be found resting throughout the day so there is no restriction on time of day in which the adults may be collected. The collector should spend the same amount of time in each house, e.g., about 15 to 60 minutes. This process allows collection density to be expressed per house and per man-hour. The captured mosquitoes must be identified by species and sex. Collection stations can be selected at random, or they can be located at predefined sites. It should be remembered that collecting depletes the population, thus, the same house should not be sampled every day.

Adult indices could be expressed in a manner similar to the larval indices:

- Adult House Index = percentage of houses infested,
- Adult Room Index = percentage of rooms infested, and
- Adult Breteau Index = number of infested rooms per 100 houses.

The Adult House Index is usually similar to the Larval House Index, although this depends on the amount of time spent searching for the adults.

If the captured adult mosquitoes are counted, then the number of adults per 100 houses, the number per 100 rooms, and the number collected per hour can also be computed.

Ovitrap Surveys. Ovitraps provide an indirect method of assessing the presence and size of adult Aedes aegypti populations. This method is particularly good for detecting the presence of Aedes aegypti where the density is so low that larvae are difficult to find. Materials needed for this investigation are black jars, fiberboard paddles, clips, paper towels, tote bags, and forms.

The standard black jar used for an oviposition trap is a 500 milliliter, wide-mouthed, glass jar painted black on the outside. As an alternative, 12 ounce aluminum drinking cans, available locally, can be painted black on the inside. A dark brown fiberboard paddle (2 x 12 cm) is inserted vertically into the jar, rough side up, and fastened to the lip of the jar with a butterfly clip (see Figure 13). Paddles may also be made from filter paper or cloth wrapped around a tongue depressor; from stiff, felt-like filter paper; or from any other absorbent material. Dark colors, especially dark red, are most attractive. Two or three milliliters of clean water are added to the jar, which is placed in a low, quiet, shaded spot in the house or garden where it is protected from rain, children, and animals. Gravid females are attracted to this dark container and oviposit on the rough side of the fiberboard paddle just above the water line. As many other containers on the premises are just as attractive for oviposition as the ovitrap, the trap should not be placed too close to other containers. The paddle is removed each week, wrapped in paper towelling or toilet tissue to avoid contamination from other paddles, and sent to the laboratory. The jar is washed to remove any eggs that may have been deposited inside, and fresh water and a new paddle are added.

To obtain the full benefit of ovitrap surveys, the area should be fairly extensively covered. Local maps should be consulted to determine where to place the traps, and visits should be made to the area for selecting specific sites. A system of grids should be used in the survey. It is recommended that sites be from 100 to 200 meters apart and that the traps be placed within 30 meters of the grid line.

Points to be considered with regard to ovitrap placement are as follows:

- Traps should be placed at or near ground level because females usually fly near the ground.
- Traps should be visible to the mosquitoes that fly over them.
- Traps should not be placed where they will fill with rain water.
- All traps should be placed where children, cats, dogs, and other small animals do not have access to them.
- Ovitraps should be located in partial or total shade and in adult resting places such as shrubbery or junk. It is preferable to place them in the rear rather than the front of a house.
- Ovitraps should not be located where tires are piled up because females tend to prefer tires to ovitraps.

Ovitrap jars should be assigned numbers or otherwise marked with an identifying code. Paddles should be dated and also marked according to the code. If collectors miss a site, noting the date will help the laboratory technician in recording information. Paddles ought to be placed in a plastic bag or wrapped in toilet tissue or other soft paper for transport to the laboratory. Some workers have designed carrying cases similar to microscope slide boxes for transporting paddles.

The occurrence, distribution, and changes in population density of Aedes aegypti in an area are revealed by the presence of eggs on ovitrap paddles. In the laboratory the paddles are unwrapped and allowed to dry in a place protected from ants. The eggs can be seen easily under a stereoscopic microscope at 10 to 20x magnification. The number of positive paddles is recorded and, optionally, the number of eggs per paddle. All mosquito eggs found on the paddle might not, however, be Aedes aegypti.

Periodically at each survey site, paddles should be submerged in water and larvae bred to allow for a definitive identification of the species.

Ovitrap indices can be expressed as percentage of positive houses and number of eggs per paddle. A sample from 50 houses is sufficient. At first, traps should be placed both indoors and outdoors to determine where most oviposition occurs, as this varies among regions.

Biological Assays

Bioassays evaluate the effect on caged mosquitoes in the field of treatment with insecticides. These assays are not a substitute for, but rather a supplement to, evaluation of the natural population level as determined by larval, adult, or ovitrap surveys. Bioassay tests can be done with area-wide sprays, perifocal sprays, and larvicides.

Bioassays with Space Sprays. Approximately 10 to 25 adult females, from an Ae. aegypti colony, that are less than 3 days old and that have been fed within the previous 24 hours are put in small, screen or net cages. Before the treatment the cages are hung in the house or garden. Glass microscope slides, treated with silicone or magnesium oxide, may be placed next to the cage to obtain additional information on droplet size and density. The cages should be placed at 30 to 100 meter intervals across the path of an aerial spray or at right angles to the path of ground equipment. Any dead mosquitoes and any affected by the insecticide are noted initially and two hours after the treatment. The live mosquitoes are then transferred carefully by means of an aspirator to clean, cardboard containers with net tops and transported to the laboratory where they are given food and water and left for 24 hours in an uncontaminated, dimly lit place with high humidity. Then, the final count of dead and live mosquitoes is recorded. Mortality rates should be plotted by cage site.

In general, the closer the mosquitoes are to the source of the spray, the higher the mortality rate. The results should give an indirect indication of mortality rates of the natural populations, breadth of the spray zone, unsatisfactory coverage and other flaws in application of the insecticide.

If information is required on penetration of insecticide droplets under beds, behind furniture, and into closets, cages can be located at these sites in each house. For reliable results the observations must be replicated using at least five cages for each type of site (e.g., five houses would require one cage per house). If only general conclusions are required, such as comparison of the effectiveness of one treatment to others, 1 or 2 cages may be located in each of 10 or more houses in standard sites, (e.g., one in the sitting room and one in the back yard). For control purposes, cages should also be placed in at least two untreated houses.

Bioassays with perifocal residual sprays. To evaluate perifocal spray deposits, standard plastic bioassay cones of the type used for measuring residual effectiveness of intradomiciliary wall spraying in malaria and Chagas' programs can be used. Document WHO/VBC/81.5 gives a complete description of this technique. As with the evaluation of space sprays, if information is desired on the relative effectiveness of the water dispersible powder on various surfaces, the test must be replicated on at least five surfaces of the same type.

Bioassays with Larvicides. Bioassays with larvicides can be conducted by inserting floating, fine mesh cages, each containing 25 larvae from a colony into the containers treated with larvicide or, in laboratory bioassays, with treated water from the field. Observation on mortality takes place after 24 hours exposure. Third or fourth stage larvae are generally used because there is high mortality among early stage larvae in untreated control cages.

Evaluation of Campaigns against Aedes aegypti

Evaluation of the status and progress of the campaigns against Ae. aegypti depends on the phase of the campaign. Traditionally, the same terminology as that used for malaria campaigns has been used for Ae. aegypti eradication campaigns: Preparatory Phase, Attack Phase, Consolidation Phase, Maintenace Phase and Vigilance Phase. For the present control programs, the Attack Phase is subdivided into Early Attack and Late Attack, to distinguish between different activities to be carried out depending on different levels of risk of virus transmission.

Preparatory Phase. This phase involves the organization, management, and planning needed prior to beginning the control phase. The first step in vector evaluation is to determine distribution and density.

- Determining Distribution. If the sole objective is to determine the presence or absence of Ae. aegypti in various regions of the country, it is possible to select urban centers within these regions and to inspect the most likely breeding sites, such as tire stores, cemeteries, and garbage dumps. On a country map the sampled localities are simply marked as positive or negative.

- Determining Density. Rates of larval infestation, adult infestation, or oviposition may be measured. The most commonly used method is the larval survey because it requires less time, equipment, and expertise and usually yields higher infestation indices. A 1% sample of the houses, or at least 100 houses, spread throughout the locality is usually sufficient; however, larger samples will yield more accurate results. Traditionally, every third house was inspected in eradiction campaigns.

  Adult collections are especially important during epidemic control and during the last stages of eradication. Human bait collections provide an estimate of the degree of human-mosquito contact while resting collections yield a sample of all adults, including males and gravid females, instead of only hungry females. In these collections, a sample of 30 to 50 houses suffices.

  The insecticides chosen for the campaign must be tested to determine the susceptibility of larval and adult mosquitoes (see WHO/VBC/81.805, 806, and 807). These tests, conducted in the major urban centers before purchasing the insecticides, will ensure that the mosquito population is not resistant to these compounds.

Attack Phase

Early Attack Phase. This phase covers any attempt to reduce the mosquito population. Decrease in the population may be negligible or may reach 1% of infested houses, at which time the campaign enters the Late Attack Phase.

For routine evaluation of the Early Attack Phase of the campaign, larval surveys should be done in at least 1% of the houses or a minimum of 100 houses immediately before each treatment at 8 to 12 week cycles. For eradication campaigns, "verification" (larval search) is done in every house before each treatment. In addition, larval surveys may be made from 1 to 10 days after some treatments to determine the immediate effect of the treatment. Ovitraps also can reflect immediate changes in the adult female population. If sufficient numbers of ovitraps are used, they may reveal population recovery and indicate where there were flaws or where coverage was inadequate.

Special evaluations may be required for new methods of treatment and of insecticides, dosages, formulas, equipment, or intervals between treatments. These special evaluations require at least two pre-treatment surveys, preferably with two or more sampling methods (e.g., larval and adult surveys). The time interval between surveys depends on the expected duration of the effect of the treatment. It is advisable to sample the population several times during the recovery period, not only immediately before and after each treatment as is done in routine evaluations. The effect of a treatment with larvicides can be assessed by conducting larval and adult surveys and larval bioassays in 100 houses before treatment, a week after treatment, and every 2 to 4 weeks thereafter. The expected effect of a space spray with an adulticide will be much shorter because new adults will emerge from pupae soon after the treatment. Adult and larval indices should be measured the day after treatment and at least weekly thereafter. Adult and larval bioassays, to detect any direct effect of the adulticide on the larvae, may be done on the day of the treatment.

Late Attack Phase. When the House Index has reached 1% or less, a level at which virus transmission is considered unlikely, the Late Attack Phase begins. Larval surveys must continue and, when a positive house is found, a concentrated search is made for larvae and resting adults in 100% of the houses within 300 meters. Ovitraps are also used to assist in finding other possible sources of infestation. Susceptibility tests are repeated during these intensified treatment activities to detect any build up of resistance before it reaches a level high enough to prejudice the campaign's success.

The Consolidation Phase of eradication campaigns begins when all of the localities of the country or operational area had obtained at least one negative verification, and the Maintenance Phase starts when all of the localities are negative simultaneously in any one verification cycle.

Vigilance Phase. To enter the Vigilance Phase, an area must be negative for two years with inspections of 100% of the houses occurring at the end of the first and second quarters and at six-month intervals thereafter. If one infestation is found during the year, the area returns to the Consolidation Phase.

As part of this vigilance, greatest attention should be paid to the possible ports of entry of the mosquitoes, including airports, seaports, border crossings, and bus or train stations. Oviposition traps are particularly useful here because they can be left continuously in high risk areas, and a large number of traps can be serviced by a few field workers in a large area. Periodic larval inspections should also be made in the same area.

The importance of vigilance against reinfestation cannot be too heavily stressed. The cost of maintaining vigilance is much less than the cost of eradication if Ae. aegypti is reintroduced and becomes established. After many years without finding Ae. aegypti, the vigilance squads tend to become apathetic and may even forget how to identify Ae. aegypti. To maintain their enthusiasm they should attend annual refresher courses and be given other incentives. The material they send to the laboratory should be identified and the results returned immediately.

CONTROL

Vector control is discussed in detail elsewhere in other volumes in this series. The following section only addresses issues of particular importance for control of Ae. aegypti.

Control with Insecticides

In the early 1900's the first successes in the control of this species were realized by reducing sources, applying oil, and adopting legal measures to prevent people from keeping objects on their premises that serve as potential mosquito habitats. With the discovery of the insecticidal properties of DDT, Ae. aegypti campaigns became DDT spraying campaigns with this insecticide applied not only around containers but also inside them. The considerable progress made toward the eradication of Ae. aegypti in the western hemisphere during the fifties and sixties was due mostly to this control strategy. As Ae. aegypti became resistant to the hydrocarbon insecticides, the alternative was to use more expensive compounds of shorter residual effect and usually of greater human toxicity.

Focal Treatments. The cornerstone of most present day campaigns is the larvicide temephos (Abate R). It may be applied to potable water in the form of sand granules coated with insecticide (1%) at a dosage of 1 ppm., which is very safe for use in potable water containers such as drums and tanks. Temephos usually provides two to three months of control if the water in the containers is not changed; for the most part, an eight-week treatment cycle is recommended. Other promising larvicides for control of

Ae. aegypti are methoprene (Altosid R, which acts like the juvenile hormone) and Bacillus thuringiensis H-14 (a larvicide bacteria). At present both products demonstrate very short residual effectiveness and are thus less effective in programs organized to complete cycles every 8 to 12 weeks.

Perifocal Treatments. When DDT was used for Ae. aegypti control, "perifocal" referred to application both around and inside the container. Now insecticides used inside the containers ("focal") are different from those used around them ("perifocal"). Usually wettable powder formulations of malathion or fenitrothion are applied at one gram per square meter on the surface of the container and within a radius of one meter around it.

Residual Treatments. Although most adult Ae. aegypti are found resting on clothes, pictures, bedspreads, curtains and other hanging objects, most of the mosquitos eventually rest on the walls for a sufficient time to be affected by residual deposits of insecticides. However, the residual treatment of all wall surfaces, as done in malaria and Chagas' programs, is slow and expensive and not necessary for the control of Ae. aegypti because the faster and more economical methods of aerosol and fog applications are available.

Space Spray Treatments. Space spray treatments in conjunction with larvicides will cause a more rapid decrease in population. Space spray treatments may be conducted with thermal foggers, mistblowers, and aerosol generators all of which may be portable hand-carried or backpack machines; land-based vehicle-mounted equipment; or aircraft-mounted equipment. Portable machines can be operated inside the houses, and they produce better penetration of the mosquito habitat. The vehicle-mounted machines give faster coverage but are sometimes deficient in penetration, especially if there is little space between houses and few openings (e.g., doors and windows) in the houses. With this equipment, coverage is frequently poor at the rear of dwellings and inside the rooms.

Application of aerial sprays by single- or multi-engine planes or helicopters is the only method rapid enough to contain an epidemic. However, there is often a penetration problem, and much of the insecticide is deposited on roof tops and vegetation. Yet application by plane or helicopter does reach interior gardens and the back rooms of houses that the vehicle-mounted equipment frequently misses.

Considering the activity cycles of the mosquitoes, the optimal time for aerial spraying would be early or mid-morning and mid- or late afternoon. Atmospheric conditions, which are best during the early morning and late afternoon hours for outdoor application, have little effect on indoor application with portable machines.

Biological Control

Some organisms are natural enemies of Aedes aegypti larvae; these include several fungi, bacteria, aquatic bugs (Notonectidae), dragonfly naiads, copepods, and mosquito larvae (Toxorhynchites). Natural enemies of adult Ae. aegypti are spiders, adult dragonflies, lizards, birds, and bats. Enemies of Ae. aegypti include ants, mites, and booklice (Psocidae). Although laboratory tests and small-scale field trials have been conducted with several of these predators and parasites, none has as yet been used operationally in large-scale campaigns. However, some success has been achieved with the top feeding guppy (Poecilia reticulata) in cisterns and drums in the Caribbean Region.

Source Reduction

Removal or destruction of discarded containers and covering of water storage containers result in elimination of breeding sources without insecticidal contamination. To be effective, efforts to reduce infestation sources must be carried out at least as often as control with insecticides because waste containers accumulate rapidly in most developing countries where garbage collection services may be infrequent and inadequate. Some containers present special problems in a source reduction campaign. For example, people often do not want to part with their used tires because of their commercial value, and they may move the tires indoors temporarily to avoid confiscation; however, during the next treatment cycle, the tires will again be found outside. Covers for tanks and drums often do not fit snugly enough to prevent entry and exit of mosquitoes, and they are almost never placed squarely on the containers. Although source reduction is extremely important, alone it is not sufficient to eliminate *Ae. aegypti*; it must be integrated with other control methods.

Cleanup Campaigns

Different sectors of the community can be involved in one- or two-day campaigns to eliminate breeding sources and other garbage. Usually, the enthusiasm needed to stage these campaigns can only be generated once a year at most in each community; this is insufficient to maintain low mosquito densities.

Sanitary Education

Public instruction via newspapers, radio, television, public meetings, and courses in the schools is very important in order to change a community's sanitary habits. This process must be continuous and intensive and should be viewed as an investment that may not pay significant dividends for many years.

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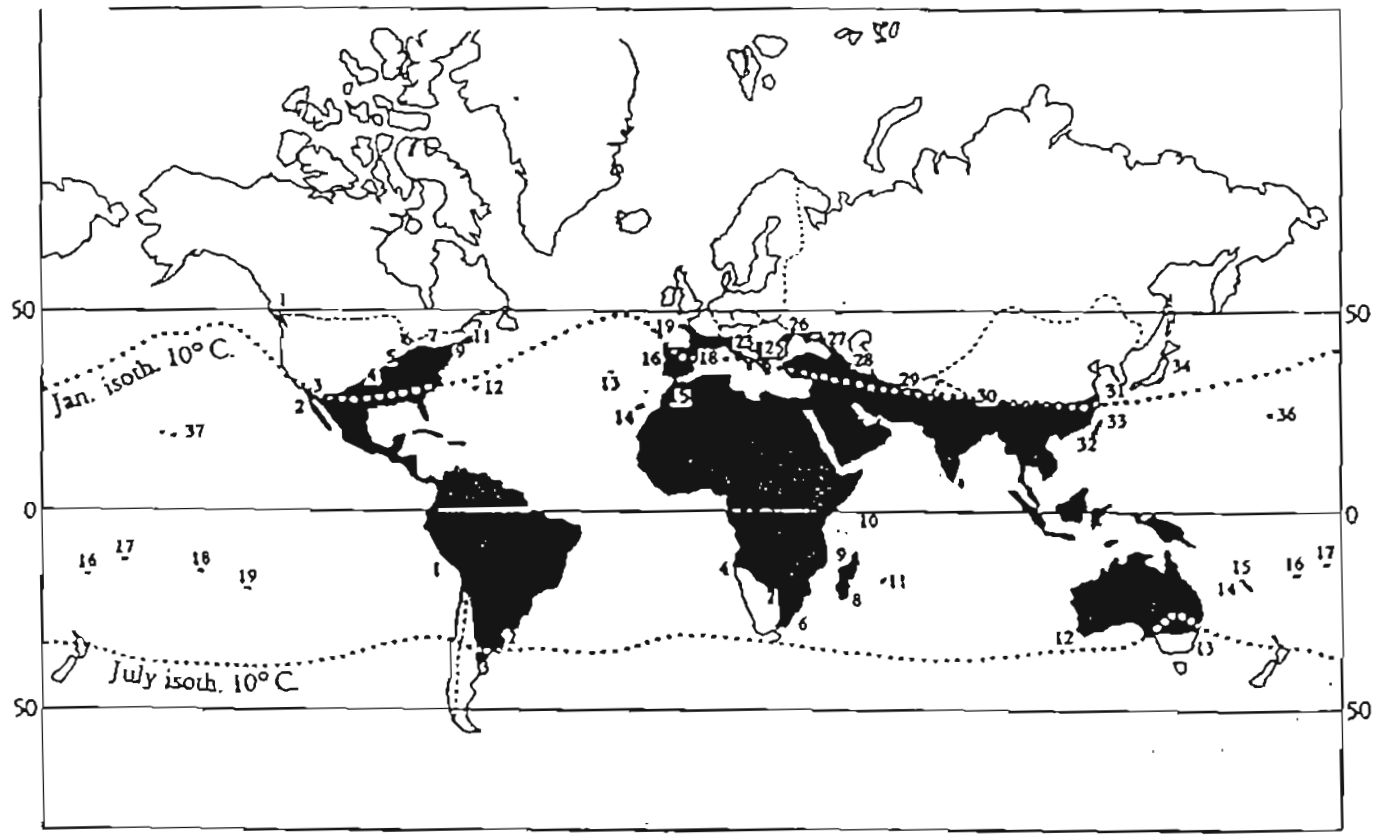
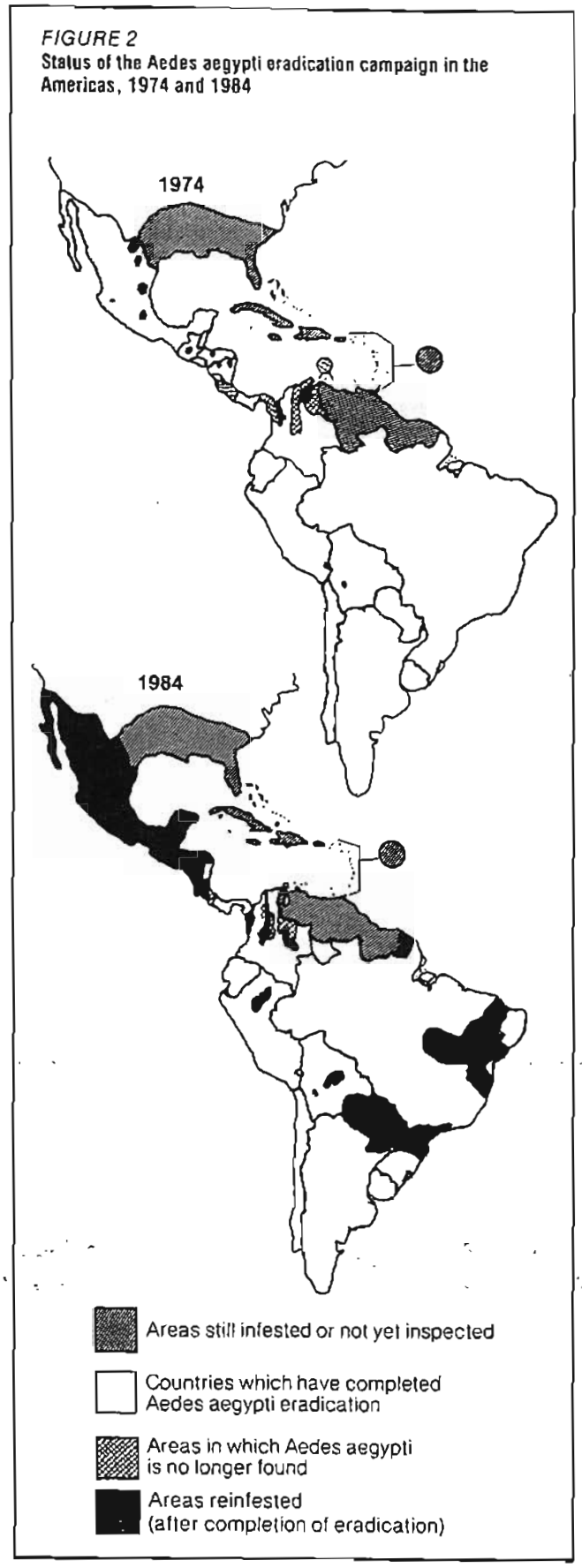
Figure 1. Map showing limits of distribution of areas originally infested by Aedes aegypti in the world



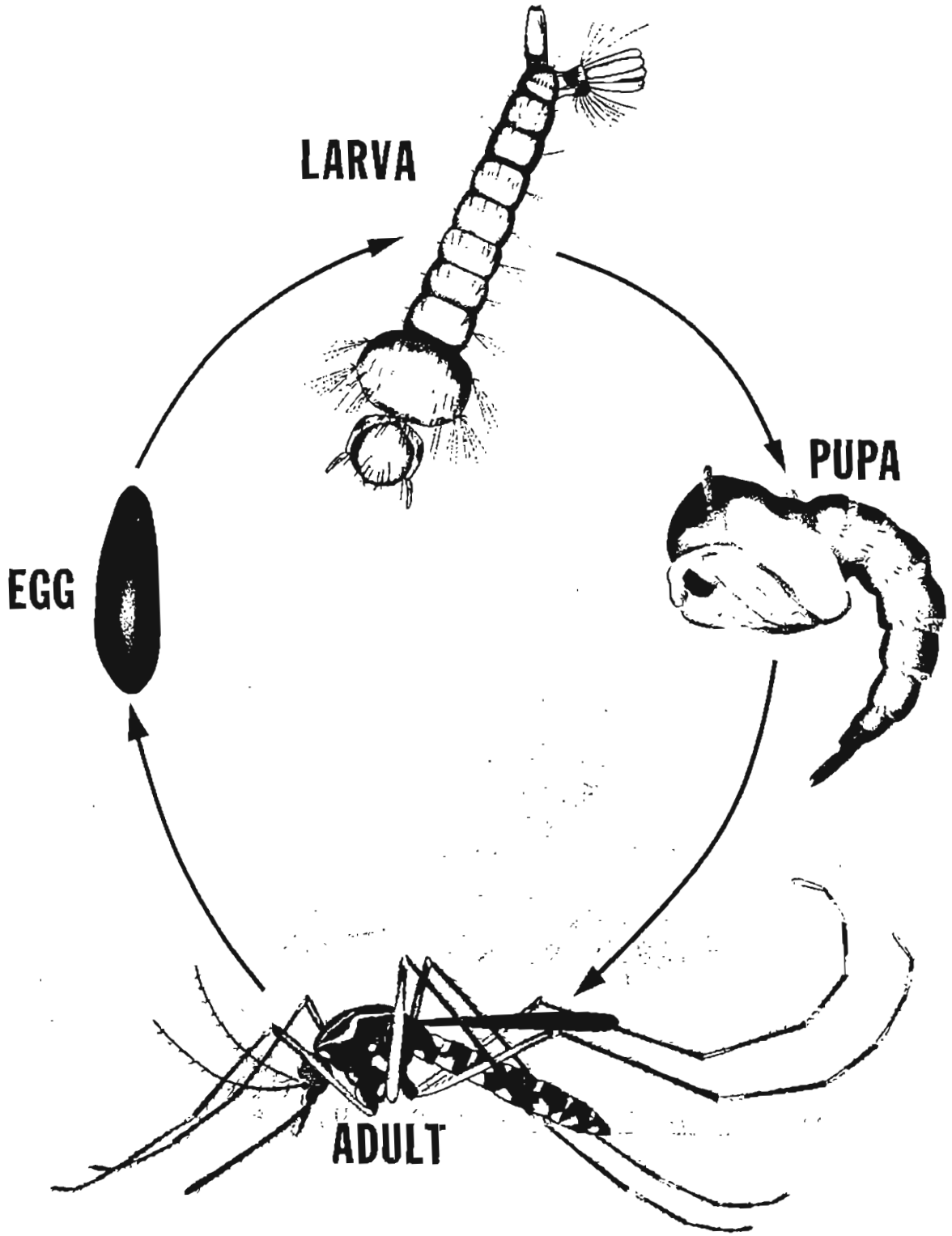
Source: Christophers, S. R. Aedes aegypti (L.) The Yellow Fever Mosquito. Cambridge University Press, London, p.36, 1960.

FIGURE 2
Status of the Aedes aegypti eradication campaign in the Americas, 1974 and 1984



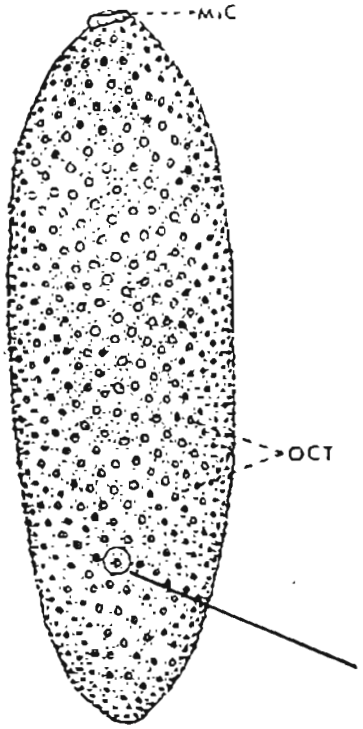
Source: Communicable Diseases Program, PAHO, Washington, D.C.

FIG. 3
LIFE CYCLE OF AEDES AEGYPTI



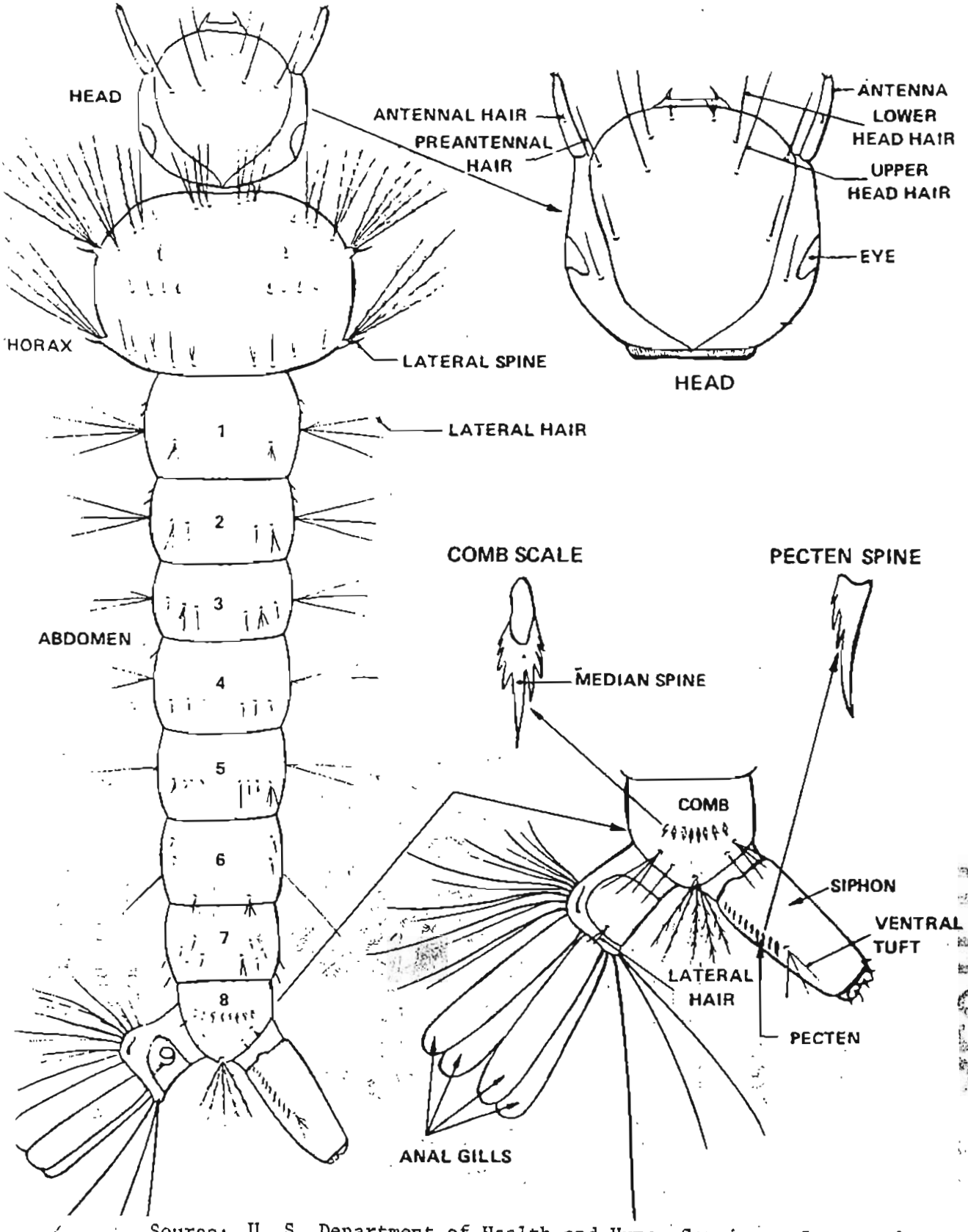
Source: Litting, K. S., and H. D. Pratt. Biology and Habitats of the Yellow Fever Mosquito Aedes aegypti. U.S. Department of Health & Human Services, Centers for Disease Control, Atlanta, Georgia

Figure 4. Egg of Aedes aegypti



Source: Mosquito Systematics 10 (2): 281, 1978.

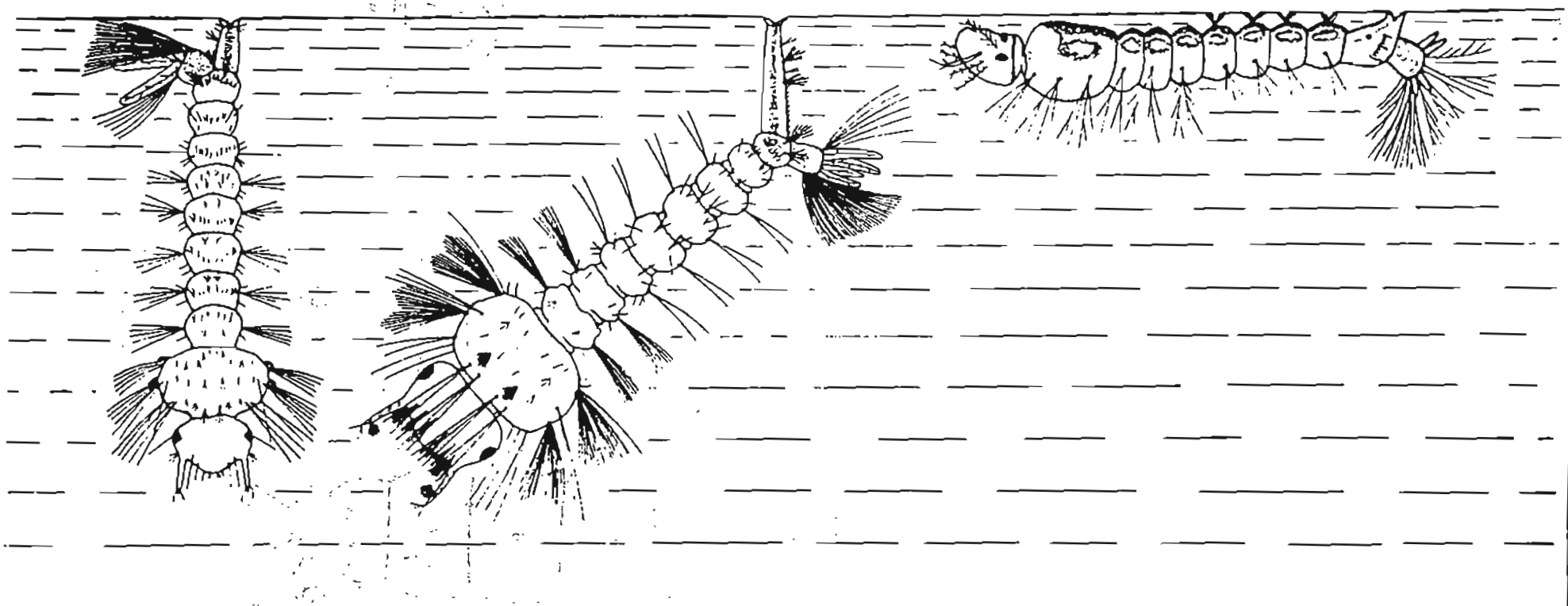
Figure 5 Diagram of *Aedes aegypti* larva



Source: U. S. Department of Health and Human Services, Centers for Disease Control, Atlanta, GA. Vector Topics No. 4. Biology and Control of Aedes aegypti. Illustration 1, p.45, 1980

AËDES AEGYPTI

FIGURE 6

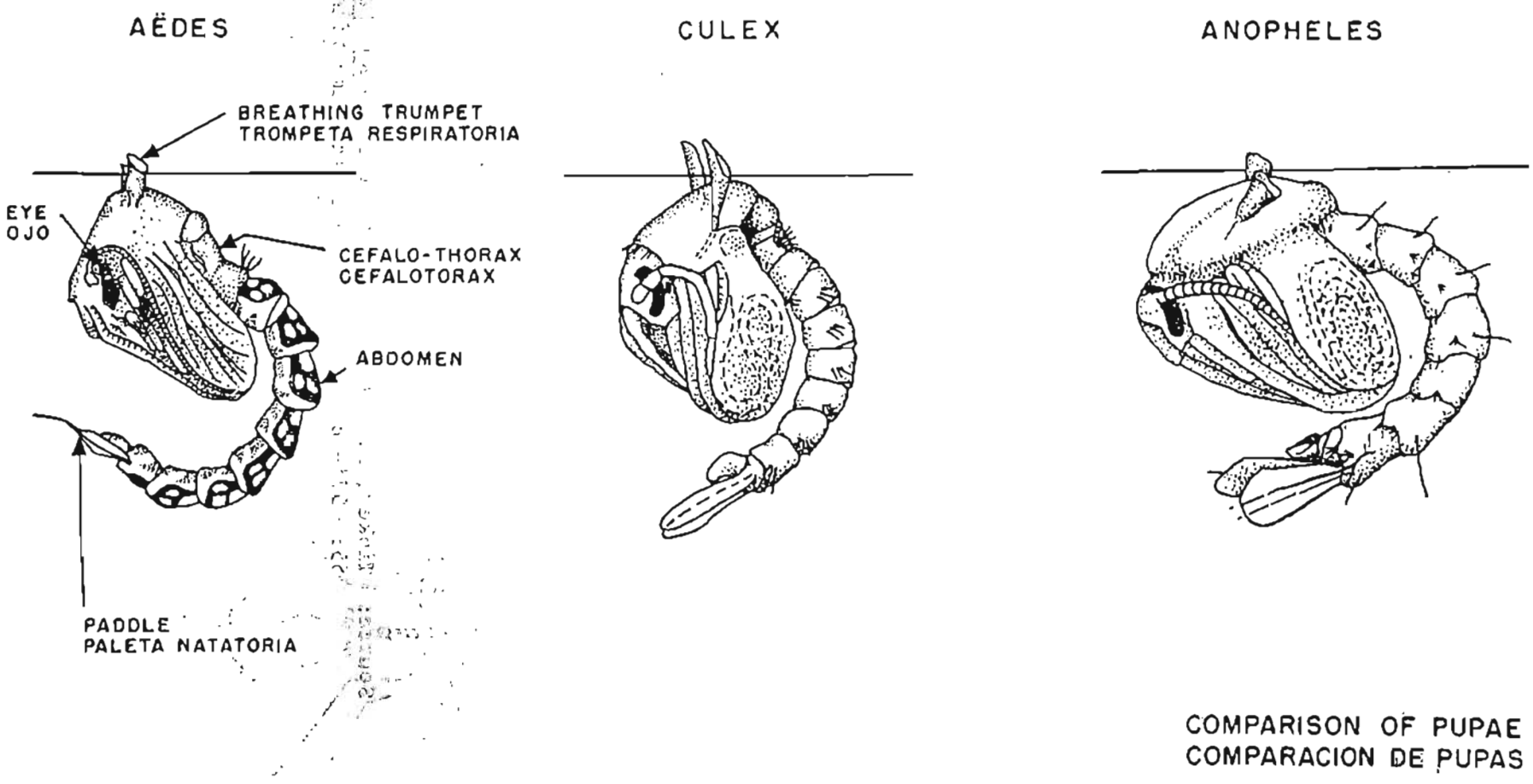


COMPARISON BETWEEN BREATHING POSITIONS OF LARVAE AT SURFACE OF WATER
COMPARACION ENTRE LAS POSICIONES DE LAS LARVAS RESPIRANDO EN LA SUPERFICIE DEL AGUA

Source: PAHO/WHO. Manual of Operations for an Aedes aegypti Eradication Service, Fig. 5, 1957.

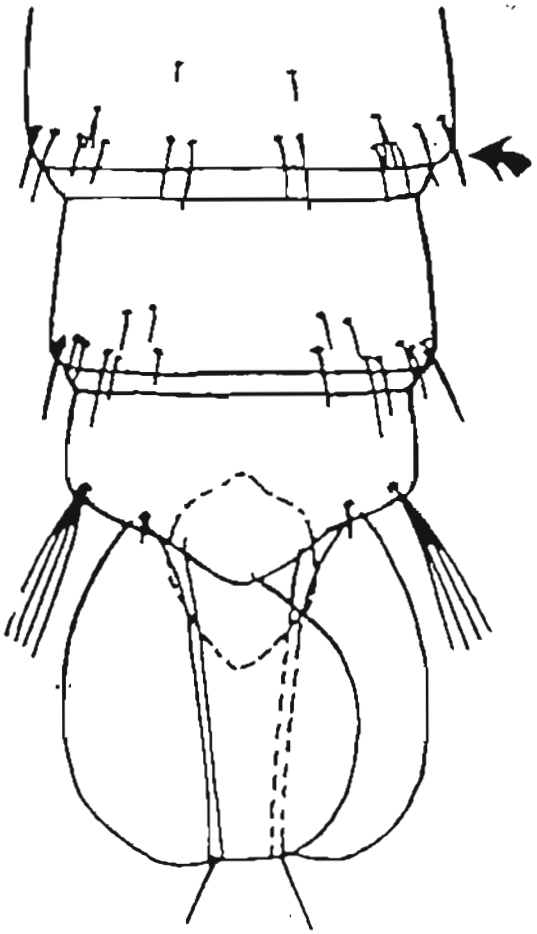
AËDES AEGYPTI

FIGURE 7



COMPARISON OF PUPAE
COMPARACION DE PUPAS

Figure 8 Pupa of Aedes aegypti



Source: Tinker, M.W. and C. J. Stojanovich - Am. Entomol. Soc. America 55: 571-582, 1962 (Original plate amplified).

FIGURE 9

CHARACTERISTICS OF ANOPHELINES AND CULICINES

Kent S. Littig and Chester J. Stojanovich

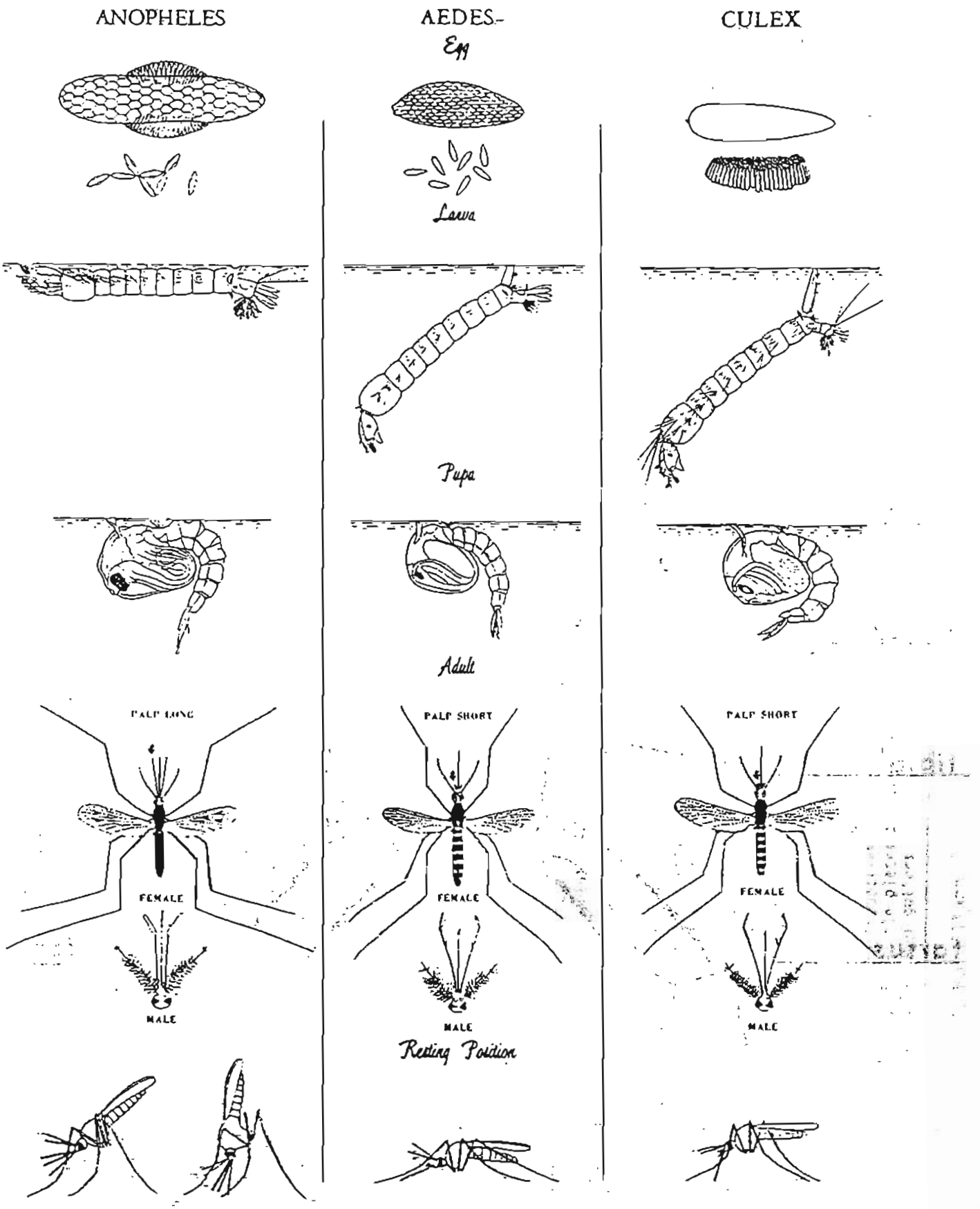
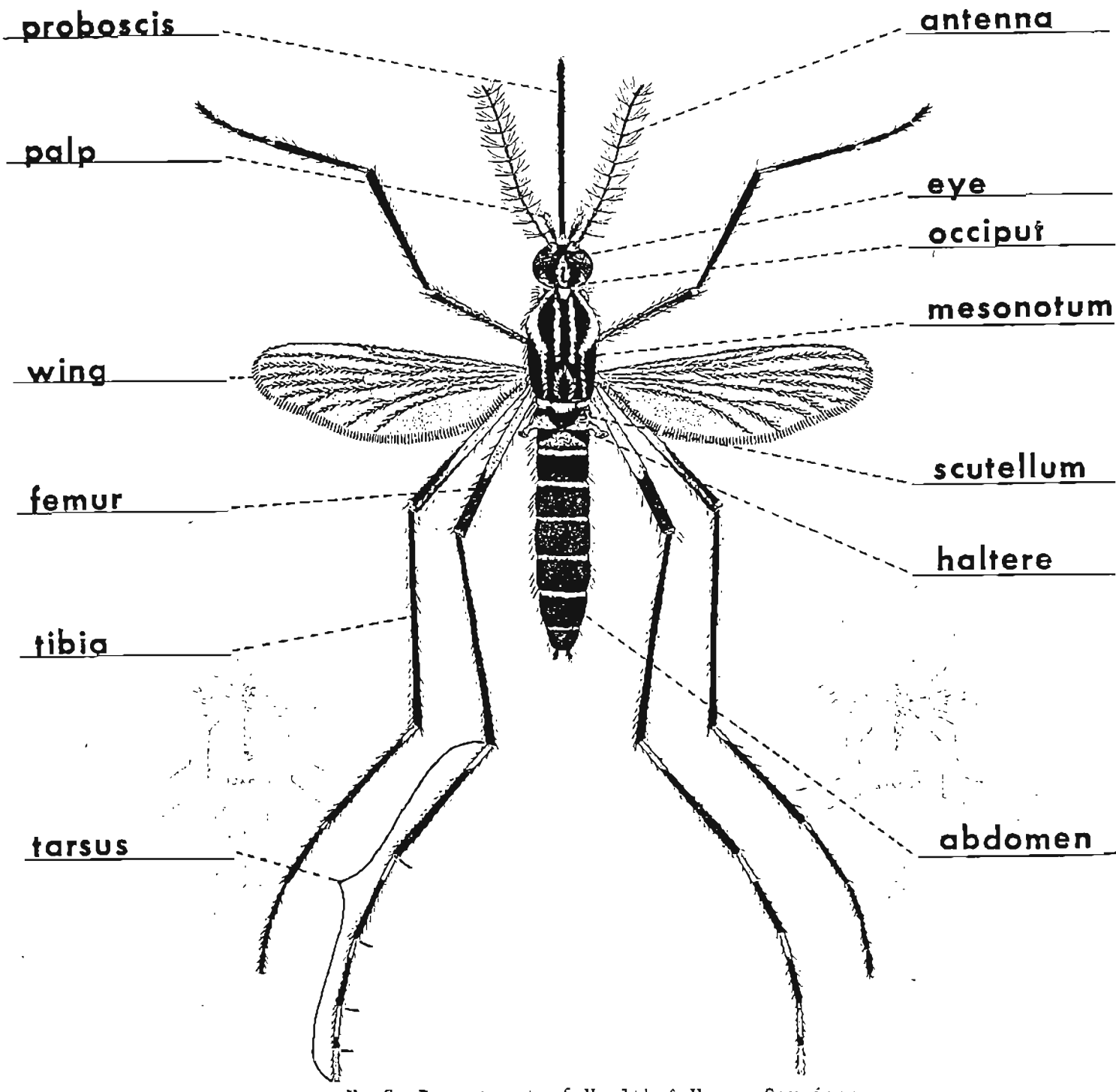
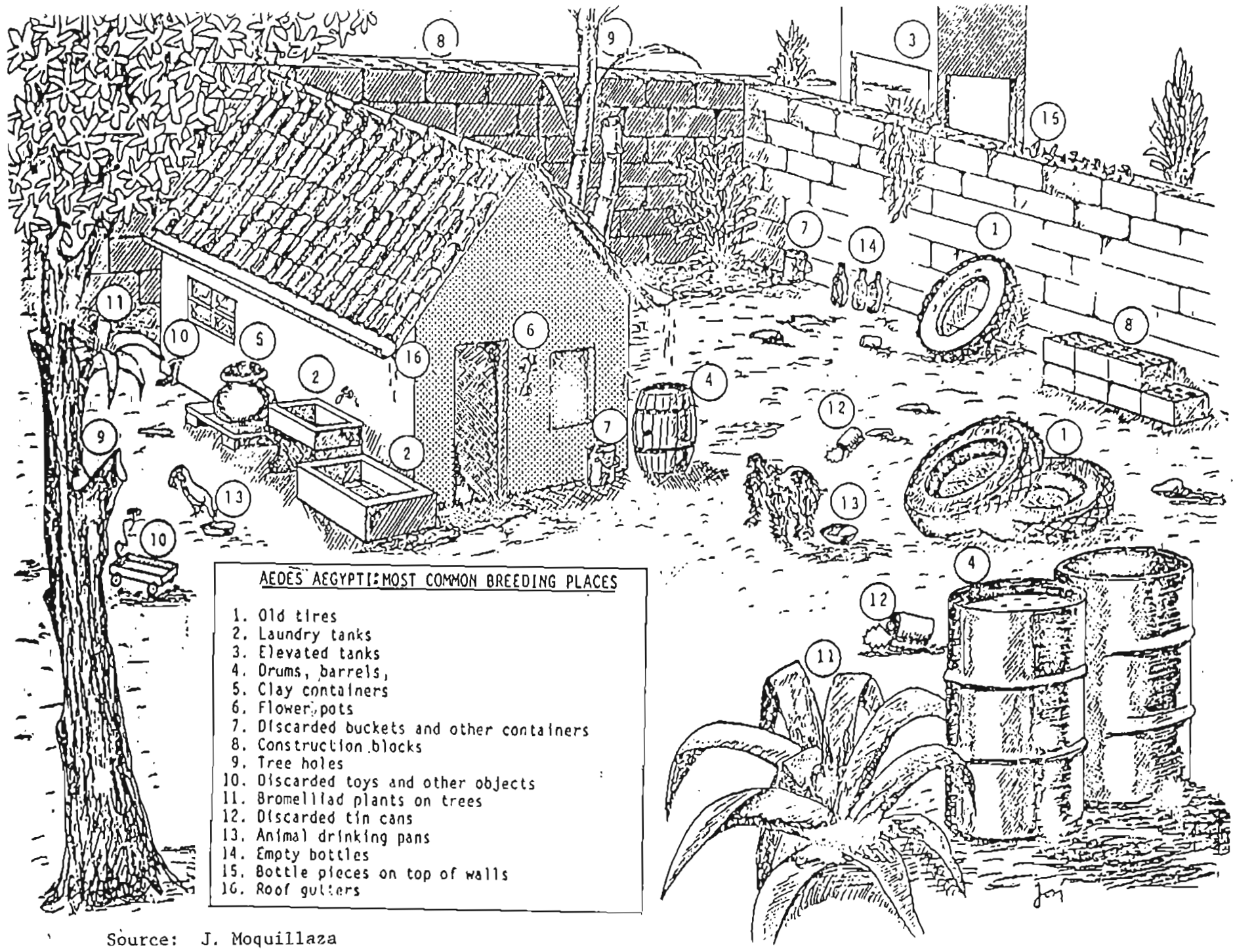


FIGURE 10

DIAGRAM of ADULT MOSQUITO

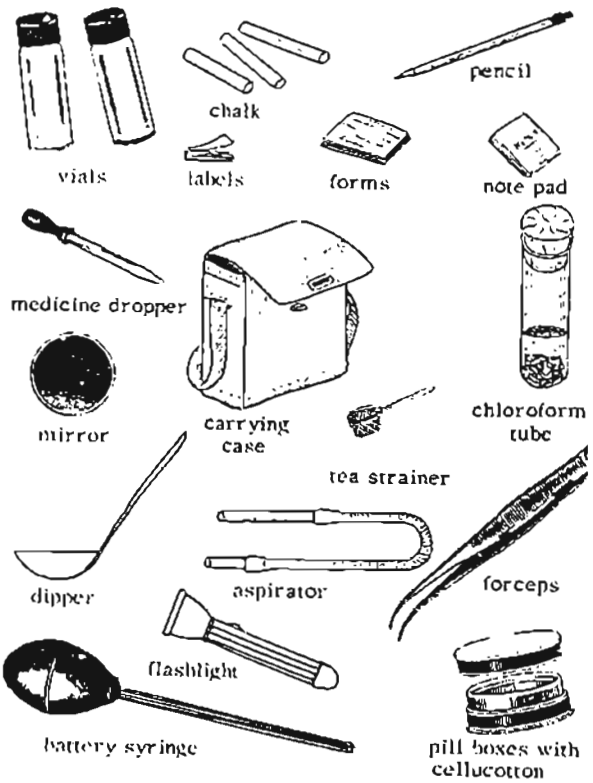


U. S. Department of Health & Human Services,
Centers for Disease Control, Atlanta, GA.,



Source: J. Moquillaza

Figure 12. Materials for larval surveys



Source: U. S. Department of Health & Human Services, Centers for Disease Control, Atlanta, GA. Aedes aegypti Handbook Series No. 2, Entomological Handbook, p.14, 1966.

Fig. 13 Oviposition Trap

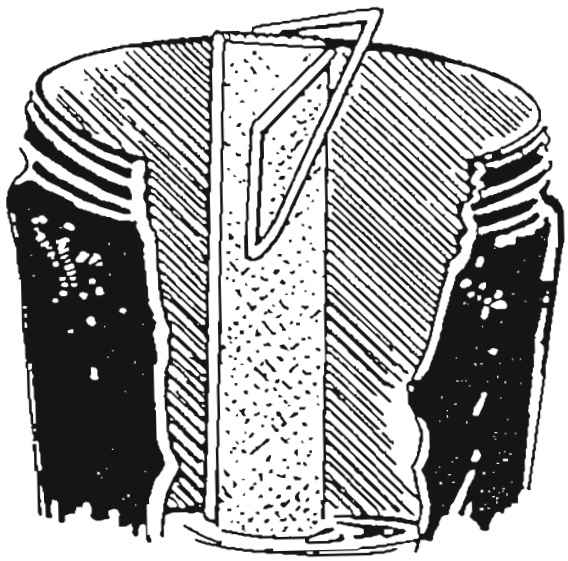


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Table 1. Status of Aedes aegypti eradication in the Americas

April 1986

Extension in km2

COUNTRY AND OTHER POLITICAL UNIT	TOTAL	INITIAL INFESTED AREA	PERCENTAGE OF THE TOTAL AREA	ACTUAL STATUS	ACTIVITY PROGRI
Antigua, Barbuda and Redonda	442	280	63.3	Infested	+
Argentina	2.779.741	1.000.000	36.0	Eradication completed	v
Aruba	190	174	91.6	Reinfested	+
Bahamas	11.405	11.405	100.0	Infested	+
Barbados	430	171	39.8	Infested	+
Belize	22.965	22.965	100.0	Reinfested	+
Bermuda	53	53	100.0	Negative	v
Bolivia	1.098.581	100.000	9.1	Reinfested	+
Bonaire	281	246	87.5	Reinfested	+
Brazil	8.511.965	5.358.822	63.0	Reinfested	+
Cayman Islands	259	259	100.0	Eradication completed	v
Chile	756.945	100.000	13.2	Eradication completed	v
Colombia	1.138.338	280.000	24.6	Infested	+
Costa Rica	50.700	20.000	39.4	Reinfested	+
Cuba	114.524	100.000	87.3	Infested (almost negative)	+
Curacao	472	448	94.9	Infested	+
Dominica	789	789	100.0	Infested	+
Dominican Republic	48.734	42.020	86.2	Infested	+
Ecuador	283.561	69,454	24.5	Reinfested	+
El Salvador	21.393	18.675	87.3	Infested	-
French Guiana	91.000	91.000	100.0	Reinfested	+
Grenada-Grenadines (Carriacou, Little Martinique, and Union	344	344	100.0	Infested	+
Guadeloupe (and part of St. Martin)	1,779	1.619	91.0	Infested	+
Guatemala	108.889	36,423	33.4	Reinfested	+
Guyana	214.969	4.662	2.2	Infested	+
Haiti	27.750	27.750	100.0	Infested	-
Honduras	112.088	69.929	62.4	Reinfested	+
Jamaica	11.424	11.424	100.0	Infested	+
Martinique	1.102	1.000	90.7	Infested	+
Mexico	1.972.546	1.000.000	50.7	Reinfested	+
Montserrat	103	103	100.0	Infested	+
Nicaragua	130.000	65.263	50.2	Reinfested	+
Panama	75.650	56.246	74.3	Reinfested	
Paraguay	406.752	200.000	49.2	Reinfested	
Peru	1.285.215	638.000	49.6	Reinfested	+
Puerto Rico	8.896	8.896	100.0	Infested	+
Saba, St. Eustatius	29	29	100.0	Reinfested	+
St. Christopher and Nevis, Anguilla	396	396	100.0	Infested	+
Saint Lucia	616	259	42.0	Infested	+
St. Martin (Netherlands part)	60	60	100.0	Infested	+
St. Vincent	388	332	85.6	Infested	+
Suriname	142.822	48.000	33.6	Infested	+
Trinidad and Tobago	5.128	3.108	60.6	Infested	+
Turks and Caicos Islands	430	430	100.0	Infested	+
United States of America	9.359.781	1.536.819	16.4	Infested	
Uruguay	186.926	186.926	100.0	Eradication completed	v
Venezuela	912.050	710.000	77.8	Infested	+
Virgin Islands (UK)	153	153	100.0	Infested	+
Virgin Islands (US)	344	344	100.0	Infested	+