Chapter 1

Sampling the Egg Population

Mosquito eggs are found in many different habitats, e.g. small pools, large marshes, rock pools, tree-holes, plant axils, flower bracts, fallen leaves, fruit husks, empty snail shells, bromeliads and a variety of man-made containers. While some species lay their eggs singly, others lay them in egg rafts or in sticky masses glued to the undersides of floating leaves. Many species deposit their eggs on the water surface, but a few lay them on the upper surface of floating vegetation, and a large number oviposit not on the water surface but at varying distances from the water's edge amongst leaf litter, mud and debris or on the walls of man-made containers, plants, tree-holes and bamboo.

Because of this great diversity of oviposition sites many different sampling techniques would be required if the eggs of the different species were to be adequately sampled. However, apart from the use of ovitraps relatively little attention has been devoted to sampling egg populations; consequently few methods have been developed. It is disappointing that there has been so little effort to study the biology and ecology of the eggs, because much valuable information can be obtained from the egg population. For example, the detection of eggs in aquatic habitats gives more reliable information on the types of oviposition sites selected by females than can be obtained from larval collections. The presence or absence of larvae cannot necessarily be taken as synonymous with the recognition of oviposition sites because some eggs may be laid in habitats from which they fail to hatch, but nevertheless these eggs represent part of the input of the adults. Egg surveys are particularly useful with species which remain in the egg state for many months, because potential larval habitats can be identified and enumerated without waiting for the larvae to appear. Furthermore, a careful study of the distribution and number of eggs in different habitats should make it possible to predict the probable size of future larval populations. Lopp (1957) emphasised the usefulness of egg surveys in predicting the potential size of pest populations of mosquitoes. In the USA Buzicky (1965) found that being able to collect aedine eggs from habitats during the winter months was invaluable in delineating breeding sites that would later require insecticidal treatment. In genetic control programmes, which result in the production of sterile eggs by field populations, the ability to sample the egg population will enable the proportions of sterile eggs laid at varying distances form the centre of control operations to be assessed. Finally, the ability to sample eggs and get population estimates in

M. W. Service, *Mosquito Ecology*

natural habitats is of paramount importance in ecological studies concerning population dynamics. If there is also information on the size of the emergent adult population, then the probability of a viable egg giving rise to an adult mosquito can be estimated. The importance of this parameter in predicting population size and the impact of genetic control measures has been stressed by Cuéllar (1969*a*,*b*).

Apart from sampling eggs already present in natural habitats, useful information can be obtained by collecting eggs from artificial oviposition sites. Such techniques have frequently been used in surveillance of *Aedes aegypti* and *Aedes albopictus* (Chadee & Corbet, 1987; Evans & Bevier, 1969; Fay & Eliason, 1966; Freier & Francy, 1991; Jakob & Bevier, 1969*a*,*b*; Jakob *et al.*, 1970; Pratt & Jakob, 1967; Subra & Mouchet, 1984; Thaggard & Eliason, 1969) and with other aedine species ovipositing in small container habitats such as domestic utensils, tree-holes and snail shells (Buxton & Hopkins, 1927; Corbet, 1963, 1964*a*; Dunn, 1927; Goettel *et al.*, 1980; Kitron *et al.*, 1989; Lambrecht & Zaghi, 1960; Lewis & Tucker, 1978; Philip, 1933; Service, 1965; Tikasingh & Laurent, 1981; Yates, 1979).

Other types of traps have been developed to sample *Culex* (Haeger & O'Meara, 1983; O'Meara *et al.*, 1989*b*; Reiter, 1983; Reiter *et al.*, 1986; Strickman, 1988; Surgeoner & Helson, 1978), *Haemagogus* (Chadee *et al.*, 1984), *Toxorhynchites* (Schuler & Beier, 1983), *Eretmapodites* (Lounibos, 1980) and *Trichoprosopon* (Lounibos & Machado-Allison, 1986).

Specific identification of the eggs obtained in surveys may sometimes be difficult because eggs have been described for only a comparatively few species, and some species cannot be separated on egg morphology. This can usually be overcome by either identifying 1st instar larvae dissected out from the eggs or by soaking the eggs and identifying the resultant 4th instar larvae or adults. A disadvantage of sampling the egg population is that it is usually more difficult and time consuming than larval surveys, especially when eggs have to be extracted from samples of soil and debris.

Procedures for sampling mosquito eggs can be divided into two main categories. The first involves the detection and collection of eggs from natural habitats while the second method uses artificial habitats such as bamboo pots, tin cans and glass jars, which are placed in a variety of different situations to attract ovipositing females.

NATURAL OVIPOSITION SITES

Anopheles

Few methods have been developed to sample *Anopheles* eggs, but Barber (1935) seems to have been the first to have seriously proposed a collecting method. He successfully collected eggs by skimming the water surface of larval habitats with a collecting bowl and straining the contents through a white muslin bag or mitten placed over the hand. Sometimes several hundred *Anopheles* eggs were collected by this method. Both Bates (1940) and Lewis (1939) successfully used this

technique to collect eggs of the *Anopheles maculipennis* complex from natural habitats in Albania. The latter collected as many as 5719 eggs in 332 dips from ditches, pools and margins of rivers. In collecting eggs of the *Anopheles gambiae* complex I have found it more convenient to use the modification proposed by Bates (1941), which consists of replacing the mitten by a piece of muslin stretched over a small wooden hoop (Fig. 1.1*a*). Several such sieves can be made and placed at the edge of larval habitats and a known number of dips strained through them. A plastic wash bottle is used to wash fine silt through the sieves and also to wash off any eggs stuck to pieces of wood or debris. Eggs can be collected from the sieve, or the contents floated off in water. An alternative method is to use a metal dipper with the bottom removed and replaced by a fine metal gauze, and after a number of dips, or sweeps through the water, the dipper is turned upside down and the contents washed into a bowl. Individual eggs can be picked out with fine forceps or with a glass pipette and sorted into tubes for later counting and identification. A palette, consisting of wire bent round to form a circle about 25 cm or less (5–10 cm) in diameter and covered with fine nylon gauze and fitted to a wooden handle, is recommended by WHO (1975) to collect both eggs and larvae of *Anopheles* from puddles, cattle hoof-prints and other small habitats.

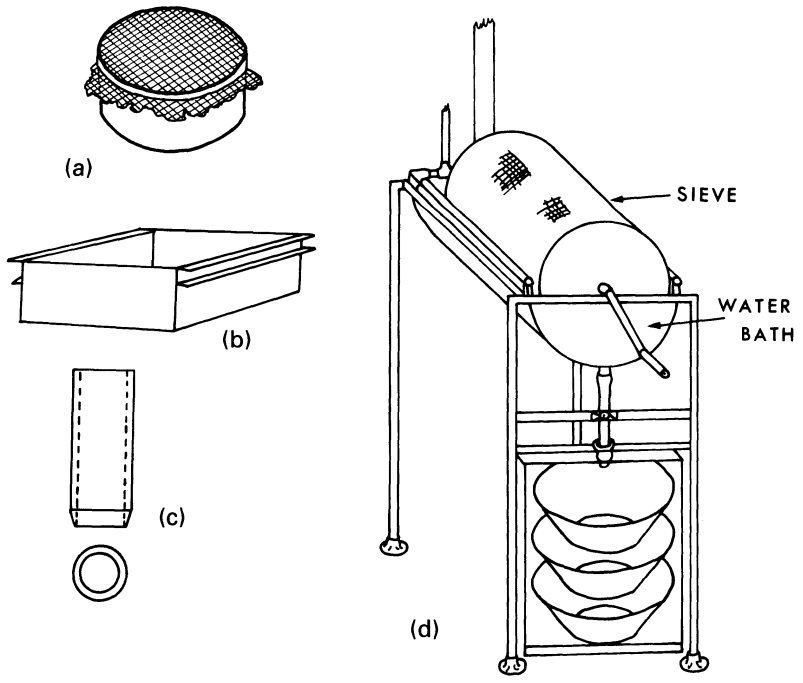

FIG. 1.1. *(a) Muslin hoop; (b) sampling square; (c) tubes (after McDaniel & Horsfall, 1963); (d) Horsfall's soil washing machine (after Horsfall, 1956).*

Earle (1956) described an automatic strainer for concentrating larval collections and as he mentioned that *Anopheles* eggs were also retained this might prove a useful piece of apparatus for removing eggs from collections made with a dipper. A description of the method is given in Chapter 2.

On the other hand Swellengrebel & de Buck (1938), Aitken (1948) and Rozeboom & Hess (1944) found it unnecessary to strain their samples and simply counted *Anopheles* eggs on the surface of the water collected in a dipper. In Holland Swellengrebel & de Buck (1938) collected as many as 242 eggs of the *Anopheles maculipennis* complex from 10 dips. Muirhead-Thomson (1940*a*,*b*) found that eggs of certain Indian and African malaria vectors, such as *Anopheles minimus* and *Anopheles funestus*, were so small that they were washed through the usual type of muslin mitten or sieve. He therefore collected eggs by skimming the surface with a white enamel tray and reported that *Anopheles* eggs were easily seen against the white background of the tray. The method proved successful in still waters but in streams, even where *Anopheles minimus* was breeding abundantly, no eggs were collected. To try to overcome this difficulty Muirhead-Thomson (1940*a*,*b*) removed pieces of vegetation and scraped surface mud from the edges of streams, and washed the material in a bowl. Although very successful in still waters, with the stream breeding *Anopheles* it was only partially successful (Muirhead-Thomson, 1940*a*). With pool breeders Muirhead-Thomson (1940*a*) found a good correlation between the abundance of eggs and larvae of different *Anopheles* species collected from the same habitats.

Similarly, Rozeboom & Hess (1944) found very good correlations between the numbers of eggs and larvae of *Anopheles quadrimaculatus* collected by skimming the water surface of reservoirs containing differing amounts and types of vegetation. Aitken (1948) compared the incidence of both eggs and larvae in various habitats in Albania. He found that of the 546 collection stations having immature stages of *Anopheles* both eggs and larvae were collected from 81%, while eggs but no larvae were collected from 13% of them. These results demonstrate the value of egg surveys.

In Sierra Leone Muirhead-Thomson (1945) collected eggs of both *Anopheles gambiae* and *Anopheles melas* from pools, puddles and partially dried up streams by using either a white enamel scoop or bowl, or directly from the water with a wire loop. He obtained as many as 1057 *Anopheles gambiae* eggs in 16 visits, and altogether collected several thousand *Anopheles* eggs. In India both Muirhead-Thomson (1940*a*) and Russell & Rao (1942) found that dipping was unnecessary, *Anopheles* eggs could be collected by lifting them from the water surface with a small wire loop.

In Thailand it appears that *Anopheles dirus* sometimes lays her eggs on damp soil above the water line. Rosenberg (1982) succeeded in finding eggs by sluicing the banks of a larval habitat with 5–10 litres of clean water and then quickly ladling the water draining back into the centre of the pool and passing it through a 150-μm cloth sieve. Examination under a microscope revealed unhatched eggs of *Anopheles dirus*. A high proportion of eggs (5/7 and 21/33) recovered up to 10–12 days after a breeding site was drained remained viable and hatched in the laboratory after flooding. In Kenya Beier *et al.* (1990) collected dry soil from

habitats and obtained *Anopheles gambiae* and *Anopheles arabiensis* larvae when the samples were flooded with water in the laboratory. The authors argue that further investigations are needed to determine the degree of desiccation to which *Anopheles* eggs can withstand. They point out that there may be greater tolerance to desiccation in populations of a species living in dry areas than exhibited by eggs of the same species collected from wetter areas.

In India, Russell & Rao (1942) working with small well-defined pits were able, by lifting *Anopheles* eggs directly from the water surface with a small wire loop, to collect most of those that had been laid the previous night. It is unlikely, however, that the total egg population can be removed and counted from many natural habitats. In most instances *Anopheles* egg surveys will only detect the presence of eggs in a habitat, or give relative population indices such as the number of eggs per dip or the number collected with a wire loop within unit time. However, even with these methods there may be sampling problems. For example, if the water surface in one habitat is clean most eggs will be stranded along the edges, but if in another site floating vegetation and debris are present a number of eggs will cling to these objects and occur away from the edges. The distribution pattern of the eggs will consequently differ in the two habitats and this will most likely be reflected in the numbers caught, although the actual number of eggs present in both habitats could be the same. Furthermore, if the same number of eggs are present in two different sized pools, it is likely that more will be collected per dip, or unit time, from the smaller pool. Such factors have to be taken into consideration if the numbers caught from different habitats are to be compared.

An estimate of the size of the egg population might be obtained by employing the removal method of Zippin (1956), which has been used by Wada (1962*a*,*b*) to estimate the size of mosquito larval populations. In this method the number of eggs collected within a short time interval, say 2–3 min, although this will largely depend on egg density, or within a standard number of samples, is recorded over a period of say an hour. The decline in numbers of eggs collected in successive time intervals, or samples, is plotted against the previous total catch. An approximate estimate of the size of the egg population is given by the intercept on the absisca of a straight line fitted to the points on the graph. The procedure is explained in greater detail in Chapter 2 in connection with estimating larval populations. In Nigeria, to test the possibilities of the method, 618 eggs of the *Anopheles gambiae* complex were placed in a small pool of about 260 cm^2 surface area, which previously contained no eggs of *Anopheles gambiae*. The total population estimated from 12 dips was 537 (Fig. 1.2), which represents 86·9% of the total population. A disadvantage of this method is that a relatively high proportion of the total population must usually be removed before a reliable estimate is obtained. In the present experiment 62·3% of the eggs placed in the pool were collected. Despite this limitation the method merits further investigations.

Although *Anopheles* eggs were collected by workers in North America, Europe, Africa and India over 30 years ago there have been very few attempts to improve or devise new sampling techniques, whereas with *Aedes* mosquitoes there has been renewed interest in egg surveys.

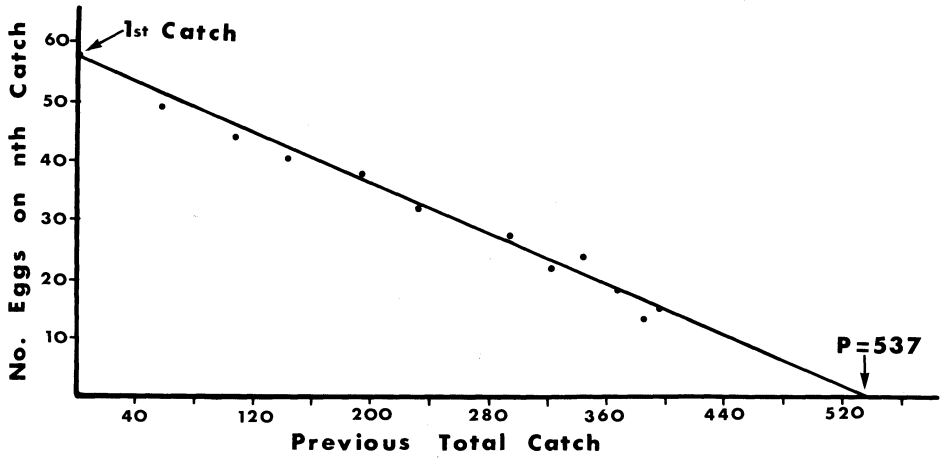

FIG. 1.2. Estimation of the egg population of Anopheles gambiae *by the removal method.*

Aedes: Ground pools, floodwaters, marshes etc.

Direct observations

Occasionally aedine eggs have been recovered from the field by locating the sites in which they are laid. Corbet (1964*b*, 1965, 1966), for example, found that in the Arctic *Aedes nigripes* lays her eggs on the mossy slopes of the northern banks of ponds, whereas eggs of *Aedes impiger* are deposited some 5–20 mm below the soil surface in cracks. Corbet (1964*b*) reported that oviposition sites of *Aedes nigripes* were often rendered conspicuous by the corpses of individuals that died after oviposition. Wesenberg-Lund (1921) located eggs of *Aedes communis* amongst leaf litter of a dried up pond. By removing layers of leaf litter from small woodland pools I have found that eggs of *Aedes cantans* and *Aedes rusticus* are mainly deposited on the undersides of the top layer of leaves resulting from the previous autumn. Very few eggs occur in soil beneath the leaf litter. Smith (1904), quoted by Mattingly (1969), was able to detect eggs of various *Aedes* species in cut out sods of earth by examining the cut edges with a hand lens. James (1966) also observed the location of *Aedes* eggs in soil samples by visual inspection.

Soaking soil samples

The detection of eggs in either natural habitats or in samples removed from oviposition sites gives some information regarding the actual site in which the eggs are laid, but the method is very time consuming and cannot be used quantitatively. One of the commoner techniques for both detecting and determining the relative abundance of aedine eggs in oviposition sites is to count the numbers of larvae that hatch when soil and leaf litter samples from oviposition sites are soaked in water (Bidlingmayer & Schoof, 1956; Bradley & Travis, 1942; Breeland & Pickard, 1963; Buxton & Breland, 1952; Dunn, 1926; Elmore & Fay, 1958;

Enfield & Pritchard, 1977; James 1966; Micks & McNeil, 1963; Ritchie & Addison, 1991; Service 1965, 1970; Wallace *et al.*, 1990; Wilkins & Breland, 1949). Filsinger (1941) studied the vertical distribution of eggs of *Aedes vexans* in sod samples removed from oviposition sites. Vegetation that grew above 2 in from soil level was cut and discarded, then vegetation that reached from 1–2 in was cut and soaked in water, and vegetation that grew up to an inch from the sod samples was similarly cut and soaked. Then the sods were sliced and separated into the 1st inch below soil level, the 2nd inch and finally the remaining 4 in. All samples were soaked in water for 24 hr and the larvae that hatched counted. Based on larval counts he concluded that only 4% of the eggs were located above the soil, 14% were contained within the 1st inch of soil, 20% in the 2nd inch and 47% below this. 'Trimmings', which represented material remaining after the sod samples had been cut up, contained about 12% of the eggs. Filsinger (1941) then placed sod samples on the top of a nest of 20-, 40-, 60-, 80- and 100-mesh sieves and washed them for 1 hr with water from a sprinkler. None of the eggs was retained by the top sieve, most were collected by the second sieve. From this simple experiment he concluded that in the field, rain was important in washing eggs down to lower depths.

In studies on *Aedes taeniorhynchus* and *Aedes sollicitans* Bradley & Travis (1942) removed sod samples with an iron ring, 1 in deep and 3·3 in in diameter, mounted on a 3-ft hoe handle. The 8-in square samples obtained were soaked in water and the number of larvae that hatched after 24 hr used to indicate the relative abundance of these two species. Elmore & Fay (1958) also studied the oviposition sites selected by these mosquitoes, but undertook a more critical evaluation of the method for determining their relative abundance. They conditioned soil samples at either 15, 21, or 26°C (60, 70 or 80°F) for 1–12 days before flooding them with water at the temperature at which they were conditioned. After soaking, the water was poured off and each sample stored for 10 days at 26°C (80°F) and 70% R.H. before being resoaked. It was found that temperature greatly influenced egg hatching. Fewer eggs hatched on the first soaking from samples that were stored at 15°C (60°F) than those that were held at higher temperatures. Furthermore, the proportions of the two species obtained varied according to temperature and storage time (1–12 days). Less than 3% of the larvae that hatched from samples stored and flooded at 15°C (60°F) were *Aedes taeniorhynchus*, and samples conditioned at 21°C (70°F) for 1–3 days before flooding produced no larvae of *Aedes taeniorhynchus*. At 26°C (80°F) very few larvae of *Aedes taeniorhynchus* hatched from samples for 1–2 days, but with increasing storage time the number of *Aedes taeniorhynchus* that hatched increased and rapidly exceeded those of *Aedes sollicitans*. They also found that the prevalence of *Aedes sollicitans* calculated from the numbers of 4th instar larvae obtained from soaking soil samples was greater than when identification was on 1st instar larvae. Evidently their rearing procedures favoured *Aedes sollicitans* more than *Aedes taeniorhynchus*. Bidlingmayer & Schoof (1956), however, did not experience this difficulty. Soil samples from salt marshes were held for a week at 26°C (80°F) then flooded and the numbers of larvae, mainly *Aedes taeniorhynchus* and *Aedes sollicitans*, that hatched within 24 hr counted. The propor-

tions of these species were about the same when based on identification of 1st instar larvae or reared adults.

Ritchie & Johnson (1991*a*,*b*) used a 10-cm diameter golf-core sampler to take 10-cm deep soil cores in mangrove forests to study the distribution of *Aedes taeniorhynchus* eggs. Soil samples were soaked in water for at least 3 days to allow newly oviposited eggs to mature, after which the core samples were flooded with dilute yeast solutions to promote hatching. In other surveys Ritchie & Addison (1991) collected soil samples from mangrove forests with modified 6- and 60-ml plastic syringes. The tips of the syringes were cut off and the front edge of the barrel bevelled to form sharp cutting edges. It was apparently necessary to cut off the tip of the plunger to enable it to be inserted into the barrel with minimum resistance. When the syringes were pushed 2·5 cm into the soil, cores of 3- and 15-ml volume were obtained by the smaller and larger syringes.

Fallis & Snow (1983*b*) reported that with *Aedes punctor* and *Aedes cantans* a slow reduction in oxygen content such as achieved by immersing eggs in 0·1% Bacto Nutrient broth (Novak & Shroyer, 1978) induced hatching, whereas transferring eggs directly to deoxygenated water failed to stimulate hatching.

James (1966) also flooded soil samples to identify oviposition sites of *Aedes trichurus* and *Aedes stimulans*. Similarly Buxton & Breland (1952) collected and flooded samples of mud, soil leaf litter and debris from a wide range of different types of habitat to detect oviposition sites. Although they recorded over 19 species, less than a third of their samples yielded larvae, and they concluded that this procedure did not provide a routine method for detecting mosquito breeding sites. However, although samples from tree-holes and rock pools were repeatedly soaked, larger sod samples were soaked only once, and this would increase the likelihood of negative results. In Panama Stone & Reynolds (1939) did not collect samples, but flooded small damp depressions in natural sites, and by this procedure identified the oviposition sites of several *Culex, Anopheles* and *Psorophora* species.

About 80% of the eggs of *Aedes detritus* hatched from mud collected from salt marshes on the 2nd-5th soakings, but a few remained unhatched until the 18th soaking (Service, 1968*a*). When gourds, which have been used in Nigeria to study mosquitoes breeding in tree-holes, were repeatedly soaked a few *Aedes* eggs failed to hatch until the 7th soaking. Similarly, in Panama a small number of eggs of *Haemagogus* species in bamboo pots remained unhatched until the 10th flooding (Galindo *et al.*, 1955). Buxton & Breland (1952) soaked tree-hole litter 13 times and obtained an egg hatch of *Aedes triseriatus* on 12, and of *Aedes zoosophus* on 9 occasions. With the rock pool species *Aedes vittatus*, most of the eggs contained in mud samples hatched during the 2nd and 3rd soakings, but a few hatched on the 6th soaking (Service, 1970). Although the detection and estimation of egg populations by soaking soil samples might appear an attractive and simple procedure a number of difficulties exist. For reliable results each sample must be flooded a relatively large number of times, and this makes the procedure time consuming. Another disadvantage is that there is no guarantee that the proportions of two, or more, aedine species present in an oviposition site will be accurately measured by the species composition of the

larvae that hatch. Eggs of some species may hatch more readily than others. Despite these limitations the method can still in many instances be usefully employed in mosquito surveys.

In studying the spatial distribution of the immature stages of *Culicoides variipennis* Vaughan & Turner (1987) used a simple plastic sampler that was thrust into the mud and which had sliding flexible partitions inserted to divide the sample from the top of the mud downwards into sections that were 0–1, 1–2, 2–3 and 3–5 cm deep. The sampler was then eased out of the mud and the various sections washed into separate containers. If certain modifications were made to this apparatus, such as making the sliding partitions of metal having a cutting edge, it might prove useful for studying the depth distribution of aedine eggs.

Extraction method: Husbands

Husbands (1952) proposed an unusual method for extracting *Aedes* eggs from irrigated pastures. First the grass was closely cut and the soil surface raked loose to a shallow depth, then a vacuum cleaner, connected to a portable generator mounted on a jeep, was used to sweep the vegetation and suck up mosquito eggs and other loosened debris. The vacuum cleaner was modified by replacing the corrugated extension tube by a smooth piece of hosing and by fitting a small cloth bag to its end inside the cleaner body to collect the sample.

The soil samples were dried and then sieved through 60- and 80-mesh screens. The *Aedes* eggs retained by the 80-mesh screen were further sorted from soil debris by slowly passing the samples through a funnel onto a white microscope stage rotated by a small electric motor. The regular trickle of soil which was deposited on the revolving stage was automatically spread out and examined under a microscope. When eggs were seen the stage was stopped and they were removed. Husbands (1952) also proposed the separation of *Aedes* eggs from fine sand and soil particles by placing the sample in a small bowl, covering it with about 1 in of water and rotating the bowl either by hand or on a mechanical rotator at about 60 rev/min, which should result in a stratification of different sized soil particles with the eggs settling out in the uppermost layer.

A generator-driven modified vacuum cleaner was used by Husbands & Rosay (1952) to collect *Aedes* eggs from the top layers of soil in irrigated pastures. Husbands (1952) claimed that the main advantages of the method were that samples could be collected from specific sites without destroying the root growth of the plants, and that only about a pint of soil was collected for each square foot of surface swept. These methods using vacuuming of turf and soil are now very rarely used.

Extraction method: Gjullin

Stage & Yates (1939) refer to the development of a machine for sifting out *Aedes* eggs from soil particles but give no description of their machine or method. The earliest published description of an egg separating machine appears to be given by Gjullin (1938). His machine was made by adapting a 24-in wide commercial grain cleaner. Soil samples were firstly dried until they became almost dusty then large debris was removed by passing the soil through an 8-mesh sieve. The

sample was then placed in the hopper of the grain cleaner and fed down over a series of 14-, 30- and 40-mesh shaker sieves to remove particles. Eggs and fine debris which passed through these sieves were collected on an 80-mesh sieve from which they were shaken onto a 60-mesh roller sieve. As they dropped on the roller sieve they were subjected to a gentle draft of air from a fan which blew away unwanted light material. Eggs were shaken from the 60-mesh roller sieve and collected underneath in a small pan. Gjullin (1938) successfully used this equipment to extract eggs of *Aedes sticticus, Aedes vexans* and *Aedes dorsalis.* He reported that about 90% of the eggs were removed from his samples, and that if the collected waste materials were run through the machine 'several times', nearly all eggs could be removed. Different sized mesh on the roller sieve might be required for separating eggs of other mosquito species. Although employed by Stage *et al.* (1952) in their studies on the mosquitoes of the northwestern states of America the apparatus has been little used by others. This last paper gives a detailed description and drawing of the apparatus and is more readily obtained than the original account.

Extraction method: Horsfall

Horsfall (1956) described a method of wet sieving to remove eggs of floodwater mosquitoes (*Psorophora* and *Aedes*) from soil and leaf litter samples. Samples are removed from oviposition sites by a 'cutting square'. This consists of a sharpened metal band bent into a 6-in square fixed to a wooden square with a handle on top. It is pushed into the ground to a depth of about 1 in and the sample cut from the soil below with a spade after which it is placed in a bag and taken to the laboratory. The sod of soil is then placed in the inner of three concentric cylindrical metal screens, having 4, 8 and 18 meshes per in respectively. The lower halves of these cylinders are immersed in a water bath (Fig. 1.1*d*). A central shaft runs through the middle of the inner cylinder and when its handle is turned the three cylindrical sieves rotate and pass through the water bath. The operator turns the handle at a rate of about 50 rev./min, first in one direction then in the opposite direction to complete about 125 revolutions. This treatment breaks up the soil sample in the sieves and flushes the eggs, and other comparatively small particles, through into the water bath. During the final 25 turns, a bottom tap on the water bath is opened and the contents empty into the first of three metal sieves (40, 60 and 100 mesh/in) placed one above the other. A strong jet of water washes the eggs through the top two sieves onto the screen of the bottom sieve, from which they are washed on to a small cylindrical fine mesh 'transfer' screen. Further separation from soil particles is achieved by flotation. Eggs are washed with about 1·5 litres of saturated sodium chloride solution from the transfer screen into a 2-litre conical funnel. The solution is stirred for 1–2 min with a glass tube through which air is passed from a pump. This causes the eggs, together with other fine organic particles, to float to the top, while soil particles sink to the bottom and are removed by opening a drain tap. The eggs are then filtered through a fine sieve, and washed with tap water into a small dish. Floating debris and most of the water is decanted as waste and the residue, which contains the eggs, reflooded with saturated salt solution. Eggs float to the

top and are poured on to another fine mesh sieve, from which they are washed with water into a dish which is scanned with a microscope and the eggs removed and identified.

Horsfall (1956) reported a recovery rate of eggs of 81–89% with this method, and later cites a recovery rate of about 80% (Horsfall, 1963). Rioux *et al.* (1967) used the machine in France to extract *Aedes* eggs from samples collected from different ecological zones of salt marshes. Leftkovitch & Brust (1968) also used Horsfall's machine to extract eggs of *Aedes vexans* from soil samples. In Canada in surveying oviposition sites for *Aedes vexans*, Novak (1981) extracted eggs from soil samples by the method of Horsfall (1956), while in the USA Meek & Olson (1976) also recovered eggs of *Psorophora columbiae* by this method followed by flotation.

Chambers *et al.* (1979) took 15 × 15 × 2·54-cm soil samples from Louisiana rice fields to collect eggs of *Psorophora columbiae, Psorophora ciliata, Psorophora discolor* and *Aedes sollicitans*. A modified version of the egg separator of Horsfall (1956) and Meek & Olson (1976) was used to extract eggs from the samples. (No details of the modification are given). This was then followed by the salt-water flotation method. Lopp (1957) recognised the value of extracting eggs and identifying breeding places in mosquito control programmes for forecasting the probable size of mosquito populations the following season; and also for undertaking surveys of pre-adults before the larvae hatched. To test the efficiency of the machine, which he mechanised to cope with large numbers of samples, he placed a single mosquito egg in each of five soil samples. An egg was recovered from four of the samples after they had passed through the machine.

Pausch & Provost (1965) used Horsfall's extraction method to calculate the average number of eggs of *Aedes taeniorhynchus* per sod sample so that approximate estimates could be made of the total eggs present in different areas. The machine was also used by McDaniel & Horsfall (1963) and Horsfall (1963) to study the local distribution of *Aedes* eggs of floodwater mosquitoes. McDaniel & Horsfall (1963) investigated the location of eggs at different levels in the soil. They selected areas known to contain concentrations of *Aedes* eggs and which were free from sticks and stones which could interfere with the removal of the samples. A 6-in square metal frame with sharpened cutting edges was placed on the ground and 25 metal tubes, 1 in in diameter and 3 in long with the bottom edges sharpened, were placed within the frame (Fig. 1.1*b*,*c*). Both tubes and the frame were carefully hammered into the soil. The soil outside the frame was removed on three sides to allow a sheet of galvanised steel to be driven horizontally underneath the frame. This prevented the soil falling out of the tubes when the frame together with the tubes was carefully lifted and transported to the laboratory. A square of plywood was placed on top of the tubes to enable the frame to be removed without disturbing the tubes. The soil cores were expelled from the upper end of the tubes by slowly inserting a cylindrical cork plunger. Slices 5-mm thick were cut and isolated, and eggs extracted by sieving and flotation. Most eggs of *Aedes stimulans* and *Aedes vexans* were found to be within about the upper 25 mm, but a few eggs of *Aedes vexans* were recovered from a depth of 71–75 mm. Checks were made to ensure that the metal tubes had not forced the eggs down to unnatural depths.

After processing soil samples with the sieving method of Horsfall (1956) eggs and organic debris can be poured into a porcelain dish, then the water containing eggs and debris can be carefully poured off and about 100 ml warm water (60–70°C) added. After a few seconds the contents are poured through a 100-mesh sieve. The retained eggs and organic debris are washed with the minimum amount of water into a dish lined with paraffin wax. About 100 ml of a 0·3% (or stronger) hydrogen peroxide solution, prepared from commercial 3% stock solution, is poured into the wax-lined dish. The hydrogen peroxide results in bubble formation within debris particles and causes them to float to the surface. After such separation the debris can be poured off, and the eggs which remain on the bottom removed with a pipette. Recovery of aedine eggs ranges from 94–100%. If viable eggs are required the pre-separation heat treatment with warm water should be omitted, but this may reduce the recovery rate to 89–98%. Although I have tried this method I prefer the much simpler method of flotation in salt or magnesium sulphate solution.

In studying the effect of tillage on the distribution of *Aedes vexans* eggs in floodplains, an 8·3-cm diameter commercial grass plugger was used to take core samples to a maximum depth of 12·7 cm (Cooney *et al.*, 1981). In the field the core samples were forced from the plugger into quart-sized cylindrical milk cartons of the same diameter as the corer. In the laboratory a hand-operated mechanical jack was calibrated so that each stroke of the piston forced the core 6 mm up into the carton and out at the top. An electric carving knife was used to slice off these 6-mm sections, which were sealed in plastic bags until processed by a modification of the Horsfall (1956) method. Eggs recovered from 0–6·0 mm represented 61·2% of the total retrieved, from 6·0–12·0 mm 22·4%, from 12·0–18·0 mm 11·2%, from 18·0–25·0 mm 4·4%, from 25·0–31·0 mm 1%, no eggs were recovered from a depth of 31·0–106·0 mm. Scotton & Axtell (1979) used a 15 × 15-cm stainless steel tray with 3 sides upturned to a height of 5 cm, with the remaining side protruded as a 5-cm lip to take soil samples from dredge spoil. To sample surface soil the lip was pushed down 2 cm into the soil and then the tray thrust horizontally to obtain 15 × 15 × 2-cm (450 cm^3) samples. Soil samples were tipped into water and when necessary broken up by 15-s use of an electric blender, having the blades covered with rubber tubing to reduce the risk of damaging the eggs. Wet sieving and flotation methods modified from Horsfall (1956) and Service (1968*b*) were applied, and eggs of *Aedes taeniorhynchus* and *Aedes sollicitans* floated off in a 1.1 sp. gr. solution of magnesium sulphate. The recovery rate was 71 ± 8%, with only 8·5% of recovered eggs being damaged.

Generally the Horsfall (1956) method, or a modification of it, remains the most commonly used system for extracting aedine eggs from soil.

Extraction method: Service

A criticism of Horsfall's method is that it necessitates the construction of a special, and fairly elaborate, piece of apparatus. The extraction technique is also time consuming. To simplify and speed up the removal of aedine eggs from soil samples a Salt–Hollick soil washing machine (Salt & Hollick, 1944) was used by Service (1968*b*). The machine is available commercially as a standard piece of

equipment used by soil zoologists to extract nematodes from soil samples, but if it has to be constructed it is more easily made than Horsfall's machine.

Soil samples are removed from oviposition sites and transported to the laboratory in plastic bags. They can be processed immediately or stored in a refrigerator until required. Freezing followed by thawing may be useful for helping break up lumps of clay in samples; alternatively chemical dispersing agents such as sodium citrate (d'Aguilar *et al.*, 1957), sodium hexametaphosphate and sodium carbonate (Raw, 1955) or sodium oxalate (Seinhorst, 1962) can be used. Fisher (1981) believed that freezing soil samples for at least a day helped to break up soil aggregates. He emptied his samples into a bucket of warm water containing the water softener Calgon, the mixture was then stirred and allowed to settle. In extracting *Culicoides* larvae from soil samples Mullens & Rodriguez (1984) found that the addition of a commercial flocculating agent (2 drops, i.e. about 0·05 ml of 0·5% solution, of 'Separan NP10' from Dow Chemical Co.) speeded up settling of mud particles and made sorting and counting easier when there was flotation in NaCl or $MgSo_4$. Following any preliminary treatment the sample is placed in a white bucket and flushed with a strong jet of water. When about three-quarters full the contents are vigorously stirred to break up the sample and dislodge eggs from plant debris, after which it is washed with a strong jet of water from a hose through 7- and 2·5-mm sieves which are mounted above each other in the Salt–Hollick machine (Fig. 1.3*a*). Small particles, together with the eggs, are washed through the finer sieve into the settling can beneath, then the sample is tipped into the 'Ladell can' which has a 0·2-mm phosphor bronze mesh screen at the bottom. After all the water has drained through, a rubber bung is inserted into the bottom of the can which is then filled with a solution of magnesium sulphate, sodium chloride or almost any other solution, including cane sugar, having a specific gravity of 1·2. The contents are stirred, and after allowing about 5 min for soil particles to sink and organic matter and the eggs to float to the surface, the top half is decanted through a small 0·2 mm sieve (Fig. 1.3*b*). Eggs are dislodged from the sides of the can and from the debris with a washbottle filled with magnesium sulphate. Finally the material collected on the sieve is washed into a convenient container. Samples can be stored in a refrigerator or a deep freeze, or processed immediately. Each sample is tipped into a white porcelain evaporating dish and examined under a stereoscopic microscope, and the eggs, which float to the top, removed and counted. Sometimes a lot of plant debris floats on top of the sample and further separation is required. The sample is poured into a 200-ml narrow-mouthed centrifuge bottle containing saturated sodium chloride and spun at $700 \times g$ for 10 min. Mosquito eggs settle out at the top and are decanted into an evaporating dish. Very occasionally when the samples contain excessive amounts of plant litter, such as small seeds and pieces of leaves, it may be necessary to tip the sample into a glass jar and place it under partial vacuum for 2–3 min in a vacuum desiccator, prior to centrifuging. As pressure is reduced plant debris sinks to the bottom. Care must be taken not to reduce the pressure too much otherwise the desiccator may implode; for this reason it is recommended that the desiccator is placed within a strong metal wire cage.

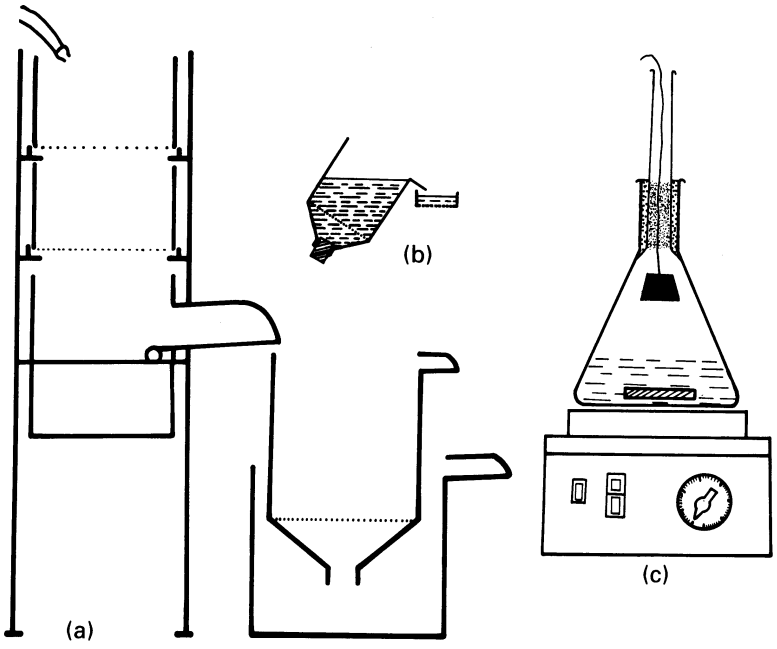

FIG. 1.3. (a) Salt–Hollick type of soil washing machine (after Salt & Hollick, 1944); (b) decanting eggs and surface debris from Ladell can into a fine sieve; (c) Erlenmeyer flask with magnetic stirring bar.

Fallis & Snow (1983*a*) found that placing samples under a vacuum was ineffective in recovering eggs of *Aedes punctor* from leaf litter because many became entangled on sinking organic matter and were not recovered. They also reported that flotation and centrifugation did not separate eggs from organic matter; they just washed their leaf litter samples with a water jet through a number of sieves (5·6 mm, 710 μm and 80 μm). Ritchie & Johnson (1989) pointed out that even with wet sieving and flotation methods it can sometimes still be difficult to identify aedine eggs from background debris. This is especially so in mangrove soils rich in peat deposits, because low density peat fragments are not adequately separated from the eggs. To make separation easier, they placed the filtered material in 2·5% sodium hypochlorite (50% commercial bleach) for 3–5 min, stirring occasionally until the soil and peat particles turned brownish yellow. The solution was then poured through a 0·15-mm sieve and washed for 30 s with water to remove much of the peat which has been partially dissolved by the bleach. The darker unbleached eggs should be removed or counted immediately because with time they will tend to bleach, making separation more difficult.

It has been shown that the Salt–Hollick soil washing machine removes about 83% of the eggs from soil samples (Service, 1968*b*). Eggs of many *Aedes* species

and also those of *Culiseta morsitans* are still viable after samples have been stored in a refrigerator, processed through the soil washing machine and centrifuged in sodium chloride. This method has been used to extract eggs of several *Aedes* species from hundreds of soil and leaf litter samples collected from woodland habitats and fresh and salt water marshes.

Lawson & Merritt (1979) described modifications to the Salt–Hollick soil washing machine (Fig. 1.4*b*). Basically these comprise an electric motor to drive a paddle to agitate the soil sample when flooded with magnesium sulphate, and an electric pump to bubble air through the sample to cause further agitation. Another modification allows the magnesium sulphate flotation solution to be recycled after filtering, so as to flood a subsequent soil sample.

Fisher's modification of Salt–Hollick's machine

Fisher (1981) constructed a modified Salt–Hollick (1944) soil-washing machine to extract Coleoptera larvae from soils (Fig. 1.4*a*). Basically the apparatus consists of two upper sieves, 45 cm in diameter at the top, 36 cm in diameter at the bottom and 20 cm high, having respectively 1·3-cm and 0·64-cm meshes (A). Both sieves are mounted on a revolving shaft turned by a 1/10-hp electric motor (F). The samples are washed through the sieves with jets of water from flat-type spray nozzles incorporating pressure gauges and shut-off valves (G). The flotation and settling tank (B) is constructed from a 211-litre galvanised dustbin, having a 32-mm sieve (K) and underneath an air line (J) that bubbles compressed air into the water to assist flotation. The tank is tilted forward so that insect material flows into the final catch sieve (C) which is positioned over a fine-soil settling tank (D) which overflows into a drain. Material collected on this sieve is emptied into a beaker and mixed with about 100 ml of hexane, water is then added and the contents stirred and allowed to separate. Insect material that floats to the interface of the hexane/water mixture is removed. This type of apparatus might prove useful for mosquito workers if they wanted to process large numbers of soil samples, up to 50–75 samples (0·5–1·4 litres) per day, the mesh size of the sieve might need to be modified to suit conditions. Tests with beetle larvae showed that it has an overall 93·4% retrieval rate.

Extraction method: Trpis

In this method proposed by Trpis (1974) soil samples are placed in a stainless steel mesh (2 meshes/cm) cylinder, which is 205 mm in diameter and height, and suspended in a metal frame placed at the bottom of a domestic washing machine. Water is poured into the washing machine up to two-thirds of the height of the mesh cylinder so that the soil sample is completely submerged. The turbulence produced by the propeller of a washing machine which is located on the bottom, breaks up the sample so that soil particles mix with the water, but larger debris remains in the mesh cylinder. Water containing the soil particles and mosquito eggs is drained through the outlet hose of the machine and flushed through a series of nested cone sieves, having 8, 16, 24 and 40 meshes/cm. Eggs and silt retained on the bottom sieve are washed into a dish, strained on to another 40-mesh sieve and then transferred to a glass separating funnel having a rubber

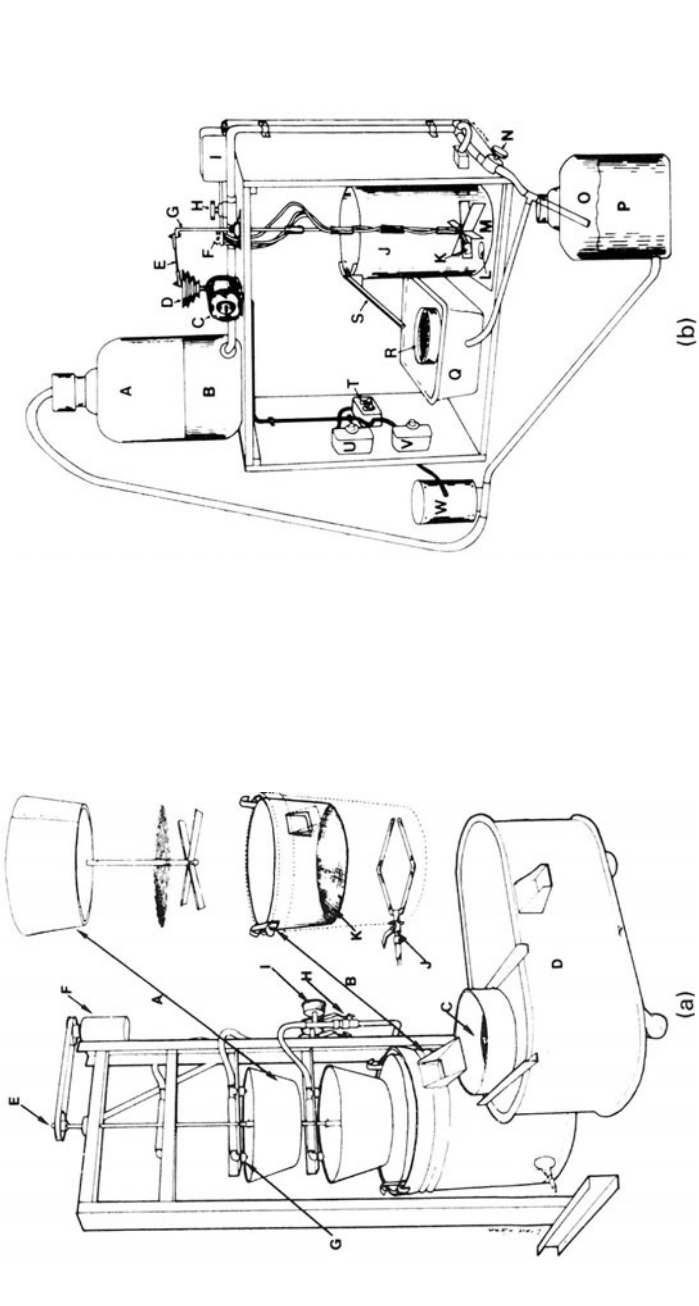

FIG. 1.4. (a) Modified Salt–Hollick machine of Fisher (1981); A — rotating sieves, B — flotation and settling tank, C — collecting sieve, D — fine soil settling tank, E — 7·6-cm diameter pulleys, F — ⅙ hp electric motor, G — spray nozzles, H — water shut-off valves, I — pressure gauge, J — air valve, K — 32-mm sieve; (b) Modified Salt–Hollick machine (Lawson & Merritt, 1979) A — storage reservoir, B — magnesium sulphate solution, C — agitator motor, D — three step sheave, E — connecting rod, F — gang valve, G — agitator shaft, H — solution flow valve, I — air pump, J — flotation cylinder, K — air stone, L — drain hole, M — agitator blade, N — cylinder drain valve, O — aspirator bottle, P — filter floss, Q — collection basin, R — retention sieve, S — sluice gate, T — on-off switch, U — agitator speed control, V — filter pump speed control, W — filter pump.

bung in the bottom and containing saturated sodium chloride or magnesium sulphate. The mixture is stirred up for 2–3 min by bubbling air through a piece of glass tubing. After settling, floating debris is removed and the mixture drained through a 40-mesh sieve and washed into a porcelain dish of tap water. Eggs sink to the bottom enabling floating debris to be discarded. The sample is then placed in another dish filled with saturated sodium chloride and the eggs which float to the top decanted into another porcelain dish and collected from the surface. This series of flotations and decantations seems excessively laborious and it should in many instances be possible to omit some of them. No details concerning the efficiency of this extraction technique are presented.

Extraction method: Miura

Because eggs of some *Aedes* species, such as those of *Aedes nigromaculis* readily hatch on the first flooding (Husbands, 1952), Miura (1972) considered that methods using water for separating eggs from soil samples might not be reliable, presumably because some eggs might hatch during processing. To overcome this he used a sonic sifter (Allen–Bradley, Model L3P), originally designed for particle size analysis, to separate *Aedes nigromaculis* eggs from air dried soil samples. Samples measuring 25 × 25 mm were cut to a depth of 10 mm from oviposition sites and processed through a sonic sifter having 14-, 40-, 60-, 80- and 100-mesh sieves stacked on top of each other. The amplitude and pulse of the sifter were set at values of 5 and 4, respectively and the samples sifted for 5 min. Most eggs of *Aedes nigromaculis* (which were 0·664 ± 0·004 mm in length and 0·182 ± 0·001 mm in width) were retained by the 80-mesh sieve, but about 10% passed through to the 100-mesh sieve. The efficiency of extraction was tested and found to be on average 91·68 ± 1·34%, but this varied according to the operator. Miura (1972) pointed out that the number and mesh sizes of the sieves, the amplitude and pulse rate of the sifter, and the time required to sift a sample largely depends on the type of soil being processed. With fairly clear sandy loams about 1·75 hr was needed to examine each sample. There was no detectable effect on the viability of about 8000 eggs of *Aedes nigromaculis* sifted by the machine for 1–10 min.

Extraction method: Ritchie & Addison

Ritchie & Addison (1991) processed soil samples from a mangrove forest by either just wet sieving (0·185 and 0·170 mm) or by coarse sieving and flotation. For the latter soil samples were passed through nested 0·30- and 0·15-mm sieves and retained soil rinsed on to 0·15-mm screening. The screen was placed on paper towelling to remove water and then completely dried in an oven for 24 hr at 50°C. The dry soil was gently broken up in a mortar and pestle, rinsed through a 0·15-mm sieve and flushed into a 1-litre separating funnel containing about 100 ml water. After 1 min the stopcock was opened and the settled soil drained out. A wash bottle rinsed down debris clinging to the inside of the funnel, and the stopcock was reopened to let out more settled soil. The residue was then filtered through a 0·15-mm sieve and the debris flushed into containers for identification of eggs of *Aedes taeniorhynchus* under a microscope. Ritchie & Addison (1991) concluded that their flotation method recovered more eggshells

(62·0%) than the sieving procedure (33·8%). They believed the method could be used to process large soil samples. They also used it to recover eggshells of *Aedes infirmatus* and *Aedes vexans*. However, they nevertheless believed that the sieving and bleaching method of Ritchie & Johnson (1989) and hatching method of Bidlingmayer & Schoof (1956) require less labour and are more efficient for recovering aedine eggs.

Alternative extraction methods

A much simplified extraction technique can be employed with samples collected from marshes, muddy ground pools, rock pools and tree-holes etc., so long as they contain little leaf litter and vegetation. The sample is placed in a beaker of water vigorously stirred and after any lumps have been broken up it is poured through ¼-in, ⅛-in and a phosphor bronze sieve stacked on top of each other. The sample is washed through the sieves with a jet of water, and the eggs retained on the phosphor bronze sieve are floated off in a solution of sodium chloride (sp. gr. 1·2). I have successfully extracted eggs of *Aedes vittatus* from many rock pool samples with this procedure as well as *Aedes* eggs from soil samples from salt marshes and woodland pools.

Several relatively simple methods for extracting nematodes worms and cysts (Fenwick, 1940; Goodey, 1957) and a wide variety of soil arthropods (Murphy, 1962) from soil samples have been described, and some of these could probably be used to extract mosquito eggs. For example, Matteson (1966) described a simple flotation technique for extracting eggs of *Diabrotica* (Coleoptera) and other insects from the soil. A 1-litre, or larger, Erlenmeyer flask with a 45/50 ground glass neck and containing a 2⅞ in metal stirring bar and the soil sample flooded with 2·6 M cane sugar solution is placed on a magnetic stirrer. A suitable sized rubber bung suspended by a piece of wire through a 45/50 ground glass joint is fitted into the flask (Fig. 1.3*c*). Additional sugar solution is added until the level rises up some 2 in in the glass joint. After about 5 min agitation the suspension in the flask is allowed to settle for 10 min, resulting in eggs and other organic debris floating up into the sugar solution in the glass joint. This small volume of liquid containing the eggs is isolated by pulling the rubber bung up into the base of the joint, which is then removed from the flask, and the contents washed through a series of sieves. Recovery rates of 79 and 86% were obtained when a known number of *Diabrotica* eggs were placed in samples and processed.

Differential water flows (elutriation) have been used to extract nematodes from soil samples (Southwood, 1978). Basically the technique uses differences in settling rates based on shape and weight of the organisms required (e.g. mosquito eggs), and soil particles and associated debris, that occurs against a water current flowing in the opposite direction. This approach has not commonly been used in entomological studies, and not, I believe, to extract mosquito eggs, but the procedures described by Blank & Bell (1988) for extracting eggs of crickets (*Teleogryllus commodus*) from soil samples might prove useful. They firstly vigorously washed and wet-sieved their soil samples, then placed the residue in an inverted cone-shaped funnel with water entering from the bottom. This

agitated the mix of soil debris and eggs and carried the eggs over to the elutriation column. Here most of the debris was flushed out while the remaining fine debris and eggs were tipped through a fine sieve and the eggs floated to the surface in a saturated magnesium sulphate solution. This method would only be suitable if large numbers of samples were to be regularly processed, because considerable effort is required to 'build' the extraction system.

Montgomery *et al.* (1979) present a relatively simple washing–flotation method for extracting insect eggs and larvae from various types of soil. Basically there is a wash tank (26-cm diameter and 30-cm deep) containing the soil sample. Water is passed into the bottom of the tank through a short length of rubber hose fitted to a pipe fixed to an inlet fitting, which is a threaded end-cap with three 0·32-cm holes drilled in the sides. This results in directing jets of water in an upward and circular motion so as to break up and mix the soil sample. The floating material is then passed from the overflow of the wash tank into a stacked series of graded sieves. Material collected on the sieves is flushed into another container with a small hand-sprayer connected to the mains water supply. Flotation is in magnesium sulphate having a specific gravity of 1.15. The recovery of eggs of *Otiorhynchus sulcatus* (black vine weevil) from 1 litre of sandy silt loam was 95·0%, but was reduced to 87·3% when 2·4-litre volumes were processed. Recovery of the smaller eggs of *Diabrotica longicornis* (northern corn rootworm) from 0·5-litre samples was 96·4%. After extraction and flotation eggs were still viable.

Speight (1973) describes an extraction method for removing mites and other arthropods and their fragments from soil that might under certain conditions be suitable for removing mosquito eggs. In this method the soil sample is broken up, placed in water and allowed to flow on to a nylon bolting-cloth 6-in wide belt which is greased with petroleum jelly that has been 'thinned somewhat' with liquid paraffin. The belt travels (9–12 m/min) over a series of rollers. Water pours off but both animal and plant debris stick to it. Use is then made of the different capacities of plant and arthropod material to adhere to a grease film. The roller passes through a water trough which causes most plant debris to be dislodged, after which the belt passes underneath a powerful spray of water which removes the arthropod material.

Whatever extraction method is used not all eggs will be recovered from samples. The efficiency of any extraction method should therefore be assessed by determining the percentage recovery of a known number of eggs processed through the apparatus. The number of eggs extracted from samples can then be corrected for 100% efficiency. For example, in a population study of *Aedes cantans* in England extending over several years, one hundred 10 × 10-cm soil samples, representing about 5% of the total oviposition area of a habitat, were collected in September, when all the eggs of the year had been laid. The number of eggs extracted by processing through a Salt–Hollick machine was adjusted for total recovery and multiplied by 20 to get an estimate of the total egg population of the habitat. Another egg estimate was made in late December, just prior to egg hatching to determine egg loss during the intervening months. This second estimate was then corrected for the percentage of eggs that fail to hatch,

due to sterility or other factors, to give an estimate of the number of viable eggs available for hatching.

Local distribution of aedine eggs in habitats

In Canada Enfield & Pritchard (1977) took frozen core samples (15 × 15 cm and 2 cm deep) at 2-m intervals along transects radiating from the centre of a pond when it was not flooded. When, however, the pond was flooded core samples were taken by cutting the earth from inside 15- and 10-cm diameter PVC pipes which were used as templates. The numbers of eggs of *Aedes cinereus* and *Aedes vexans* per sample were estimated by repeated flooding (3) of the core samples and identification of the larvae. The pond was divided into six strata (Fig. 1.5*a*), and the mean numbers of eggs per sample unit was divided by the sample area (0·0225 m^2 or 0·00785 m^2) to give the mean density of eggs within each stratum. From these values, and the areas of the strata, estimates of overall mean egg densities and their standard errors were calculated for each stratum, and also the total egg population in the entire pond was estimated.

In their pond Enfield & Pritchard (1977) could find no evidence that the distribution of eggs was related to any physical features. Precision, as judged by the size of the standard errors, was reasonably good (7·8 and 10·8%) but there were larger standard errors when egg densities were low, a time when many sampling units had no eggs. A larger core sampler might have resulted in better estimates at these low densities. A disadvantage of this, and related methods, is that estimating egg numbers from larvae hatched on repeated soakings can be laborious, and of course will not work if the eggs are in diapause. Moreover, if the soil is frozen hard it can be very difficult to cut samples from the ground.

In Florida citrus groves Curtis & Frank (1981) removed 100-cm^2 soil samples from three zones, namely the bottom of furrows, from a distance of 1–2 m from the bottom on sloping banks and from an area between 2 m to the crown of the furrow. The samples were processed according to Horsfall (1956). The mean numbers of eggs increased from 0·3, 2·0 to 43·3 in the three zones, increasing with distance from the bottom of the furrows.

Leftkovitch & Brust (1968) studied the distribution of *Aedes vexans* eggs in a pond to determine their distribution and the best procedures for egg sampling. The pond was divided into 10-ft squares and a 1-ft square soil sample was cut to a depth of 1 in and taken from each square. Eggs were extracted from the samples by Horsfall's method. The heights below the level at the edge of the pond from which the samples were taken were measured so that an inverted contour map of the pond could be drawn, taking the highest point as zero. The mean number of eggs per sample was 33·92, the variance was 2890·31. In a random distribution the mean is an estimate of the theoretical variance, i.e. the mean and variance are equal. Now, because in this instance the variance was much greater than the mean it shows that the eggs were highly aggregated, that is they occurred in clumps in the pond. Since the eggs were not evenly distributed it was decided to find out whether their distribution was related to topographical features of the pond. First, the numbers of eggs extracted from the samples were grouped into categories corresponding with the successive heights

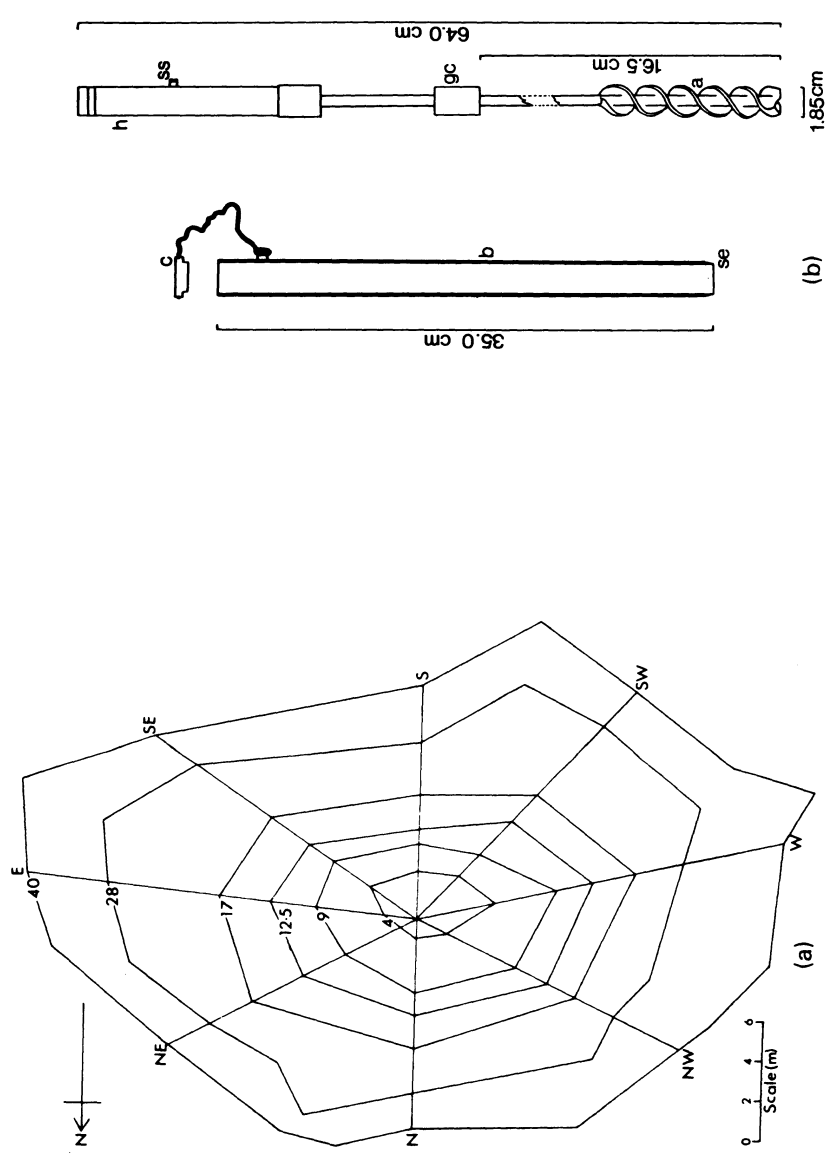

FIG. 1.5. (a) Map of a pond showing positions of transects and area covered at the different centre depths shown (cm) (Enfield & Pritchard, 1977); (b) Core sampler for tree-holes, a — auger, b — barrel of corer, c — cap, gc — guiding collar, h — wooden handle, se — sharpened edge; ss — stop screw (Kitching, 1971).

TABLE 1.1
NUMBERS OF EGGS OF *AEDES VEXANS* IN RELATION TO THE LEVELS AT WHICH THEY WERE FOUND (LEVELS MEASURED FROM HIGHEST POINT) (AFTER LEFTKOVITCH & BRUST, 1968)

Level (in)	*Number of eggs*	*Mean of transformed values*	*A*[a]	*B*[b]
0·5	3	321	5036	28029
1·5	9, 3, 33	522	4715	22993
2·5	10	555	4193	18278
3·5	4, 9, 24, 20, 23, 13, 4, 56, 1, 6, 20	525	3638	14085
4·5	1, 37, 141, 40, 8	549	3113	10447
5·5	15, 120, 1, 250, 19, 10	582	2564	7334
6·5	16, 19	634	1982	4770
7·5	19, 147, 15, 112	724	1348	2788
8·5	2	216	624	1440
9·5	—	—	408	816
10·5	7, 3	408	408	408
	Sum	5036	28029	111388

Mean level = 28 029/5 036 = 5·566 in position, i.e. at 5·07 in.
Standard error = $\sqrt{([2(111\,388/5\,036) - 5{\cdot}566 \times 6{\cdot}566]/10)} = 0{\cdot}876$.

[a] A is obtained as the cumulative sum of the column of means, beginning at the bottom.
[b] B is obtained from A in the same way that A is obtained from the means.

(in inches) in the pond from which they were collected (Table 1.1). There was a significant relationship between the mean numbers of eggs in each group and its variance. For statistical reasons this relationship must be removed by a suitable transformation before the data can be analysed. The transformation $z = 10^3 (1 - y^{-0{\cdot}352})$, where y is number of eggs obtained by the power law of Healy & Taylor (1962) and then a scale factor, was found to remove this association. The mean values of the transformed data for each depth group were calculated, and the mean level (5·07 in) and standard error (0·876 in) calculated by the summation method described by Elderton (1953). The 95% confidence limits of the mean were calculated as 3·11–7·01 in. These results show that with the correct sampling procedure relatively few samples need to be collected to elucidate the vertical distribution of eggs in a habitat.

While it is not anticipated that such a mathematical approach to sampling will be generally adopted by mosquito workers, the data clearly show that the distribution of *Aedes* eggs in natural habitats is likely to be highly aggregated. This necessitates the use of suitable transformation before the results can be statistically analysed. In practice, however, it may not be worth the effort to obtain precise transformations; in many instances converting field counts to $\log (x + 1)$ will suffice. Miura (1972), however, used a modified square root transformation, $\sqrt{(x + 0{\cdot}5)}$, for comparing the mean number of eggs of *Aedes nigromaculis* in different parts of oviposition sites.

Special habitats and species

Tree-holes and bamboo

Tree-holes are probably the most widespread class of natural habitats, species of several genera of mosquitoes breed in them. It is well known that *Aedes* can be collected from tree-holes by removing dry debris from them (Buxton & Breland, 1952; Dunn, 1926; Lounibos *et al.*, 1985; Trpis, 1972; Wilkins & Breland, 1951). In addition to collecting material from the bottom of tree-holes Dunn (1926) carefully scraped the inside walls and bottom with a metal spoon to recover the maximum number of eggs. Arnell & Nielsen (1967) also obtained eggs by scraping the walls of tree-holes. Although eggs are often collected by such methods it is difficult to obtain quantitative results. Kitching (1971), however, standardised the collecting method by developing a small core sampler for collecting semi-fluid substrates from tree-holes. One part consists of a 35-cm long piece of brass tubing 1·85 cm in internal diameter and with the distal end sharpened. A loosely fitting pierced cap fits over the opposite end (Fig. 1.5*b*). The other part fits into the brass tubing and consists of a commercially produced steel drill bit with the small screw part of the tip and squared upper part of the shaft cut off. The remaining section is fitted into the lower half of a solid brass collar, the upper half of which is fitted by a brass shaft to a wooden handle. Both the collar and drill bit fit closely into the length of brass tubing. A screw projects out of the side of the wooden handle so that when the auger is pushed through the tubing as far as the screw permits, the auger and shaft project from the bottom of the tubing with the bottom of the brass collar level with the sharpened lower edge of the tubing. In taking a sample the tube is placed in a tree-hole and first worked in by hand, then with the cap on the top it is hammered through the substrate until hard underlying wood is reached. The auger is then carefully screwed down the tubing. In practice it was found that when the auger had penetrated part way into the tree-hole the whole apparatus could be lifted out together with the sample. When the auger is pushed through the tubing the brass collar scrapes the side and ejects the sample into a plastic bag. Tree-hole debris still attached to the auger is washed into the bag. Knowing the diameter and length of the core, its volume and surface area sampled are readily calculated.

When attempting to compare or estimate the egg population in different sized tree-holes the area of the bottom of the tree-holes being sampled must be known. Furthermore, many mosquitoes lay at least some of their eggs on the inner walls of tree-holes, and these will be missed unless the walls in addition to the bottom debris is sampled (Jenkins & Carpenter, 1946). It is more difficult to collect eggs from the walls of tree-holes, especially when they are deposited in cracks and crevices. In tree-holes with narrow openings it will even be difficult to collect eggs from the bottom.

Several tropical mosquitoes oviposit in water-filled sections of bamboo, and these are generally more easily sampled than tree-holes. It is not so difficult to remove bottom debris, and eggs can usually be collected more easily from the smooth walls than from those of tree-holes. Sides of growing bamboo are sometimes punctured by insects and birds, and in some regions, especially Latin

America and Malaysia when these bamboo sections become filled with rainwater, certain mosquito species lay their eggs in them. It is difficult to remove debris from these habitats unless the bamboo is cut across, thereby destroying the habitat. In tree-holes and bamboo where it is difficult or impossible to remove bottom debris, eggs can sometimes be collected by filling habitats with water and then siphoning or pumping out the contents. This method is still not very effective in collecting eggs adhering to the inner walls. The presence of mosquito eggs in debris collected from tree-holes and bamboo is usually detected by soaking it in water and collecting the larvae that hatch out, but this may not give a reliable indication of either the number of eggs present or species composition (p. 8). A better approach is to extract the eggs from the debris by sieving and flotation.

Plant axils

Some mosquitoes oviposit in plant axils such as those formed in banana plants, pineapples, *Ravanela*, bromeliads, *Nepenthes*, grass, and in cavities of pitcher plants (Lounibos *et al.*, 1985). There is little information on the recovery of eggs from these habitats, but eggs can sometimes be located *in situ* by pulling the plants apart. For example, in Canada eggs of *Wyeomyia smithii* were collected from pitcher plants *(Sarracenia purpurea)* by dissecting the plants under a stereoscopic microscope. Alternatively, accumulated debris in the axils can be flushed out, sieved and eggs recovered by flotation techniques, or their presence detected by soaking the debris and removing the larvae that hatch.

Rock pools

Large numbers of eggs of *Aedes vittatus* have been recovered from mud collected from rock pools by sieving and flotation (Service, unpublished data). Eggs have also been detected in rock pools by repeatedly soaking mud samples and identifying larvae that hatched (Service, 1970). In coastal areas of East Africa where *Aedes aegypti* breeds in coral rock holes, eggs have been detected by soaking soil and detritus from the rock holes and identifying the resultant larvae (Trpis *et al.* 1971).

Crab holes

Evans (1962) collected a few eggs of *Psorophora confinnis* by scraping the walls of burrows made by crawfish.

Miscellaneous container habitats

Included under this category are peridomestic containers such as clay pots, tin cans, water butts, tyres, and bottles, and also natural ground containers such as split fruit husks, coconut shells, dead leaves lying on forest floors, flower sheaths, rotting fallen tree trunks, snail shells and a variety of other container habitats. Larvae are commonly collected from these habitats but egg collections are not often made. With species that lay eggs in rafts or masses, both the numbers of egg rafts and total number of eggs can be counted. The number of egg rafts may not, however, represent the number of gravid females that oviposited, because

while females usually deposit all eggs in a single raft, a number of smaller rafts will be laid in the same or different containers if she is interrupted during egg laying. Egg rafts are also easily broken and incomplete rafts may be recorded as complete ones. Because they break easily rafts collected in the field should be stranded on wet filter paper in individual tubes when they are transported to the laboratory. Simple population estimates can be made by multiplying the number of 'intact' rafts by the mean number of eggs per raft; intact rafts will represent the number of females that have oviposited in the habitat.

In Nigeria Lambrecht & Peterson (1977) used ladles to scrape mud and debris from earthen water-storage pots, and on soaking the materials hatched out larvae of *Aedes aegypti, Aedes fowleri, Aedes bromeliae, Aedes luteocephalus, Aedes apicoargenteus* and *Aedes unilineatus* in that order of abundance. They also used various sized spoons to scrape the debris from the inside of tree-holes, and after soaking obtained larvae of *Aedes aegypti, Aedes stokesi, Aedes bromeliae, Aedes luteocephalus, Aedes ingrami, Aedes apicoargenteus, Aedes africanus*, and *Aedes dendrophilus.* In both collections *Aedes aegypti* was by far the most common species.

Aedine eggs can sometimes be seen in containers. Eggs of both *Aedes vittatus* (Service, 1970) and *Aedes aegypti* deposited on the inner surface of clay pots have been counted. Furthermore, it has also been possible to count the eggs of *Aedes vittatus* laid on the walls of small rock pools and emergent plants (Service, 1970). Eggs could probably be located and counted in other container habitats, but breeding in them is usually detected by larval collections.

In a Mexican cemetery Arredondo-Bernal & Reyes-Villanueva (1989) collected eggs of *Toxorhynchites theobaldi* from containers with a simple plastic scoop. By firstly removing all the eggs in this manner from 25 artificial oviposition containers, followed by the collection of eggs at 2-hr intervals (0600–2100 hr) from these containers the diel pattern of oviposition was determined. The lowest mean number of eggs per container (approx. 8) was recorded at 1500 hr, while a peak mean of 80·9 eggs was recorded at 1900 hr, just 1 h before twilight. The numbers of eggs laid in a container showed a positive correlation ($r = +0{\cdot}70$) with surface area, and was expressed by the regression line $y = -14{\cdot}12 + 0{\cdot}126x$, where y is the mean number of eggs per sample and x is surface area in cm^2. The slope of the equation ($b = 0{\cdot}1266$) means that for each 1-cm^2 of water the oviposition rate increased by 0·13 eggs, thus an increase of 7·9 cm^2 allows 1 more egg to be laid in the container.

Chadee & Small (1988) used the following scoop for collecting the hydrophobic eggs of *Toxorhynchites moctezuma*, from small natural and artificial container habitats. A white plastic teaspoon (122 mm long with a bowl 40 mm long, 30 mm wide and 8 mm deep) has a 20-mm hole removed from the centre by pressing down with a piece of copper piping heated from a bunsen flame. The bowl of the spoon is placed in chloroform for a few seconds and then while still soft a small piece of nylon mesh (aperture 660 μm) is stuck to the underside. This spoon was also useful for collecting adult *Trichoprosopon digitatum* guarding her egg raft after a glass tube had been placed over her. Such a simple scoop may be useful for collecting other mosquito eggs that float on the water surface.

With small container habitats, e.g. snail shells, fruit husks, fallen leaves, the presence of eggs can be detected by immersing them in water and counting the larvae that hatch.

Mansonia (Mansonioides) species

Species of the subgenus *Mansonioides* and some species of *Mimomyia* (e.g. *Mimomyia hybrida*) lay their eggs in clusters glued onto the undersides of leaves of floating aquatic plants, such as *Pistia stratiotes*. Many years ago Dyar & Knab (1916) collected egg masses of *Mansonioides* from *Pistia* plants, and Wanson (1944) reported that it was comparatively easy to collect eggs of *Mansonioides* from their natural habitats. Bonne-Wepster & Brug (1939) also had little difficulty in finding *Mansonioides* eggs on the underside of leaves. In Florida Lounibos & Dewald (1989) inspected leaves of *Pistia stratiotes* for eggs of *Mansonia* species. In Sri Lanka Laurence & Samarawickrema (1970) collected *Mansonioides* eggs from the undersides of floating leaves. They recorded the presence or absence of egg masses daily on specific plant leaves and compared their distribution, resulting from overnight oviposition, with a Poisson model to determine whether the egg masses were randomly distributed. They were found to exhibit a distinctly aggregated distribution, and there was a marked preference for oviposition on leaves that had already been selected for egg laying. In addition to selection for individual leaves there was also preference for specific areas of the habitat. Laboratory experiments, however, failed to confirm these field observations. In Thailand Gass *et al.* (1983) successfully collected egg masses of *Mansonia annulifera, Mansonia indiana* and *Mansonia uniformis* by removing plants including *Pistia, Eichhornia, Salvinia, Jussiaea, Nymphaea* and *Marsilia* from either randomly selected 15 × 15-cm or 1 × 1-m plots within selected areas of 100–200 m^2. The aggregative distribution of eggs fitted a negative binomial distribution.

Other culicine species

Egg rafts of *Culex, Culiseta, Uranotaenia* and *Coquillettidia* and also some other genera are usually readily seen on the water surface, and in fact are often collected in larval surveys. Buxton & Breland (1952) successfully collected egg rafts of *Culiseta morsitans* from natural habitats, while Barr (1958) used his hand to submerge aquatic plants so that egg rafts floating on the water could be more easily seen. Armstrong (1941) could collect only a few egg clusters of *Coquillettidia perturbans*, but in Canada to collect egg rafts of this species Allan *et al.* (1981) constructed floating oviposition frames by bending plastic tubing into a circle to enclose about 0·05 m^2 of water. Care was taken to ensure that no egg rafts were present at the beginning of the exposure period, then at weekly intervals over a period of about 10 weeks all egg rafts were removed from a series of 15 frames. Only nine of the oviposition frames contained egg rafts, the mean number being 2·73 rafts/frame, 86% of which were in circles near or enclosing *Typha latifolia* and 13% in frames with and near *Carex* spp.

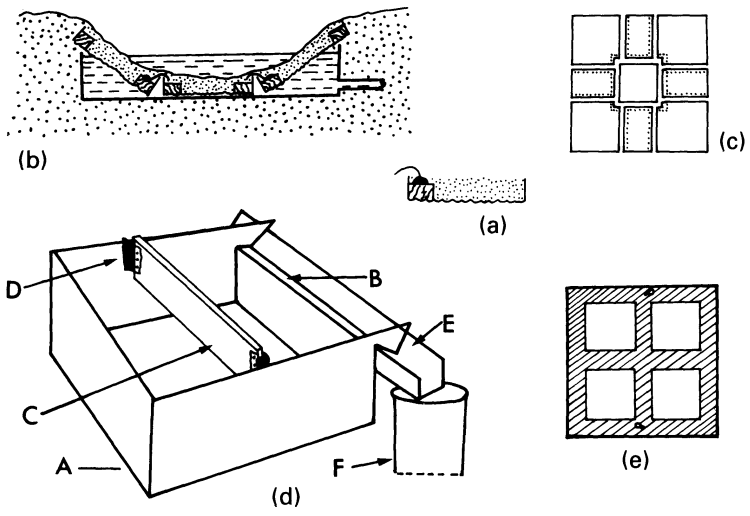

FIG. 1.6. Artificial oviposition trap for Anopheles *eggs; (a) a soil tray; (b) soil trays in metal box sunk in small pool; (c) exploded plan of soil tray arrangement, dotted lines show flanges joining trays; (d) apparatus for skimming* Anopheles *eggs from water surface showing: A — box-like water container, B — lip, C — plastic boom, D — rubber flanges, E — sloping gutter, F — collecting can (after Christie, 1958); (e) plan of 18-in square 'styrofoam' block with four 6-in square sections cut out to serve as oviposition sites for* Culex *species (Smith & Enns, 1967).*

ARTIFICIAL OVIPOSITION SITES

Artificial pits: *Anopheles*

Small water-filled borrow pits have sometimes been used as artificial oviposition sites for *Anopheles* species. In India Russell & Rao (1942) studied the effect of mechanical obstructions and shade on egg laying by *Anopheles culicifacies* by digging a number of oviposition pits, 9–12 in deep and 2 or 3 ft square. They filled the pits with seepage water to about 2–3 in from the top and kept them free of macroscopic vegetation. Eggs of *Anopheles culicifacies*, *Anopheles subpictus* and *Anopheles vagus* were collected from the water surface of the pits by lifting them off with a wire loop. In studying the oviposition behaviour of *Anopheles melas* in relation to salinity in West Africa, Muirhead-Thomson (1945) also dug a number of artificial pits to attract ovipositing females.

Christie (1958) devised complicated artificial oviposition sites for *Anopheles gambiae*. A metal box containing suitable pool water was placed at the bottom of a shallow pool about 18–20 in square. Metal boxes in several pools were connected by piping via a cistern and ballcock to a reservoir of water thus ensuring that they remained flooded. A number of small soil trays were made by tacking wooden slats to 1-in wide iron banding and covering the bottoms with 16-mesh plastic mosquito gauze (Fig. 1.6*a*). These trays were filled with soil and placed

side by side in the metal box (Fig. 1.6*b*,*c*) with peripheral trays positioned at an angle of about 30°. Flanges connected the trays together and prevented the soil from being washed down between them. After a night's exposure water was drained from beneath the trays, first by emptying the cistern, then by lifting one of the corner trays and pumping the remaining water out of the metal box. As the water drained through the trays *Anopheles* eggs were retained on the waterlogged soil. Eggs were either removed from the trays in the field (Christie, 1958) or the trays were placed in individual plastic bags and taken to the laboratory for egg extraction. Eggs were recovered by gently lowering the tray into a metal box (Fig. 1.6*d*) containing water (A). More water was carefully added so that it lapped the lip (B) at one end of the box. A plastic boom (C) with rubber flanges (D) at the ends was used to sweep floating debris and eggs into a gently sloping gutter (E) from where they were flushed into a small can (F) having a 100-mesh bottom. Material collected at the bottom of the can was washed through a 16-mesh sieve onto a 100-mesh one, and then finally into a conical vessel which had an 8-cm opening and a 2·5-cm diameter base covered with 100-gauge mesh. This inverted conical vessel was lowered into a container of water so that debris and eggs floated to within about 1 cm from its rim. Floating debris containing eggs was transferred by a small paint brush to a small piece of paper waterproofed with cellulose paint and folded up like a concertina to give a series of gutters. Debris in the gutters was flooded with clean water, examined under a stereo-microscope and the eggs lifted out by a fine wire loop. After all eggs had been collected they were transferred to filter paper having a 3-mm grid to facilitate counting.

In three field trials 373, 392 and 579 eggs of *Anopheles gambiae* were recovered from the trays. A recovery rate of 60–69% was obtained when the efficiency of the extraction technique was tested by placing a known number of eggs of *Anopheles gambiae* in the artificial oviposition sites in the field (Christie, 1958).

Christie's procedure of producing artificial oviposition sites and extracting the eggs is unnecessarily complicated. It could be simplified by excavating small shallow depressions, lining them with plastic sheeting, placing small amounts of soil on the bottom and flooding with suitable pool water. After mosquito oviposition the water can be siphoned or baled out and passed through a fine mesh sieve to retain any eggs. The waterlogged soil at the bottom of the pool together with the plastic lining can be removed and taken to the laboratory for washing through a series of graded sieves. Final separation of the eggs could be achieved by flotation in magnesium sulphate or sodium chloride (sp. gr. 1·2).

Oviposition pools: *Culex*

In Florida Smith & Jones (1972) constructed artificial oviposition pools for *Culex nigripalpus* by stapling black plastic cloth to a wooden frame 30 in long, 18 in wide and 3 in deep. These artificial pools were embedded in the ground in shaded sites near large collections of water, with the tops of the frames level with the ground. About 2·5% of the egg rafts collected from them failed to hatch, but of those that did about 89% were *Culex nigripalpus* and the remainder other *Culex* species. There was no difference between the numbers of egg rafts laid in

pools containing water or hay infusion, but about three times as many eggs were laid in pools containing crushed 40% hog supplement ('Purina') which was added at the rate of approximately 8 g/gal of water.

In studying the influence of soil fermentation on the selection of oviposition sites in California Gerhardt (1959) dug a series of shallow 2-ft square pits and lined them with polythene sheeting. A 6-in layer of soil was placed at the bottom of each pit and covered with 6 in of tap water. These pits failed to attract ovipositing mosquitoes, but eggs of *Culex stigmatosoma* and a few also of *Culex tarsalis* and *Anopheles freeborni* were laid in pits which were supplemented with 2 lb prepared dog meal. Egg rafts of *Culex stigmatosoma* were also collected from pits to which 11 lb of either sucrose, casein or hydrogenated vegetable oil was added.

De Meillon *et al.* (1967) studied the oviposition cycle of *Culex quinquefasciatus* in Yangon by creating an attractive artificial oviposition site consisting of a shallow galvanised tray (0·9 × 0·6 m) containing septic tank water together with scum and floating debris. To study the diel pattern of oviposition two collectors worked 3-hr periods and removed egg rafts from the water with a piece of stiff white paper as soon as they were laid. A roof of plastic sheeting and palm leaves was erected about 1·2 m over the artificial pool to protect the collectors from rain. In calm weather peak oviposition was around sunset and sunrise, corresponding to the principal arrival times of gravid females, but wind and heavy rain delayed oviposition and caused an irregular cycle. The arrival time of gravid females was investigated by placing on a septic tank a very simple trap consisting of a wooden frame (53 × 51 cm) 71 cm high at the front having a sloping plywood roof. The top compartment was covered with mosquito gauze while the sides of the lower section were made of stiff plastic sheeting. The bottom was covered with mosquito gauze. A 1·3-cm wide louvre-type entrance between the two compartments allowed gravid mosquitoes to enter the trap. These were removed hourly (see pp. 33–9 for other 'gravid traps').

From field trials testing the larvicidal properties of *n*-capric (decanoic) acid on mosquitoes (Maw & House, 1971) it was discovered that the acid eventually turned pools into abnormally attractive oviposition sites (Maw, 1970). The acid acted as a 'fertiliser' to bacteria of the family Pseudomonodaceae which then generated certain properties that proved to be attractive to gravid mosquitoes. Maw & Bracken (1971) used these properties in developing effective artificial oviposition sites for *Culex restuans.* Eight 1-m square pools made from 5 × 15-cm sections of wood and having a bottom of 4-mm thick hardboard were lined with polythene plastic sheeting. A series of 21 smaller pools measuring 30 cm^2 and 10 cm deep were also constructed. The pools were filled with water collected from nearby streams or temporary pools. To each large pool was added 40 ml capric acid dissolved in 15 ml 95% ethanol, 10 ml saturated ammonium nitrate, and 1 litre of water collected from pools that had been treated with capric acid the previous year and which were known to be attractive. This water which was kept frozen until required, contained the necessary bacteria. The smaller pools had proportionally less attractants added. When the water in the pools lost its turbidity, reflecting a decrease in bacteria level, further amounts of

capric acid were added to maintain maximum attractiveness. Egg rafts were collected from the pools daily from June to September.

A total of 7115 egg rafts, presumably all of *Culex restuans*, were collected from the eight large pools ($\bar{x}$ = 889·4) and 1962 rafts from the smaller pools ($\bar{x}$ = 94·4). Initially no eggs were deposited in a series of 16 untreated pools, but from mid-August to the conclusion of the trials in September 11 egg rafts were collected from these pools. It was thought that eggs found in early June were from females that had overwintered. If so, then these pools were probably more effective in assessing the reappearance of hibernating populations of *Culex restuans* than were the light-traps employed by Belton & Galloway (1966) as they failed to notice overwintering females in their catches. Maw & Bracken (1971) found that the seasonal incidence of the egg rafts was very similar in both the large and small pools.

In Canada Brust (1990) constructed artificial pools to collect egg rafts of *Culex tarsalis* and *Culex restuans*. Each pool was 1 m^2 and made from 4-cm thick wood to form a 1 × 1 × 0·2-m frame lined with black polyethylene sheeting. A 70 × 70 × 2-cm thick sod of lawn grass was placed in each pool as an oviposition attractant, and water added to a depth of 10 cm. Six holes (2-cm) were drilled 3 cm from the top of the pool and covered with fine netting to allow excess water to drain out. Best results were obtained when the sods were changed at 3-week intervals for *Culex tarsalis*, and every 1–3 weeks for *Culex restuans*. Buth *et al.* (1990) made similar pools to collect egg rafts of these two species and also *Culiseta inornata*. Madder *et al.* (1980) used inflated plastic paddling pools (84 cm diameter) lined with a layer of sods and filled with tap water as oviposition sites in Canada for *Culex pipiens* and *Culex restuans*. From a series of pools they collected 13 606 egg rafts over about 3·5 months. They found that the addition of decanoic acid, 95% ethanol, and ammonium nitrite added to pool water in the proportions described by Maw & Bracken (1971) did not provide any additional attractant for these for two *Culex* species.

Smith & Enns (1967) floated artificial oviposition blocks of 'Styrofoam' plastic on oxidation lagoons in Missouri. Four 6-in square pieces were cut from an 18-in square and 3-in thick block of 'Styrofoam', leaving a 2-in margin between the cut-out portions and between the outer edges (Fig. 1·6*e*). The total oviposition area in each block was 1 ft^2. A length of nylon rope was tied to two 4½-in eye-bolts inserted through two opposite ends of the block to secure it to a cement anchor. From an exposure period from April to August, 7715 egg rafts of the *Culex pipiens* complex, 79 of *Culiseta inornata* and 27 of *Culex tarsalis* were collected. Larval collections of the *Culex* species were comparable to the results of the egg survey, except that about 0·1% of the larvae collected were *Culex salinarius*, a species not represented in egg collections.

Oviposition traps: *Culex*

Clay pots, generally found outside, but sometimes also inside houses, or other receptacles such as jars, can be used as artificial oviposition sites both in (Southwood *et al.*, 1972) and away from houses (Service, 1970). Yasuno *et al.* (1973) poured about 2 litres of 1% yeast infusion into clay pots to improve their efficiency

in attracting gravid *Culex quinquefasciatus*. Other types of man-made receptacles such as bottles, tin cans and tyres can be placed in different habitats to detect the presence of ovipositing females (Bond & Fay, 1969; Dunn, 1927).

In Japan rice-straw infusions in earthen jars provided attractive oviposition sites for *Culex pipiens* form *pallens*. By collecting egg rafts from a series of pots at hourly intervals Oda (1967) was able to study the oviposition cycle of a natural population. Daily collections of egg rafts also provided useful information on their seasonal abundance. On the island of Seahorse Key, off the Gulf Coast of Florida, USA, artificial oviposition sites for *Culex quinquefasciatus* consisted of 2·5-gal plastic wash tubs containing an infusion of equal parts of liver powder, brewer's yeast and hog food supplement. Egg rafts were removed daily, and every 3rd or 4th day the tubs were emptied and refilled to prevent the formation of surface scum or the establishment of predators (Lowe *et al.*, 1973).

In India 4- or 5-litre clay pots holding 2 litres of water containing 1% baker's yeast infusion were placed as *Culex quinquefasciatus* ovitraps in courtyards of houses (Sharma *et al.*, 1976). Preliminary experiments had shown this yeast infusion was better than hay infusions, dog biscuit infusion and water from drains, but in the cool season because yeast fermentation is reduced, larval waters from laboratory colonies were added to some traps. Ovitraps appeared to be efficient from June to September with the highest mean number of egg rafts per trap, 18·5, being recorded in August. Peak densities correlated well with rainfall, but not with adult densities.

Oviposition traps have often been used to monitor the seasonal abundance of *Culex* vectors of arboviruses (Leiser & Beier, 1982; Madder *et al.*, 1980; Reiter, 1986), they have also been employed to catch older (gravid or recently oviposited) *Culex* to increase the chance of getting virus-infected mosquitoes (Reiter, 1987; Reiter *et al.*, 1986).

In Texas Strickman (1988) collected egg rafts of *Culex quinquefasciatus* from oviposition traps consisting of 6-litre plastic rubbish cans containing foul-smelling water produced by putting 32 g alfalfa pellets/4 litres water and held for 11 days at about 27°C. In field trials in Indiana (Hoban & Craig, 1981; Hoban *et al.*, 1980) it was found that fresh cow manure diluted in water was a better attractant than horse manure or commercial dehydrated cow manure for attracting ovipositing *Culex restuans*. This led to the development of a simple bucket ovitrap with the lid propped 2–3 cm open and containing cow manure in a cheese cloth sack. Altosid was added to the traps to prevent adult emergence. In some situations over 100 egg rafts, mainly *Culex restuans* and *Culex pipiens*, were recorded each day (Hoban & Craig, 1981). The numbers of egg rafts collected corresponded with the numbers of adults caught in light-traps.

Leiser & Beier (1982) compared oviposition traps with New Jersey light-traps in Indiana for monitoring *Culex pipiens* and *Culex restuans*. The Hoban & Craig (1981) type ovitrap was used (Fig. 1.7), and consisted of a 5-litre plastic bucket three-quarters full of water and with the lid propped partially open (10 cm) with a clothes peg. A cloth bag of 300 g of fresh cow manure is added; rocks placed in the bag ensure it stays submerged. An Altosid tablet is added to the water. Sixteen ovitraps were placed in the shade at various locations 5 m from a New

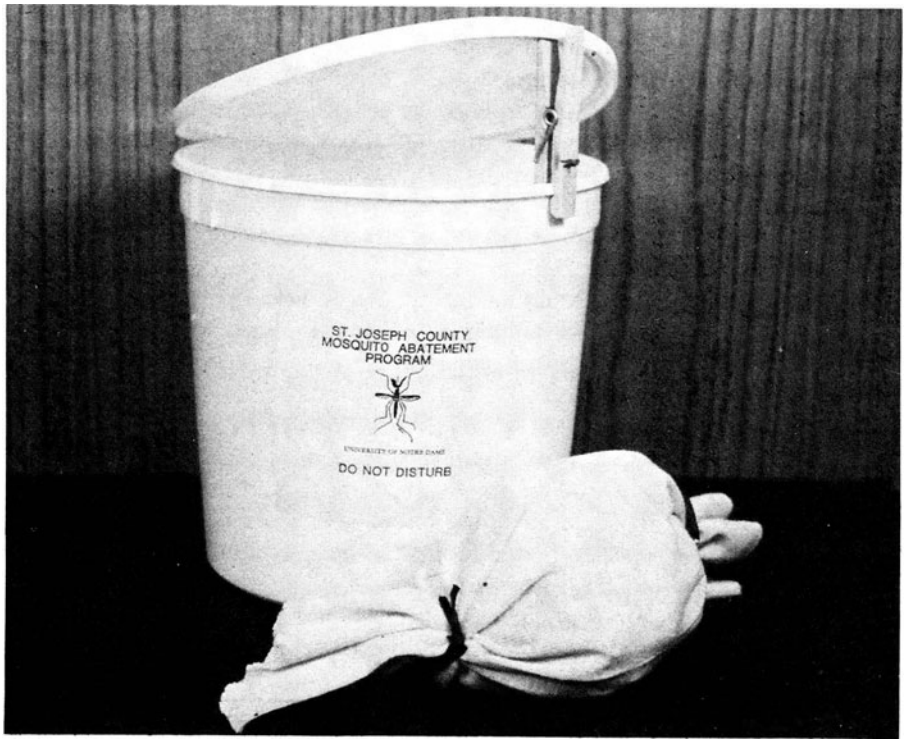

FIG. 1.7. Hoban & Craig (1981) type Culex *ovitrap (courtesy of L. Leiser).*

Jersey light-trap, which operated from 2200–0600 hr daily, from May to October. A total of 365 992 mosquitoes were collected from the light-traps, including 25 232 female and 35 051 male *Culex* spp. At the same time 4193 *Culex* egg rafts of *Culex pipiens* or *Culex restuans* were retrieved from the ovitraps. Both sampling methods showed that *Culex* populations peaked in July, when in a single week the maximum catch was 18 856 adults in the light-traps, and a week later a peak of 960 egg rafts were collected. There was a good positive correlation ($r = 0{\cdot}63$) between the numbers of *Culex* taken in light-traps and egg rafts found in ovitraps for 14 of the 16 collecting stations. It was thought that in the two locations showing a negative correlation this was likely due to a multitude of alternative breeding sites. Leiser & Beier (1982) concluded that although both methods adequately monitor changes in population size, many more ovitraps can be operated than light-traps with the same man-hours. Moreover, ovitraps are less expensive.

In Florida ovitraps have collected egg rafts of *Culex nigripalpus, Culex quinquefasciatus, Culex salinarius* and *Culex restuans* (Haeger & O'Meara, 1983). The relative abundance of such mosquitoes appears to be influenced by seasonal and geographical variations (Lowe *et al.*, 1974; Nayar, 1982; O'Meara *et al.*, 1989*b*) and by the type of trap (O'Meara *et al.*, 1989*b*; Smith & Jones, 1972). In Florida

O'Meara *et al.* (1989*b*) compared two types of ovitraps for attracting ovipositing *Culex* mosquitoes, namely 1-quart (0·95-litre) Kilner (Mason) jars inserted into concrete blocks (19·5-cm cubes). The outside of the jar and its block were painted black, and two jars in their blocks were placed side by side. The other trap consisted of a rectangular (56 × 44 cm and 8 cm deep) plastic tub painted black.

An oviposition infusion was prepared by adding about 2·5 kg of oak leaf litter to a 76-litre container filled with tap water and fitted with a lid. This infusion was left for at least 1 week before being placed in the two types of ovitrap. A few hours before sunset 0·5 litres of the infusion was poured into the jars and 3·8 litres into the plastic tubs; ovitraps were restocked with infusion on each of three consecutive nights a week over a year. Six tubs and six jar-type oviposition traps were placed alternately along transect lines.

From a total of 3720 trap-nights 4540 egg rafts were collected. Significantly greater numbers of egg rafts were recovered from tubs than from jars for *Culex nigripalpus* (7·7 ×), and *Culex restuans* (2·9 ×), but more (1·4 ×) egg rafts of *Culex quinquefasciatus* were recorded from jars than tubs. Although the number of *Culex salinarius* egg rafts collected from jars was also larger (1·2 ×) the difference was not significant. Not only did the type of trap (jar or tub) affect oviposition by *Culex quinquefasciatus* and *Culex nigripalpus* but, whereas the former species showed no preference for ovitraps placed in shaded or unshaded situations, fewer *Culex quinquefasciatus* laid eggs in shaded traps. This emphasises the effect of environmental conditions and trap location.

Gravid traps: *Culex*

In Sri Lanka Samarawickrema (1967) caught gravid females of *Culex quinquefasciatus* from 1800–2000 hr as they alighted on the walls of an open cesspit to lay eggs; but De Meillon *et al.* (1967) appear to be among the first to have constructed a trap to monitor the arrival of gravid mosquitoes—*Culex quinquefasciatus* (p. 29), since then several gravid traps have been designed, including the following.

Surgeoner & Helson trap

In Canada, Surgeoner & Helson (1978) built a trap consisting of an 84-cm diameter plastic inflatable paddling pool, the middle of which was placed in a hole so that when filled with water the depth at the centre was about 23 cm. The pool was lined with sods of earth, and 40 ml *n*-capric (decanoic) acid diluted in 15 ml of ethanol was added to improve the water as a *Culex* oviposition site. In addition about 0·6 litres of water from a nearby highly productive source of *Culex restuans* was added. About every 3 weeks a further amount of 40 ml capric acid was added to the water. A 30-cm metal container, 26 cm high and weighted with stones was placed in the centre of the pool, having the rim of the container about 3 cm above the water (Fig 1.8*a*). A CDC light-trap with the collecting bag replaced by a pint-sized plastic container with two 3 × 2-cm windows and bottom covered with netting was placed on the stones. The lid of the CDC trap was about 6 cm above the water. An altosid briquette was added to the pool water to prevent development of mosquitoes.

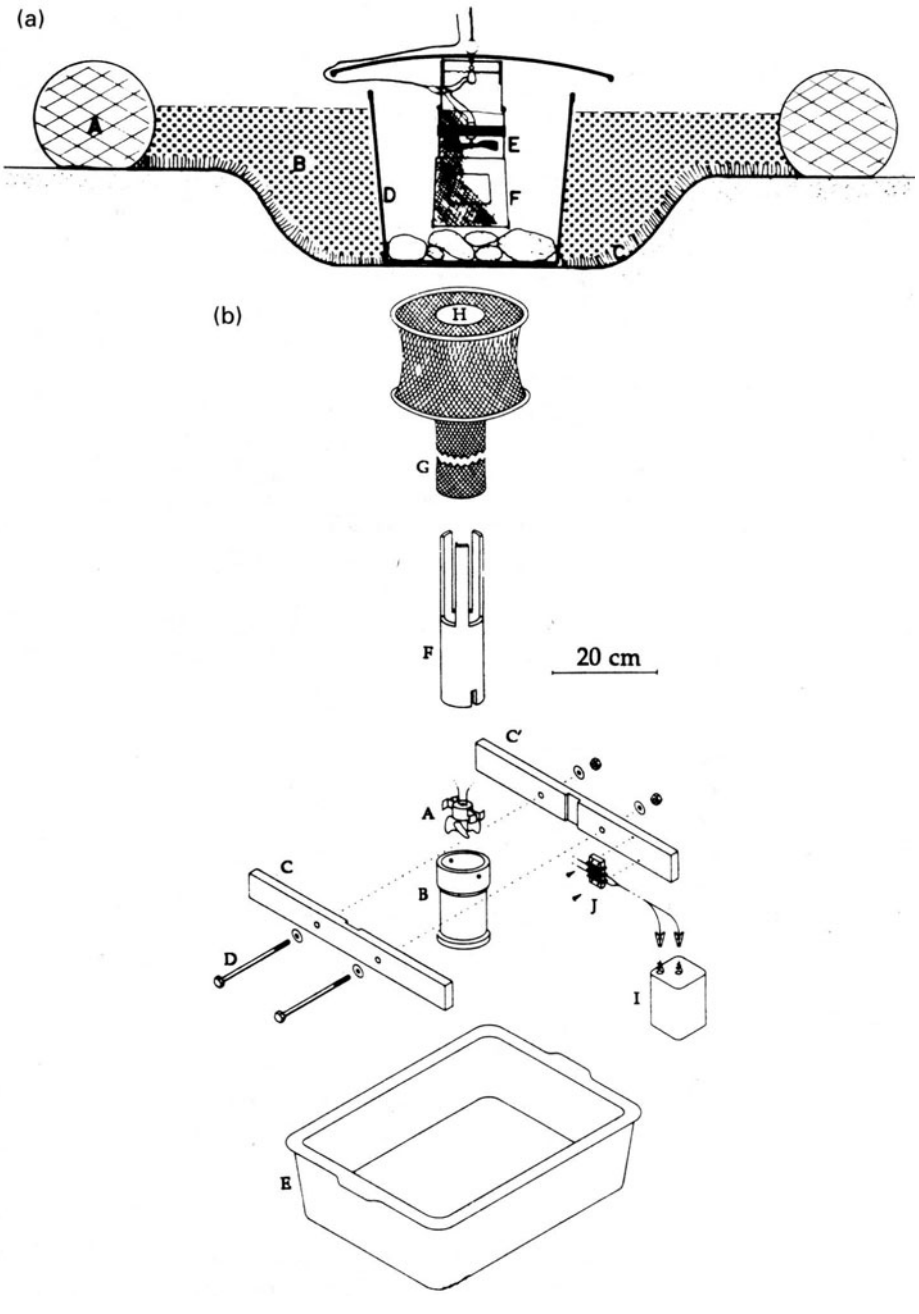

FIG. 1.8. (a) Schematic representation of oviposition trap for Culex *mosquitoes; A — plastic inflatable wading pool, B — water, C — sod, D — metal container, E — CDC trap, F — collection container (Surgeoner & Helson, 1978); (b) Reiter's (1983) gravid trap. A — motor/fan assembly, B — inlet tube, C — & C' — cross bars, F — chimney, G — collecting bag, H — reinforced support for bag, I — 6-V battery, J — connector block.*

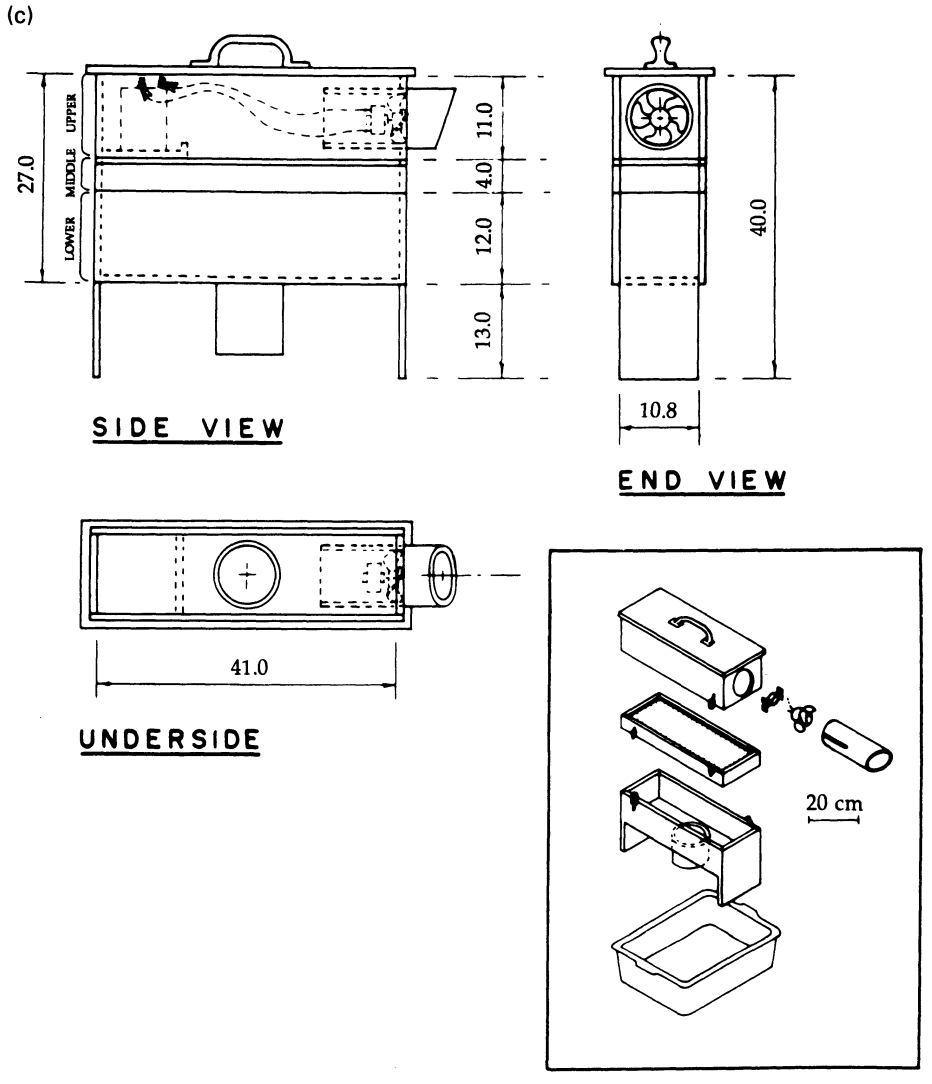

FIG. 1.8 — contd. *(c) Modified Reiter (1987) trap (see text).*

Surgeoner & Helson (1978) compared the numbers of mosquitoes collected in five of these traps, five CDC traps and five cone-traps baited with dry ice. The total numbers of females caught from about 40 trap-nights with percentages of *Culex pipiens* and *Culex restuans* in parentheses were as follows: 1199 (94·7%) in the oviposition traps, 7340 (37·4%) in the CDC traps, and just 387 (72·4%) in the carbon dioxide traps.

Although the CDC traps caught more *Culex* adults (2748) than the oviposition trap (1136) the authors believed that the former caught substantially higher proportions of nulliparous mosquitoes, and that together with the tedium of sorting the *Culex* from other mosquitoes meant that the more selective oviposition trap was better for catching *Culex* for virus isolation studies. The

oviposition trap also caught a few *Culiseta inornata, Aedes vexans*, and *Aedes triseriatus*. This trap has, however, been largely superseded by the Reiter oviposition traps (Reiter 1983, 1987; Reiter *et al.*, 1986).

Reiter's gravid traps

Frequently mosquitoes caught in light-traps with or without carbon dioxide, are predominantly nulliparous (Magnarelli, 1975; Morris & DeFoliart, 1971), so the probability of collecting infected mosquitoes in arbovirus studies is relatively small. This can be overcome by employing traps that catch gravid females, and several such traps have been designed (De Meillon *et al.*, 1967; Lewis *et al.*, 1974; Surgeoner & Helson, 1978). However, these traps are not very portable and for these reasons Reiter (1983) developed what has become known as the 'gravid trap'.

His original trap consists of a 3-in diameter PVC inlet tube housing a 6-V d.c. motor, as used in CDC traps, on which is mounted a four-bladed 3-in counterclockwise fan. (Alternatively an upward flow of air is produced by reversing the terminals of a CDC fan and motor, but this eliminates the aerodynamic efficiency of the fan and specimens may be damaged.) The inlet tube is clamped between two vertical wooden boards that fit over a black plastic box (18·5 × 14·0 × 6·5 in). A plastic 12-in long PVC chimney slots into the upper end of the inlet tube. The top half consists of three struts as shown in Fig. 1.8*b* which fit into a netting collecting bag and supports it. For this the middle of the collecting bag is reinforced with a circular patch of denim cloth. The oviposition attractant is made by adding 1 lb of hay and 1 oz each of dried brewer's yeast and lactalbumen powder to 30 gal of tap water. This infusion is allowed to mature for 5 days. Traps are placed in position 1 hr before sunset, and trapped mosquitoes removed the next morning with an aspirator. New oviposition media are used for each trap-night.

From 203 trap-nights in Memphis 28 690 *Culex* mosquitoes were caught (141·3/trap-night) which was much more than caught in New Jersey light-traps (0·33 *Culex*/collection). At least 90% of the mosquitoes caught in the gravid trap were gravid, and at least 80% were alive. Egg rafts were rarely found on the attractant media, showing the efficiency of the trap in sucking up ovipositing females.

In Tennessee 135 724 mosquitoes belonging to at least 25 species were collected in CDC gravid mosquito traps of Reiter (1983) in 954 trap-nights (Reiter *et al.*, 1986), of which 98·78% were *Culex pipiens* s.l. and *Culex restuans*, which are important St. Louis encephalitis vectors. These traps also caught reasonable numbers of *Aedes aegypti* (236), *Aedes triseriatus/hendersoni* (251) and *Culex erraticus* (544). The average catch was 142·3 mosquitoes/trap-night. At least 95% of the females were gravid and usually 80–95% were alive when the traps were emptied. This preponderance of gravid mosquitoes should increase the likelihood of catching disease-infected mosquitoes. The gravid traps caught 88 times more *Culex* than were collected by mechanical aspiration of outdoor mosquitoes resting in culverts, and underground shelters, and 96 times more *Culex*/man-hour. One operator can service at least 20–30 gravid traps/day compared to just 8–10 resting sites.

Reiter (1986) described a routine for making an oviposition attractant for *Culex* and several other raft-ovipositing genera. The equipment consists of two tapered 120-litre plastic dustbins (garbage cans) stacked one in the other, with the top bin retaining its lid. The inner top dustbin has numerous 0·6-cm diameter holes drilled in the bottom, while the outer dustbin has a tap towards the base and is mounted on a 4-wheel dolly. Grass-hay (0·5 kg) and 5 g each of dried brewer's yeast and lactalbumen and 114 litres of water is put in the inner dustbin and left to mature for 6 days. The inner dustbin is then hoisted out by an overhead pulley system while the bottom dustbin with the attractant oviposition water can be rolled up into the back of a pick-up truck and transported to field sites. The oviposition trap consists of a black plastic 'tote-box' (47·0 × 35·6 × 16·5 cm) containing 4 litres of oviposition water. Egg rafts subsequently collected from these oviposition traps are placed individually in the 24 wells of a plastic tissue culture plate, which is covered with a plastic plate lid. Several plates are stacked together and transported to the laboratory, if necessary in a cool box. First instar larvae hatching in the wells are identified to species. Later Reisen & Meyer (1990) laboratory- and field-tested eight different potential oviposition attractants for *Culex tarsalis*, namely tap water, a slightly modified Reiter (1983) medium, the modification proposed by Ritchie (1984*b*) of adding isopropanol to Reiter's (1983) medium, leaf infusion, alfalfa infusion, steer manure infusion, and water which had contained either larvae or pupae of *Culex tarsalis*. The gravid traps of Reiter (1983) were baited with these solutions, and in addition sod-baited traps of Maw & Bracken (1971) were evaluated. It was concluded that none of these attractants was of any use for trapping field populations of gravid *Culex tarsalis*, although in the laboratory there was some indication that steer manure was somewhat attractive. They also reported that the numbers of egg rafts of *Culex quinquefasciatus* collected per trap were very variable and seemed to be strongly influenced by the numbers of natural competitive oviposition sites, as well as by trap placement. This emphasises the importance of trap location in sampling.

In Sri Lanka Jayanetti *et al.* (1988) baited Reiter (1983) type oviposition traps with water that had 5 days previously had 250 g alfalfa pellets and 0·2 g yeast added to about 18 litres of water. From a total of 119 trap collections seven species of mosquitoes were caught, but *Culex quinquefasciatus* (83%) and *Armigeres subalbatus* (16%) comprised most of the catch. The mean numbers trapped per night was 32·17 *Culex quinquefasciatus* and 6·83 *Armigeres subalbatus*, of which 95 and 77% respectively were gravid females. The authors considered that in terms of collecting effort and cost, the gravid traps were much more effective than catching mosquitoes indoors with aspirators, especially as *Armigeres subalbatus* is partially, or mainly, exophilic.

Using basically the Reiter gravid trap Ritchie (1984*b*) in Florida suspended a CDC light-trap over a 29 × 34 × 12-cm deep brown plastic pan containing 6 litres of three different oviposition attractants. The basic solution was produced by adding 0·9 kg hay, 10 g brewer's yeast and 114 litres of water to a bucket and leaving it covered to mature for a week. The other attractants consisted of a 2:1 mixture of this hay infusion and industrial grade isopropyl alcohol, and

isopropyl alcohol without the hay infusion. With traps having just isopropyl alcohol the mean catch of *Culex* mosquitoes, predominantly *Culex nigripalpus*, was 168·8, the hay infusion trap caught a mean of 227·7 *Culex*, while when a mixture of both attractants were used a mean of 405·2 *Culex* mosquitoes were trapped. These increases were accompanied by increases in the numbers of gravid females collected. In paired trials, while a carbon dioxide-baited CDC light-trap caught almost 5 times as many *Culex* as the hay-isopropyl infusion trap ($\bar{x}$ of 1562·7 vs 335·1) the latter collected almost 50 times ($\bar{x}$ 3·0 vs 147·6) as many gravid females, and moreover the parity of unfed females was almost double that recorded from the carbon dioxide light-traps.

In trials in California Reisen & Pfuntner (1987) reported that surprisingly the gravid trap of Reiter (1983) performed poorly, in catching only a mean (% of total catch in parentheses) of 2·7–16·0/trap-night (0–13%) of *Culex quinquefasciatus* in an area where considerable numbers of adults were caught in carbon dioxide traps. In fact the mean number of gravid *Culex* was greater from collections from walk-in red boxes (3–7) than in their gravid traps (0·4). This contrasts with reports of Reiter *et al.* (1986) of 142·3 females/trap-night, 95% being gravid, and Ritchie (1984*b*) of 405·2 females/trap-night of which 57% were gravid.

Reiter's redesigned gravid trap

The gravid mosquito trap of Reiter (1983) suffers from certain limitations, namely up to 10% of the catch of adults is damaged by passing through the fan blades, and adults tend to die of desiccation. Moreover, ants, racoons and birds can inflict damage on the mosquitoes or traps. Reiter (1987) therefore redesigned the trap to consist of a rectangular box 41 cm long, 27 cm high and 13 cm wide made of 1·1-cm plywood. The trap is composed of an upper, middle and lower compartment (Fig. 1.8*c*) held together with suitcase latches. The upper part is 11 cm high and has a carrying handle screwed on the top, and a small shelf inside on which batteries are placed. The 4-cm high middle compartment has an 8 mesh/cm screen fastened across the entire top. The lower compartment, 12 cm high, has solid ends that extend down 13 cm and support the trap when it is not resting on the oviposition pan. A 6-V d.c. CDC-type motor and a 4-bladed 7·6-cm diameter fan is mounted in a bracket that fits into a 9-cm slot cut in an 18-cm length of 7·6-cm diameter PVC tubing. This tubing projects from a hole in the end of the top compartment, and the end is cut obliquely to prevent rain entering the trap (Fig. 1.8*c*).

A 6-V battery placed on the shelf in the top compartment operates the fan and draws air up through a length of PVC tubing that projects 10 cm below the floor of the bottom compartment. (A commercial form operates from a 12-V battery or two 6-V batteries connected in series, or has a transformer allowing operation from 120-V a.c. power.) Oviposition attractant is added to the black plastic pan which measures 47·0 × 36·0 × 16·7 cm (Reiter, 1986). The distance between the surface of the oviposition medium and the air inlet tube should be 5 cm. At the end of the collecting period a cover is placed over the inlet tube and the top compartment is removed. Dry ice or an anaesthetic is placed on the

screen top of the middle compartment to anaesthetise or kill the catch. The two compartments are then tipped upside down and mosquitoes that have fallen onto the screen are removed. From 716 trap-nights 49 471 mosquitoes (mean of 69·1/trap-night) were collected, and in most collections at least 95% were gravid females. The condition of the specimens was good, and few died even when the trap was left in the field for 2 days. Provision of cotton wool soaked in sugar water in the lower compartments prolongs survival.

Bamboo pots

Water-filled sections of bamboo, usually termed bamboo pots or cups, have commonly been used as artificial oviposition sites to attract mosquitoes that breed in tree-holes and bamboo (Bang *et al.*, 1979; Causey & dos Santos, 1949; Corbet, 1963, 1964*a*; Dunn, 1927; Galindo *et al.*, 1951, 1955; Harris, 1942; Harrison *et al.*, 1972; Laarman, 1958; Lambrecht & Zaghi 1960; Lounibos, 1979, 1981; Petersen & Willis, 1971; Philip, 1933; Sempala, 1983; Service, 1965, 1970; Yates, 1979). Suitable bamboo may not grow in areas in which the pots are to be used. In the dry savanna areas of Nigeria bamboo pots 'imported' from the rain forests of the south split in the severe dry season. It was discovered, however, that cylindrical gourds of *Lagenaria siceraria* did not crack, even when exposed to direct sunlight, and when used instead of bamboo pots they accurately reflected the mosquito species breeding in tree-holes (Service, 1965). In Zaire Laarman (1958), however, found that bamboo pots did not always give a true picture of the species breeding in tree-holes. For example, larvae of *Toxorhynchites brevipalpis* and *Culex albiventris* were rarely collected from bamboo pots although they were common in tree-holes. In England, to prevent bamboo pots from splitting, their outsides were coated with embedding wax (Yates, 1974). Furthermore, to obtain the maximum number of pots from a limited supply of bamboo, lengths which were open at both ends had a 5-mm thick piece of cork glued to one end, which was also coated with wax (Yates, 1974).

The conventional method of sampling bamboo pots is to tip out the contents and identify the mosquito larvae, but McClelland (1956) in studying *Aedes aegypti* in Uganda pointed out that *Aedes* eggs might remain undetected on pot walls without hatching for relatively long periods, during which time larval inspections would be negative. He also considered that other factors such as unfavourable conditions in the water, competition with larvae of other species, and possibly selective predation by *Toxorhynchites* larvae might result in high, or complete larval mortality of *Aedes aegypti* before the pots were examined. By inserting a 4¾-in cylinder of filter paper attached to a cellulose acetate sheet into each pot he was able to collect *Aedes aegypti* eggs on the filter paper just above the water line. Corbet (1963) dispensed with the cellulose sheet and lined the insides of bamboo pots with filter paper which was dyed grey (30 ml black 'Pelikan' waterproof ink in 4 litres water) to make the surface more attractive as an oviposition site. In Tanzania, Trpis (1972) used bamboo pots lined with paper towelling, but in addition introduced a 20 × 120-mm hardboard 'paddle' in the pots, which were placed in different ecological areas and also at different heights. *Aedes* eggs were laid on both oviposition surfaces. Williams (1962) and

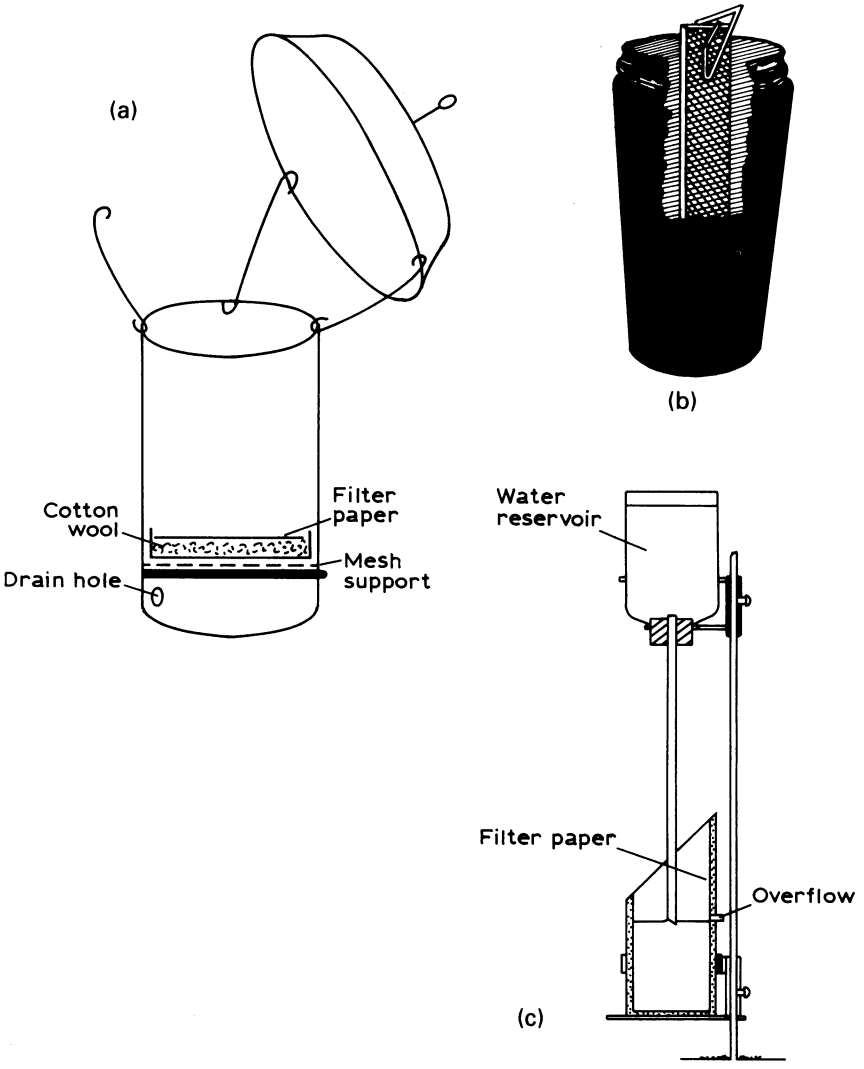

FIG. 1.9. (a) Aedes aegypti *oviposition trap of Weinbren & O'Gower (1966); (b)* Aedes aegypti *black glass jar ovitrap; (c) bamboo pot lined with paper as oviposition surface, and with water reservoir above (Yates, 1974).*

Wilton (1968) had shown in laboratory studies that the tree-hole mosquito *Aedes triseriatus* lays more eggs on dark than light coloured surfaces. Pink or green blotting paper can be substituted for dyed filter paper. Many, if not most, *Aedes* species apparently prefer to lay their eggs in cracks and crevices or at least on rough, in preference to smooth, surfaces (Beckel, 1955; Dunn, 1927; Fay & Perry, 1965; O'Gower, 1955, 1957, 1958, 1963; Penn, 1947; Wallis, 1954). Under laboratory conditions *Aedes triseriatus* laid more eggs on paper towelling that

had been embossed with a pattern from a 16-mesh hardware cloth than on smooth towelling (Wilton, 1968). It is therefore appropriate to line bamboo pots with paper having an embossed surface. This is particularly important in studies on the distribution of eggs in relation to the water level in the pots, because if smooth paper is used nearly all *Aedes* eggs are deposited along the vertical edges where the paper overlaps.

A useful technique for using bamboo pots as oviposition sites has been developed by Yates (1974) working in England. Sections of bamboo are cut across obliquely at an angle of about 50° (Fig. 1.9*c*) because it was found that more eggs of *Aedes geniculatus* were laid in these pots than those having a horizontal opening. Such oviposition preferences may not be shown by other tree-hole species. When blotting paper or filter paper is left in water-filled pots for any length of time it usually becomes difficult to remove without tearing or disintegrating. Yates overcame this by using 'laboratory bench paper' which is commercially available in large sheets (e.g. 'Benchkote') and consists of absorbent white filter-type paper backed with a thin sheet of plastic paper. This is dyed grey in an alcoholic solution of black drawing ink (1 part 'Pelikan' ink:50 parts water); then the wet paper is passed through a domestic mangle which has been modified by slipping a cylinder of hard wire mesh over one of the rollers to give an embossed pattern on the paper. Before placing the paper lining in the pot it is dried and alcohol removed by placing it in an incubator. The paper linings are numbered, placed in the bamboo pots in the field and can be replaced at regular intervals.

To study the vertical deposition of eggs in relation to the water line a constant water level must be maintained in the pots. Yates (1974) achieved this by making a water reservoir from an inverted polythene bottle. A length of 14-mm diameter glass tubing is placed through a rubber bung inserted into the neck of the bottle, and its lower end cut at 45°. A 13-mm hole is drilled through the bamboo pot and paper lining at the required water level and a short length of tube inserted. This serves as an overflow. With this arrangement a drop of only 2 mm in water level is compensated by water descending the glass tube.

Yates (1979) made good use of his bamboo pots in England to study oviposition behaviour of *Aedes geniculatus*. In summary pots were fixed to trees at heights, 0·5, 2·0, 4·0 and 8·0 m and to a tower at 9 heights, from ground level to 11·2 m at 1·4-m intervals. Regression coefficients (*b*) of the linear relationships between height and $\log_e$ (eggs + 1) were calculated. Because regression coefficients varied between individual trees, two estimates of the overall slope were made. Firstly a weighed overall estimate was made by averaging the regression estimates from all trees with the reciprocals of their estimated variance being used as weights. The other estimate was obtained after the number of eggs laid in the pots was adjusted for differences in surface area available (Fig. 1.10*a*) for oviposition due to slightly different-sized pots. Corrected egg numbers were then transformed and plotted against height. Pots of similar shape and size were used on the tower, so allowing the number of eggs in pots at the same height to be pooled and the resulting total egg numbers to be transformed to logs and plotted against height (Fig. 1.10*b*).

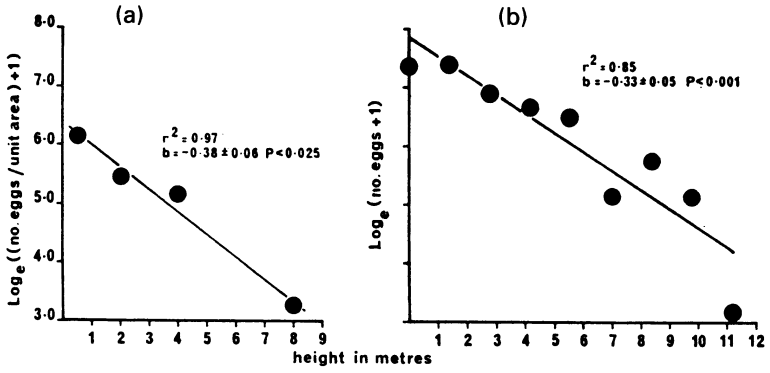

FIG. 1.10. The vertical distribution of Aedes geniculatus *ovipositing in bamboo pots as shown by the relationship between (a) $\log_e$ ((no. of eggs/unit area of oviposition surface) + 1) and height in 1972; and (b) $\log_e$ (no. of eggs + 1) and height in 1973 (Yates, 1979).*

In order to get the seasonal incidence of oviposition the numbers of eggs laid on the linings of the bamboo pots were standardised to correct for differences in the length of exposure. This was done by calculating the number of eggs per day for each period and then expressing this number as a percentage of the yearly total. By replacing the paper lining in the pots at regular, though variable, intervals Yates (1979) found that 97·8% of the eggs (1663) were laid during the daytime, with peak oviposition being 2–3 hr before sunset.

In Nigeria Bang *et al.* (1979) found that the numbers of *Aedes (Stegomyia)* species collected in bamboo cups nailed to trees at a height of 1·5 m in rural habitats were much greater than found in tin-can type ovitraps nailed alongside them. They also found that all eggs of all species hatched on three soakings in April (during the rainy season), but in November during the dry season three soakings produced only the following hatches, 8% *Aedes bromeliae*, 27% *Aedes dendrophilus*, 53% *Aedes luteocephalus*, 60% *Aedes africanus*, 61% *Aedes apicoargenteus* and 94% *Aedes aegypti*. At least nine repeated soakings were required to cause all eggs to hatch, and consequently to enable the true proportions of the species ovipositing in the ovitraps to be determined.

Before pots are used to sample the local population of mosquitoes they should be matured for 1–2 weeks in the field by filling them with filtered tree-hole water or rain water and adding a few dead leaves. If this conditioning is omitted they may be unattractive when they are first used (Harris, 1942). In a yellow fever vector survey in southern Nigeria CDC-type ovitraps (Fay & Eliason, 1966) and bamboo cups, which had been weathered for at least 4 weeks and boiled, were set up in six vegetation types. The commonest vector species was *Aedes aegypti* and about the same percentage of bamboo pots and ovitraps were positive in the different six vegetation zones (Bown *et al.*, 1980).

Paper linings in bamboo pots can be changed at regular intervals and the numbers of eggs counted and identified. When specific identification of the eggs is impossible the linings must be repeatedly soaked and the resultant larvae iden-

tified. Larval identification is usually necessary when pots are used without linings and eggs are deposited on the pot walls. Some of the difficulties associated with interpreting oviposition results by larval identification have been outlined on pp. 9 and 21. In Thailand Harrison *et al.* (1972) used unlined pots. After exposure in the field larvae were removed from the pots which were then returned to the laboratory and soaked in water and the numbers of larvae hatching recorded daily. To prevent extraneous oviposition the pots were covered with paper towels. Before pots were returned to the field they were cleaned with a wire brush, sandpaper and boiling water to remove any residual unhatched eggs.

Certain species, such as some of those belonging to the genera *Haemagogus, Sabethes* and *Armigeres*, oviposit in closed sections of bamboo that have a small hole in the side, usually made by certain beetles or birds. To attract ovipositing females that lay eggs in these habitats bamboo pots have a small hole bored in the side near the top which is covered by a lid (Carpenter *et al.*, 1952; Galindo *et al.*, 1951, 1955). The pots are sampled by removing the top and tipping the contents into a white enamel bowl. These pots could also be lined with filter paper so long as the entrance hole was not obscured. More recently in Sri Lanka Amerasinghe & Alagoda (1984) compared mosquito oviposition in two types of bamboo pots (45–50 cm long, 10–12 cm in diameter), one having the top open, and the other having the cut end covered with a removable piece of hardboard, and a 2-cm hole bored in the side for entry of ovipositing mosquitoes. Coarse blotting paper dyed grey was placed inside the pots. In some pots the modification devised by Yates (1974) was used to maintain a constant water level, so that the height eggs were laid above the water surface could be obtained. Traps of both types were placed at ground level and at heights of 3·5 and 7·0 m. The most common species were *Aedes albopictus, Armigeres subalbatus, Aedes novalbopictus* and *Culex quinquefasciatus*. The two *Aedes* species exhibited a clear preference for ovipositing in traps with open tops, the preference was not quite so marked with *Armigeres subalbatus* and *Culex quinquefasciatus* showed little choice between the two types of traps.

In southern Africa over 6 years a total of 19 species were collected from bamboo pots (Jupp & McIntosh, 1990). The most common species were *Aedes aegypti, Aedes ledgeri, Aedes metallicus, Aedes fulgens, Culex nebulosus* and *Culex horridus. Aedes furcifer/cordellieri* were known to be common in the area, but few were collected from bamboo pots during 1976–1977 and 1977–1978 (3–4% only of pots positive) or bottles in 1980 (1%). It was thought that this might have been because the openings were too large, and this supposition was substantiated when the openings of the bamboo pots were made smaller in 1980 and 1981, and the percentage of samples with *Aedes furcifer/cordellieri* increased to 14 and 48%, respectively. This agrees with findings in Senegal that *Aedes furcifer/cordellieri* prefers to oviposit in tree-holes with small openings (Raymond *et al.*, 1976).

The horizontal and vertical distribution of different species can be studied by placing bamboo pots, or cylindrical gourds, lined with paper in different ecological zones and at different heights (Bang *et al.*, 1979; Corbet, 1964*a*; Causey &

dos Santos, 1949; Galindo *et al.*, 1951; Harris, 1942; Laarman, 1958; Lounibos, 1979, 1981; Service, 1965; Surtees, 1959; Yates, 1979). Seasonal incidence and diel periodicities of egg laying can be investigated by regularly replacing the oviposition papers in pots. Alternatively the pots can be covered with lids, a few of which are removed each hour throughout the 24-hr day (Corbet, 1964*a*; Lambrecht & Zaghi, 1960). It will be more difficult to use bamboo pots to measure or compare the population size of mosquitoes in different areas or habitats, because the incidence of egg laying in the pots not only depends on population size but also on the number of alternative natural oviposition sites that are available. This difficulty is not limited to the use of bamboo pots, but is inherent in most types of sampling programmes where artificial habitats are created.

Tree-hole ovitraps

Loor & DeFoliart (1969) used an oviposition trap made from a beer can to detect the presence of the tree-hole mosquito *Aedes triseriatus*. The top of a 12-oz beer can was removed and its outside covered in beige coloured masking tape. The can was then filled to 1 in of the top with distilled water. The relative attractiveness was evaluated of cans containing: (1) only water; (2) water plus organic debris consisting of 75% dry and 25% green oak leaves; (3) a black muslin sleeve lining the interior; and finally (4) organic debris and a black muslin sleeve. Cans were attached to trees at heights of 2·5 and 5 ft. They were examined weekly and organic debris and the sleeve removed and the eggs counted. Of the total of 2394 eggs of *Aedes triseriatus* that were recovered 69% were from cans with organic debris and the black muslin sleeve, and 26% from cans with only the black sleeve. A few eggs of *Orthopodomyia signifera*, a species which normally breeds in tree-holes, were also collected from the cans.

Because of difficulties in locating sufficient numbers of easily accessible natural tree-holes of the same type and size in their studies in California Lewis & Tucker (1978) made artificial ones based on an earlier model (Lewis & Christenson, 1975). Traps were made of ¾-in thick wooden boards about 8 × 10 or 10 × 12-in cut and double bevelled at 45° angles. To prevent water leakage silicone seal ('silicone glue and seal', or 'silicone chalk and seal') were used between the joints as a gasket. Two lengths of 4·76-mm diameter galvanised steel wire with each end formed into a loop were passed round the assembled boards and tension obtained by tightening the nuts on bolts passed through these loops. A piece of plywood was then screwed onto the bottom of the trap. Another similar piece of plywood with a 2-in diameter hole cut from the middle was hinged to the top with a length of webbed strapping, and the trap kept closed except when samples were being removed. Water and alfalfa pellets were added to the traps as necessary. Oviposition paddles (ovisites) consisted of 2 × 3-in pieces of wood abutted with cork strips over which thin grooved layers of balsa wood were stapled. Adjustments were made of the amount of cork to ensure the ovisites floated on the water surface and the balsa wood remained moist but not covered with water. Ovipositions were removed weekly. These traps proved attractive to both *Aedes sierrensis* and *Orthopodomyia signifera*.

During the 1980s there were several ecological studies on *Aedes triseriatus*,

many using various ovitraps, some of which are described here. Beier & Trpis (1981) used the ovitraps of Loor & DeFoliart (1969) to monitor *Aedes triseriatus* breeding at the Baltimore Zoo. They concluded that ovitraps competed with natural tree-holes as oviposition sites, because fewer eggs were collected from traps placed near beech trees with water-filled holes, than those placed near beeches lacking holes. This and the fact that mature woods of large beech trees have most tree-holes will affect the numbers ovipositing in ovitraps. Clark *et al.* (1986) also used the ovitraps of Loor & DeFoliart (1969) but lined them with black flannel to collect eggs of *Aedes triseriatus*.

Kitron *et al.* (1989) used 12-oz lidless aluminium tins painted black on the outside as oviposition traps for *Aedes triseriatus*. They were half-filled with oak leaf infusion, provided with an overflow hole, and attached to the bases of trees. Even weekly topping up failed to prevent the traps sometimes drying out between weekly inspections. Balsa strips, as advocated by Novak & Peloquin (1981), 2·5 cm wide and 15 cm long served as oviposition paddles, they were attached to the can with a 'binder clip' to minimise damage from animals. Ovitraps of Novak & Peloquin (1981) were used by Walker *et al.* (1987) to collect *Aedes triseriatus* and *Aedes hendersoni* in Indiana ovipositing at three different heights. Paddles were soaked twice in water and 4th instar larvae identified.

In Texas Aziz & Hayes (1987) placed 400-ml plastic beakers lined with paper towels and filled with 300 ml of a mixture of tree-hole and rainwater at heights of 0·6, 1·2, 1·8, 2·7 and 3·7 m in trees to collect eggs of *Aedes triseriatus*. Tongue depressors wrapped in paper towelling and towelling lining the beakers served as oviposition sites. Although eggs were obtained at all heights most were collected from the lower ovitraps (0·6–1·2 m).

In Nigeria, Dunn (1927) found that tin cans were much less attractive to *Aedes aegypti* and other tree-hole species than bamboo pots, but apart from the addition of a few leaves no attempt was made to make the cans more attractive to gravid females. Because in many areas tin cans are more readily obtained than bamboo pots, it would consequently be worthwhile assessing the effectiveness of suitably prepared tins cans as artificial breeding sites for tree-hole mosquitoes, much as has been done for *Aedes triseriatus* in the USA. Size can be an important factor as shown in Indiana where Hanson *et al.* (1988) found that large metal can ovitraps—3100 ml capacity 18 cm tall, 16 cm in diameter and painted black—collected 3·19 times more eggs of *Aedes triseriatus* per positive trap than smaller traps—350 ml, 12 cm tall, 6·5 cm in diameter—and moreover 4·86 times as many of the larger traps were positive.

Weinbren & O'Gower (1966) constructed an ovitrap from a 4¼-in diameter, 6¾-in high tin can for studying tree-hole breeding mosquitoes in Puerto Rico. A circular metal pie dish, with sloping sides and having a basal diameter of 5½ in and an opening of 7¼ in, is held some 4 in above the tin by three stout equally spaced wires to serve as a cover (Fig. 1.9*a*). At least one wire support is easily detachable from the cover for access to the contents in the tin. Both the insides and outsides of the tin and cover are painted matt black. Two holes about 2 in apart are punched in the tin 1¾ in from the bottom, and two more holes are punched diametrically opposite. Two pieces of stout wire (e.g. plastic covered

copper wire) are passed through these holes to provide a support for a 4⅛-in diameter platform of very fine mesh. A small quantity of 2-week-old horse manure infusion is placed in the bottom of the can to attract ovipositing females to the traps. To prevent the infusion mixture rising too high in the can a 1/16-in drain hole is drilled in the can 1¼ in from the bottom. Non-absorbent cotton wool is dipped in water and placed in a 90-mm plastic petri-dish and then covered with a circle of coarse paper which has been dyed black ('Tintex' dye). This is the oviposition substrate. The petri-dish is lowered onto the wire mesh screen situated near the base of the tin. An eye-bolt is passed through the centre of the pie dish cover so that the trap can be suspended amongst vegetation.

In Illinois Lang (1990) compared oviposition by *Aedes triseriatus* in 2·8-litre can ovitraps, painted black and containing an oak leaf-litter infusion, having either horizontal (open top) and vertical (side hole) entrances. For the latter type of trap the can was closed with a lid and a 9 × 10-cm hole was cut in the side of the upper part of the can. Drain holes were punched in all traps 8 cm from the bottom. A 7·5-cm wide strip of muslin cloth attached by a paper clip to the rim of the traps and extending into the water served as the oviposition surface. Significantly more eggs were obtained from ovitraps having the standard horizontal top openings.

Beier *et al.* (1982) systematically placed 36 ovitraps made from 350-ml black aluminium cans fitted with a partial lid to keep out rain and debris in a wood to study the spatial distribution of *Aedes triseriatus*. Presoaked balsa paddles (Novak & Peloquin, 1981) were used as the oviposition substrate. Each ovitrap was partially filled with 200 ml of a 1:3 dilution of oak tree stemflow and distilled water. Traps were fixed to trees at a height of 50 m. From 7 weekly collections 13 311 eggs were collected, and based on identification of larvae hatched from eggs it is estimated that 98·4% were *Aedes triseriatus* and 1·6% *Aedes hendersoni*. There was no correlation between the numbers of eggs in the different traps and the numbers of *Aedes triseriatus* collected from the surrounding area by aspirator collections.

In studying oviposition behaviour of *Aedes triseriatus* in 300 ovitraps with balsa wood paddles in Illinois Kitron *et al.* (1989) used several measures to define the dispersion of eggs in ovitraps. They calculated: (i) prevalence, that is proportion of ovitraps with eggs; (ii) mean intensity, that is mean number of eggs per positive ovitrap; (iii) mean density (*md*), which is the product of prevalence and mean intensity (or total eggs in traps divided by number of traps with eggs); (iv) Lloyd's (1967) mean crowding (*mc*), which can be calculated as mean density (*md*) plus the variance (var.) divided by mean density (*md*) minus one, thus $mc = md + \text{var}/md - 1$, and (v) patchiness, which is mean density (*md*) divided by mean crowding (*mc*). The regression of mean crowding on mean density (Iwao, 1968, 1970) was plotted to separate the effect on aggregation of numbers of eggs per oviposition and dispersion of oviposition events among the ovitraps. The intercept measures the numbers of eggs per oviposition and is zero when a single egg comprises an oviposition. The slope measures the degree of aggregation of oviposition events and equals 1 when the distribution is random.

They found that most eggs were deposited on balsa paddles without eggs and

not on paddles with eggs that had been returned to the ovitraps. The dispersion pattern was highly aggregated, so some traps had many eggs whereas many had none. Frequently the numbers of ovipositions per trap could be fitted to a negative binomial distribution. Non-random, but selective, oviposition occurred not only spatially within weekly samples but also temporally among weekly samples. Ribeiro, Mather & DeFoliart (see Kitron *et al.*, 1989) found that the dispersion pattern of eggs among ovitraps fitted a logarithmic distribution and oviposition events were distributed spatially in a multinominal fashion among the traps.

Whereas in laboratory experiments 80–130 eggs were laid by single *Aedes triseriatus* (Mather & DeFoliart, 1983), eggs appeared to be laid in ovitraps in clumps of 29–47, suggesting that gravid females scatter their eggs in 2–4 ovitraps (Kitron *et al.*, 1989).

In Florida Mortenson *et al.* (1978) used conventional glass jar ovitraps, with hardboard paddles, fixed to trees to monitor the tree-hole species *Aedes sierrensis*. The tops of the jars were covered with ¼-in screening fitted to a Kilner (Mason) jar screw-cap ring to exclude rodents. Sometimes up to 81·3% of the ovitraps were positive after a week's exposure, the maximum number of eggs in a single trap was 495, recorded in late May.

Landry & DeFoliart (1987) wanted to age-grade female *Aedes triseriatus* by ovariole dilatations, after they had laid eggs, so they designed an ovitrap that retained females after oviposition. Their trap is illustrated in Fig. 1.11*a* and consists of a 20·4-cm length of 10·2-cm diameter PVC tubing (A) closed at the bottom with a circular piece of plexiglas (B) stuck on with ethyl dichloride. A plywood (1·3-cm thick) ring (C) having an outer diameter of 10 cm and an inner diameter of 5 cm, and with a 60° slit cut through to accommodate an oviposition paddle (D), was positioned on a ring (E) made of the original PVC tubing with a 2·5-cm piece removed so that it could be glued inside the trap body 6·35 cm from the bottom. This ring-shaped platform served as a resting site for mosquitoes. Eight overflow holes (F) were drilled below this supporting ring. Three holes (G) (2·54-cm diameter) were spaced around the body to allow insertion of an aspirator to remove adults trapped after oviposition, these are normally plugged with rubber stoppers. The entire trap is painted black except for the underside of the plywood platform, which was painted white to make eggs more easily detectable. Gravid mosquitoes enter through the lid (H) made from a PVC pipe-coupler (10·2-cm diameter) and pass through a funnel (I) of metal mosquito screening secured to it by a plywood platform support (similar to part E) inside the screen funnel (J), and attached to the coupler with screws. The stem of the funnel (2·54 cm diameter) was positioned 1·9 cm above the overflow holes. A metal ring (K) was welded to a lag-screw which can be screwed into a tree, and the trap slipped through the metal ring to rest on the three rubber stoppers.

These traps were baited with either oak infusion water made by adding oak leaves to distilled water in the bottom of the trap, or from a laboratory stock of oak leaf water, or were filled with filtered tree-hole water.

A total of 1 715 ovipositing *Aedes triseriatus* were collected over 4 years from 149 traps, which were checked three to five times a week. Live adults were

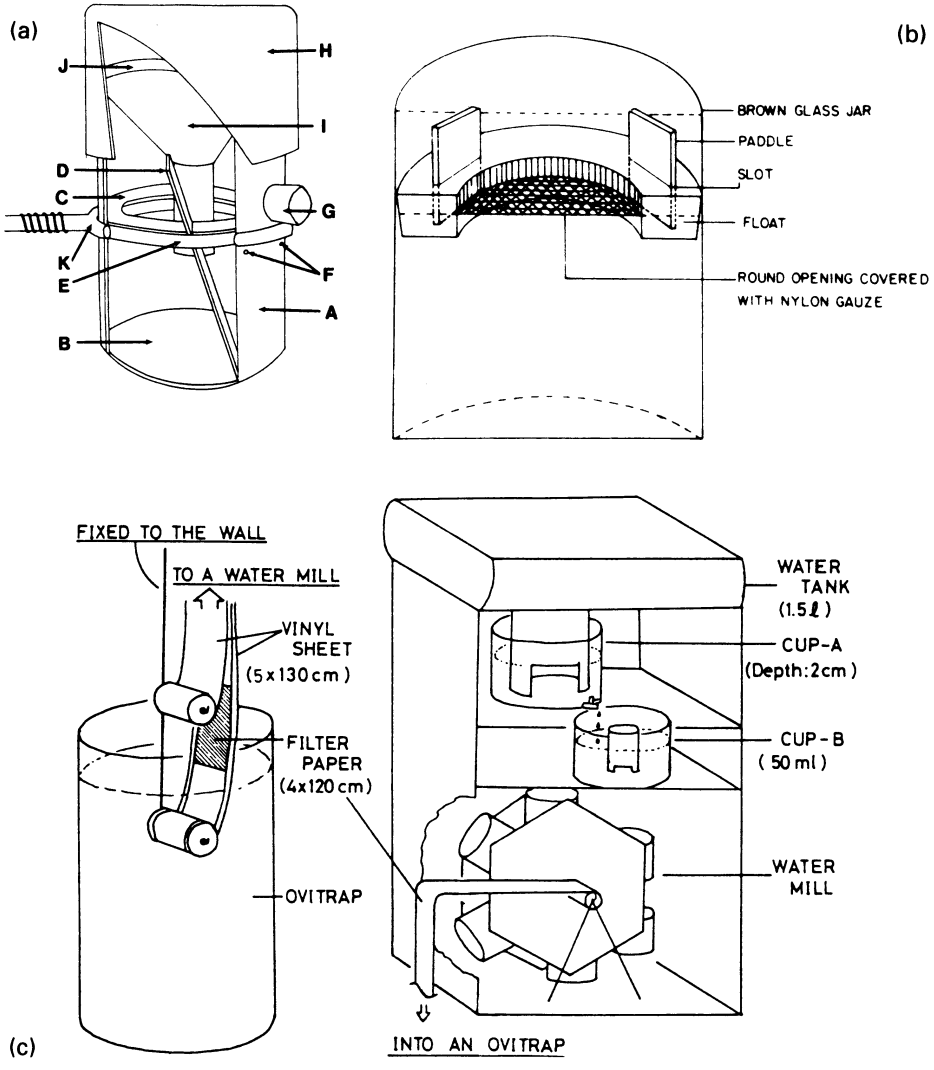

FIG. 1.11. (a) Female-retaining Aedes *ovitrap. See text for explanation of parts (Landry & DeFoliart, 1987); (b) autocidal* Aedes aegypti *ovitrap (Cheng* et al., *1982); (c) automatic recording ovitrap, the shaded part of the filter paper is the part that becomes exposed to ovipositing females for 1 hr (Tsuda* et al., *1989).*

removed with aspirators and kept for at least 28–30 hr to allow ovariole sacs to contract and for the formation of dilatations. Many mosquitoes escaped from the traps after oviposition, and a number were found with eggs but no adults. Uniparous adults formed 79·9–92·7% (mean 84·1%) of the trapped mosquitoes, means of 13·2% were 2-parous, 2·1% 3-parous, 0·5% 4-parous and 0·1% 5-parous. These female-retaining ovitraps were used later by Landry *et al.* (1988) to collect ovipositing *Aedes triseriatus*.

Schuler & Beier (1983) from a grid of ovitraps in a wood found that the eggs of *Aedes triseriatus, Aedes hendersoni, Toxorhynchites rutilus rutilus* and *Toxorhynchites brevipalpis* were highly aggregated in certain areas. Later Beehler & DeFoliart (1990) studied the spatial dispersion of *Aedes triseriatus* eggs in ovitraps in a wood. Using Taylor's (1961) power law they calculated *b* as 1·4, which indicates a clumped distribution of eggs among traps. Lloyd's (1967) mean crowding index was calculated and regressed against the mean, and a slope of greater than one was obtained, again indicating a clumped distribution. Like Beier *et al.* (1982) they were unable to explain why some traps were more attractive or less attractive. The intercept of the regression line (mean crowding vs mean) of 30·3 suggested that individual eggs are clumped in groups of 31 ± 9·8, which is taken as an estimate of the egg batch size of *Aedes triseriatus*. Beehler & DeFoliart (1990) point out that their estimate is in between estimates of 29·3 ± 16·9 and 46·9 ± 25·3 derived by Kitron *et al.* (1989). They argue that their estimate is likely to be more precise as it was calculated from data obtained from daily, not weekly, sampling as undertaken by Kitron *et al.* (1989). They concluded that despite a contagious distribution of oviposition in their ovitraps, only a few traps were needed for detecting and monitoring populations of *Aedes triseriatus*.

Field trials in Puerto Rico showed that the Weinbren & O'Gower (1966) ovitrap was effective in collecting eggs of *Aedes aegypti*, which was particularly useful as the species was not caught at light or in bait catches. However, in later trials (Haber & Moore, 1973) in the same area *Aedes aegypti* eggs were not collected in these traps when they were baited with either horse manure or rabbit food infusion, but neither were they collected from tyres, bamboo pots nor the *Aedes aegypti* traps of Fay & Eliason (1966). The mosquito was apparently absent from the area, possibly due to changes in the environmental conditions since the previous survey of Weinbren & O'Gower (1966). Eggs of *Aedes mediovittatus, Culex antillummagnorum* and *Culex secutor* were retrieved from glass ovitrap jars, but none of these nor any other species was collected from the Weinbren & O'Gower trap.

Kitching (1972) obtained eggs of the tree-hole breeding chironomid *Metriocnemus martinii*, in southern England by placing glass ovitraps at various heights in beech trees (*Fagus sylvatica*). Each trap consisted of a 9-cm diameter glass jar, 7 cm high and filled to a depth of about 3 cm with distilled water, to which was added wood chips, beech leaves and bark, to produce a bark/water/air interface. Similar traps might be useful for tree-hole breeding mosquitoes.

Rock pools

In Nigeria eggs of *Aedes vittatus* were obtained by covering the walls of small water-filled rock pools with pink blotting paper (Service, 1970). Eggs were also obtained by extracting them from the mud in the bottom of the pools (p. 18).

Glass jars, clay pots etc.

In Samoa Buxton & Hopkins (1927) used artificial test containers to study the factors controlling egg laying in *Aedes pseudoscutellaris* and *Aedes aegypti*. Their artificial containers, or pots as they were called, consisted of glass vessels 15 cm

in diameter and about 10 cm tall, and were half-filled with different types of water. Each oviposition pot was covered with a 4-gal petrol tin, 36 cm high and 24 in square which had the bottom removed and a 7·5-cm diameter hole cut in the top. The pots were inspected at weekly intervals, the *Aedes* eggs removed with a small paint brush, and the water level maintained by adding distilled water. Both the estimated number of egg layings and the total eggs laid by each species in the different pots were recorded. Apparently about 17 000 eggs of *Aedes pseudoscutellaris* and about 15 500 eggs of *Aedes aegypti* were collected from the pots.

In Florida after mass release of *Aedes aegypti* in an otherwise *aegypti*-free area ovitraps comprising twenty 3-litre half-filled buckets lined with filter paper and eleven 55-gal barrels lined with white cloth were set out. Over a 22-day period 96 652 eggs were collected from one release site. At another release site 61 954 eggs were collected from these ovitrap-buckets (Seawright *et al.*, 1977).

Aedes aegypti ovitraps

During the first 3 years of the US *Aedes aegypti* Eradication Program (Schliessmann, 1964), which began in 1964, ovitraps were developed for detecting the presence of *Aedes aegypti* (Fay & Eliason, 1966; Fay & Perry, 1965; Jakob & Bevier, 1969*a*; Pratt & Jakob, 1967). Field evaluations showed that the ovitrap was potentially a sensitive and efficient technique for detecting populations of *Aedes aegypti* (Chadee & Corbet, 1987, 1990; Evans & Bevier, 1969; Fay & Eliason, 1966; Frank & Lynn, 1982; Furlow & Young, 1970; Hoffman & Killingsworth, 1967; Nayar, 1981; Ritchie, 1984*a*; Subra & Mouchet, 1984; Tanner, 1969), even when population densities were low (Jakob & Bevier, 1969*b*). Several improvements and modifications have been made to the ovitrap designed by Thaggard & Eliason (1969).

The construction of the ovitrap, which has been extensively used in America, and to a lesser extent elsewhere, is as follows. Each trap consists of a glass jar painted glossy black on the outside, 3 in in diameter at the top, about 5 in high with tapered sides and having a capacity of about 1 pint. Water to a depth of about 1 in is added to the jar and a ¾-in wide, 5-in long hardboard paddle having a smooth and a rough surface is attached vertically with a paper clip to the inside of the jar (Fig. 1.9*b*). Identification marks can be written on the smooth side of the paddle. Eggs of *Aedes aegypti* are usually deposited just above the water line on the rough side of the paddle which faces towards the centre of the jar. Paddles should be made of the hardboard used for interior decorating as this is more absorbent than the exterior-type hardboard, and thus presents a more suitable oviposition surface (Thaggard & Eliason, 1969). Because of difficulties in obtaining hardboard having the correct absorbent properties, other materials have been evaluated as substitutes (Jakob *et al.*, 1970). After testing more than 50 different materials it was concluded that brown or grey velour paper paddles were about as efficient as hardboard paddles. Jakob *et al.* (1970) found that more than 98% of *Aedes aegypti* eggs were deposited on the face of the velour paddles, whereas only about 81% were deposited on the rough side of hardboard paddles, 19% being laid along the edges of the paddles. O'Meara *et*

al. (1989*a*) used red velour paper paddles of Kloter *et al.* (1983) in glossy black polypropylene plastic jars to collect eggs of *Aedes bahamensis* in Florida. It seems that the choice of using paddles made from hardboard or velour paper is mainly governed by the availability of the materials. Several investigators have used balsa paddles, for example Hanson *et al.* (1988) used balsa wood paddles in their ovitraps for monitoring *Aedes triseriatus* populations, while Kitron *et al.* (1989) attached their balsa strips (15 cm long, 2·5 cm wide) with a clip to black-painted can-type ovitraps to minimise animal damage. Schuler & Beier (1983), Beier *et al.* (1982) and Beehler & DeFoliart (1990) used presoaked balsa paddles in black aluminium cans to collect eggs of *Aedes triseriatus, Aedes hendersoni, Toxorhynchites rutilus rutilus* and *Toxorhynchites brevipalpis*. In Japan Toma *et al.* (1982) used paper towels as an oviposition substrate in their survey of *Aedes albopictus*, while in Tanzania Trpis (1972) lined his pots with paper towelling in addition to using hardboard paddles. Ballard *et al.* (1987) used ovitraps similar to those of Novak & Peloquin (1981) which had tongue depressor blades (15 cm) which had been scratched with a saw blade as the oviposition substrate. In the USA Berry (1986) used muslin cloth strips attached to the rims of ovitraps with paper clips as an oviposition surface. In Fiji Goettel *et al.* (1980) used ovitraps made from black plastic cups containing hardboard paddles which were removed at 3- or 4-day intervals. Each paddle was soaked in water for 2 weeks in the laboratory, when a few were soaked for 3 weeks an extra 6·4% *Aedes pseudoscutellaris* and 5·2% *Aedes aegypti* hatched. Before re-use paddles were placed in boiling water for 30 min, brushed under running water and then allowed to dry out. Rozeboom *et al.* (1973) omitted paddles from their traps which were lined with rough brown paper. They found that only about 17% of the pots contained more than 61 eggs of *Aedes albopictus*, whereas laboratory observations showed that the average egg batch size was 63 eggs. Similar observations were made on the oviposition behaviour of *Aedes polynesiensis*. They concluded that these species did not discharge all their eggs in a single oviposition site. But possibly the traps were not very attractive and thus females only deposited a few eggs in these, whereas they normally laid all their eggs in a single natural habitat. In India Reuben *et al.* (1977) found that a brown cloth strip placed in black glass ovitraps was considerably more attractive to ovipositing *Aedes aegypti* than jars having velour paper or hardboard strips. They also found that more eggs were laid on a green cloth strip (1332) than red (627), yellow (672), brown (698) or blue cloth strips (752). In addition to *Aedes aegypti* eggs of *Aedes albopictus, Aedes vittatus, Aedes unilineatus* and *Aedes micropterus* were laid in jars having green strips of cloth. Ovitraps with brown cloth strips were also successfully used in later studies (Reuben *et al.* 1978). In Trinidad ovitrap paddles were changed every 2 hr to study the diel oviposition cycle of *Aedes aegypti*. One to 43 eggs were obtained on a paddle during this interval (Chadee & Corbet, 1990).

In Puerto Rico Reiter (pers. comm., 1990) has been using paired ovitrap jars, one with 10% and the other with a full concentration (100%) of hay infusion (prepared from 1 kg hay in 120 litres of water placed in a bucket with a lid, and left in a shaded place for 7 days). As many as 200 *Aedes aegypti* eggs, and sometimes even more than 500, can be collected from a single paddle after a day's exposure.

There have been many modifications to the classical ovitrap, for example substituting black-painted tins or black plastic beakers for the glass bottle. In comparative trials in Louisiana Kloter *et al.* (1983) found that black glass jars and black plastic beakers were equally attractive to ovipositing *Aedes aegypti*, whether supplied with velour paper or fibreboard paddles. However, fibreboard paddles were better because snails and cockroaches sometimes destroyed the paper ones. Even so predators may still remove eggs from fibreboard paddles without destroying the paddles. Ovitraps are usually serviced every 7 days, but to overcome the problem of predation Frank & Lynn (1982) suggested having the shortest possible time between ovipaddle collections, but this makes surveillance labour intensive. Shorter trap exposure periods, however, have been used, including 1-day periods (Frank & Lynn, 1982; Nayar, 1981), but Ritchie (1984*a*) concluded that in most surveys a weekly exposure period was suitable. Clearly if ovitraps are withdrawn and not replaced at the end of the exposure time, then longer exposure periods will increase the likelihood of detecting *Aedes aegypti* breeding, especially when the mosquito population size is small. Another problem is the flooding of ovitrap jars with rainwater, but this can be more easily prevented with plastic beakers because of the simplicity of drilling an overflow hole in them. Ovitrap size can be important. For instance Berry (1986) found that 12-oz can-type traps collected about seven times more *Aedes aegypti* eggs as did 10-oz ovitraps.

In southern Africa 134 glass bottles painted black on the outside and containing two tongue depressors as oviposition paddles attracted 11 mosquito species in 1980 compared with 15 species collected from 49 bamboo pots in the same year (Jupp & McIntosh, 1990).

Originally a small glass vial of ethyl acetate was suspended within the oviposition jar, supposedly acting as an attractant for gravid females, but in 1967 this practice was discontinued when it was discovered that eggs of *Aedes aegypti* were obtained just as frequently in jars without ethyl acetate (Hoffman & Killingsworth, 1967; Thaggard & Eliason, 1969). In Trinidad Chadee & Corbet (1987) placed conventional ovitraps of Fay & Eliason (1966) under houses to study diel patterns of egg laying by *Aedes aegypti*, but in later studies (Corbet & Chadee, 1990) substituted black plastic jars having a top diameter of 8 cm. The oviposition liquid in the traps was a yeast mixture (15 mg dry yeast/350 ml water), and an overflow hole was drilled 7·6 cm from the top. Reiter *et al.* (1991) found that 10% hay infusion in ovitraps was a good oviposition attractant for *Aedes aegypti*, but the best procedure was to use paired ovitraps. For example, an ovitrap containing 100% hay infusion paired with another having 10% infusion collected together the highest numbers of eggs (92·2/collection), which was 8·1 times more than a single ovitrap with water. The largest number of eggs were laid in the pots containing 10% hay infusion. Other useful combinations were 100%/water in which most eggs were laid in the ovitrap with just water and the paired concentration 100%/100%. It seems that a strong hay infusion provides a powerful olfactory stimulus, but on arrival gravid females seem to prefer to oviposit in traps having a less strong hay infusion.

Occasionally ovitraps have been placed in cement half-blocks, painted black, to prevent them tipping over (Anon, 1979; Ritchie, 1984*a*). O'Meara *et al.* (1989*a*) attached black polypropylene ovitrap jars to pieces of white plywood to stabilise them, and also used a wire bar across the entrance to prevent animals drinking from them. In *Aedes aegypti* programmes ovitraps are normally inspected weekly and the paddles carefully removed and placed individually in plastic envelopes. After fallen leaves and other debris are removed from a trap, so that alternative oviposition sites are not provided and the water level adjusted, a new paddle is inserted.

Ovitraps have been used by many workers in North America as a routine surveillance method. Fay & Eliason (1966) found that one mosquito inspector could cover a three to five times larger area if oviposition surveys were made instead of larval surveys, and the costs were halved, or even quartered. Jakob & Bevier (1969*b*) reported a 17-fold decrease in working days when ovitraps were substituted for larval surveys. They, and others (Fay & Eliason, 1966; Furlow & Young, 1970; Tanner, 1969), considered that ovitrap surveys were more sensitive than larval surveys in detecting the presence of *Aedes aegypti*. In Trinidad Chadee (1986) compared the efficiency of human bait catches, larval surveys and ovitraps for detecting relatively low levels of *Aedes aegypti*. As was reported by both Fay & Eliason (1966) and Tanner (1969) ovitraps were the most sensitive sampling method for *Aedes aegypti*, but did not identify larval habitats. Only one ovitrap contained eggs of another species, (*Haemagogus janthinomys*), whereas another seven species were caught in bait collections, and four in larval surveys. Giglioli (1979) and Slaff *et al.* (1983) found that bait catches were inadequate in monitoring *Aedes aegypti* populations. Furlow & Young (1970) found ovitrap surveys about equally as sensitive as larval surveys in detecting *Aedes triseriatus*. However, in Jakarta Nelson *et al.* (1976) found they were less sensitive than human bait catches or larval surveys for monitoring *Aedes aegypti*, and in Bangkok Pant *et al.* (quoted by Nelson *et al.*, 1976) also found ovitraps the least sensitive method of detecting low populations of *Aedes aegypti* after control operations (see p. 157, Chapter 2). Ovitraps have also proved very useful in studies on the dispersal of genetically marked mutants of *Aedes aegypti* (Bond *et al.*, 1970; Fay & Craig, 1969; Fay & Eliason, 1966; Haüsermann *et al.*, 1971). The proportion of mutants that have dispersed into various areas is found by soaking eggs laid on the paddles and rearing through to adults.

Although primarily developed for *Aedes aegypti* surveillance, ovitraps when used in America have attracted ovipositing adults of other *Aedes* species, including *Aedes triseriatus*, *Aedes atropalpus*, *Aedes mediovittatus*, *Aedes zoosophus* and *Aedes albopictus*, and also *Orthopodomyia signifera* (Beehler & DeFoliart, 1990; Pratt & Kidwell, 1969). In Louisiana 1-pint capacity black ovitraps contained either distilled water, distilled water plus leaf litter, distilled water plus a 1% emulsion of fish oil fertiliser, or hay infusion. The most effective attractant for *Aedes albopictus* was the hay infusion; the fish oil seemed to attract most *Aedes triseriatus* but this needs further investigation (Holck *et al.*, 1988). In the Western pacific region ovitraps have been used in Taiwan, Guam and Okinawa, and apart from attracting ovipositing females of *Aedes aegypti* and *Aedes albopictus*,

eggs of *Aedes aureostriatus okinawanus, Aedes riversi, Aedes pandani* and *Aedes nocturnus* were collected on filter paper paddles used in the traps (Reisen & Basio, 1972). In Tanzania Trpis (1972) used ovitraps to study oviposition of *Aedes bromeliae* in different ecological zones, while in Kenya, Subra & Mouchet (1984) used conventional ovitraps indoors to study the oviposition preference of *Aedes aegypti*. In other areas ovitraps will probably sample other species breeding in container habitats.

Chan *et al.* (1971) found that the most common out-of-door habitat of *Aedes albopictus* in Singapore was discarded tin cans, and Chan (1971) made use of them as convenient ovitraps. Empty condensed milk tins were painted black and placed at ground level in shaded sites, such as under bushes and banana clumps. The oviposition surface consisted of a piece of hardboard (Bristol Board is quoted in the publications but this was due to confusion of terms) measuring $1 \times 4\frac{1}{2} \times \frac{1}{8}$ in.

Multipaddle trap

Tikasingh & Martinez (1983) developed a multipaddle trap to collect eggs of *Haemagogus equinus*, other *Haemagogus* species and *Aedes aegypti*. The modified trap consists of a 1·5-litre plastic ice cream carton about 16·5 cm in diameter and 10 cm deep. Twelve hardboard paddles (2·5 × 13·0-cm) are stood vertically in wire hoops around the inside wall of the carton. In field trials in Trinidad with four traps exposed from May 1981 to February 1982, 148 (15·8%) of the 936 paddles examined had eggs. Total eggs were 1013, giving a mean of 6·8 per positive paddle. There appeared to be no difference between the attractiveness of red cartons and those painted inside and out black. (A. B. Knudsen (Tikasingh & Martinez, 1983) found that in Anguilla multipaddle traps attracted *Aedes aegypti*.) The authors believe the employment of many paddles increases the numbers of eggs caught. I imagine the same could be achieved by lining the inside of the carton with strong brown paper towels, or embossed benchkote paper died grey-black (Yates, 1974).

Automatic recording ovitraps

To study the time of oviposition of *Aedes albopictus* in Japan Tsuda *et al.* (1989) used an ovitrap incorporating an automatic recorder (Fig. 1.11*c*). A 1·5-litre water tank allows water to drain down into a water cup A to maintain a constant depth of 2 cm, from here water drains into a cup B (50 ml) which when full overflows into one of the small cups of the water-mill wheel. This is connected to a strip of filter paper (4 × 120 cm) which has one end in the ovitrap, and advances it 11 cm, thus exposing a new area for oviposition. The part of the filter paper already with eggs is then lightly sandwiched between two strips of plastic (5 × 130 cm) to prevent further oviposition. This trap was successfully operated from 0800–1900 hr and advanced a clean strip of filter paper for egg laying every hour. Another approach would be to have a clockwork motor advance the strip of paper continuously, not at hourly intervals.

Autocidal trap

In Singapore Chan *et al.* (1977) and Lok *et al.* (1977) designed autocidal *Aedes aegypti* ovitraps that had one to two hardboard paddles inserted through a floating doughnut (American) shaped ring with nylon mesh covering the centre hole. This arrangement caused larvae to suffocate, or prevented adults from escaping from the trap. The modifications to this trap made by Cheng *et al.* (1982) for use in the USA to control *Aedes aegypti* are described here. The ovitrap jar consists of a dark bottle (approx. 10 cm high and 8 cm in diameter) and two expanded polystyrene rings having a piece of nylon mesh glued between them, which is then placed on the water in the bottle. Two short pieces of hardboard (3·2 × 6·4 × 0·3 mm) acting as oviposition paddles are inserted into the polystyrene ring with their lower edges in contact with water in the ovitrap (Fig. 1.11*b*). As in conventional ovitraps paddles can be periodically replaced, but if left undisturbed and eggs on them hatch 2nd instar larvae are unable to squeeze through the nylon mesh and eventually drown. These traps have proved to be a sensitive and reliable method of detecting and monitoring not only *Aedes aegypti* but also *Aedes triseriatus*.

Miscellaneous ovitraps

In Trinidad ovitraps of the Fay & Eliason (1966) design were found to be suitable for collecting eggs of *Haemagogus equinus* (Tikasingh & Laurent, 1981). Of 6678 oviposition paddles exposed 69% were positive and had 24 445 eggs. The number of eggs deposited per paddle per week ranged from 1 to 150, with an average of 35.

Chadee & Tikasingh (1989) used the modified ovitraps described by Chadee & Corbet (1987) to study diel oviposition by *Haemagogus janthinomys*. Paddles were removed at 2-hr intervals for 24 hr on 1 day/week for 53 weeks. They also studied diel oviposition of *Haemagogus equinus* (Chadee & Tikasingh, 1990) using the same method. Very few eggs of *Haemagogus janthinomys* (175) were caught over the entire period, but more eggs (820) of *Haemagogus equinus* were collected. In other trials in Tobago Chadee *et al.* (1984) recovered eggs of *Haemagogus equinus* and *Haemagogus celeste*, as well as those of *Aedes taeniorhynchus* and *Aedes berlini*, from their paddles.

In Trinidad ovitraps consisting of 500-ml capacity polystyrene cups (height 135 mm, basal diameter 60 mm, mouth 90 mm) painted black and filled with 275–300 ml water were evaluated as ovitraps for *Toxorhynchites moctezuma*. To prevent eggs being displaced by rain some traps had an inverted plastic petridish supported 60 mm above the cups on three wire supports, lid and supports were also painted black (O'Malley *et al.*, 1989). However, only 1·4% of the pots were colonised, compared to 6·0% of natural oviposition sites comprising fruits of the tree *Lecythis zapucajo*.

In studying the seasonal variations in relative abundance of *Aedes albopictus* and *Aedes aegypti* in Thailand Mogi *et al.* (1990*b*) used as ovitraps greenish dark-grey ceramic ant traps that formed a circular trough. Diameters of the inner and outer rims were 7 and 15 cm, respectively. The trough held about 400 ml of water. The inner side of the outer rim was lined with brown paper

towelling that had a rough surface and which remained intact for at least a week after soaking. However, a disadvantage of using such towelling was that, unlike hardboard paddles, eggs hatched without submersion, presumably because the paper gets much wetter. There was a 5-day exposure between removal of the paper towels. The distribution of eggs in these ovitraps was distinctly contagious but the data did not fit a negative binomial model with a common k (Mogi *et al.*, 1990*a*).

FIG. 1.12. *Example of a typical tyre-type ovitrap (M. W. Service).*

In the USA Bradshaw & Holzapfel (1985) placed car tyres at the base of trees and put in two handfuls of sterilised tree detritus to establish ovitraps to monitor breeding mosquitoes (Fig 1.12). They found that the relative abundance of *Orthopodomyia signifera, Aedes triseriatus, Anopheles barberi* and *Toxorhynchites rutilus* in these sentinel traps closely approximated that in actual tree-holes. In New Orleans Freier & Francy (1991) evaluated tyre traps for ovipositing *Aedes albopictus*. Firstly, an oviposition medium was made by incubating 1 g rabbit pellets for 3 days at 27°C in 3·8 litres of water. A tyre was then placed horizontally on the ground and six 7-cm diameter equally spaced holes made around the tread. A plastic container containing 1 litre of oviposition water was placed on the ground within the tyre centre, and a plywood board covering the tyre opening was placed on top of the tyre. A 2-m length of 10-cm diameter plastic tubing projected from an opening cut from the middle of the board. A motor and fan placed at the end of this tubing sucked mosquitoes that had entered the tyre trap

through the six peripheral holes, into a 0·5 litre screened carton inserted in the tubing near the tyre.

A vertical tyre trap was also made. This consisted of three tyres with their sidewalls attached together placed with the tread on the ground. The tubing and suction motor was connected to one end of the group of tyres. About 1 litre of oviposition medium was placed in the middle tyre. Neither arrangement was very successful because more *Aedes albopictus* (mean 4·2/6-hr trap-day) were caught in the gravid trap of Reiter (1983), than in the horizontal (1·5) and vertical (0·8) tyre traps. Similar decreases in numbers were observed in these three traps for *Aedes triseriatus* and *Culex salinarius.*

In Wisconsin ovitraps with balsa wood paddles sometimes contained eggs of *Orthopodomyia signifera* (Beehler & DeFoliart, 1990; Loor & DeFoliart, 1970), while in Tahiti *Aedes aegypti* ovitraps (Fay & Eliason 1966; Fay & Perry, 1965) were used to attract *Toxorhynchites amboinensis* (Rivière, 1985).

In Panama although no *Aedes aegypti* were caught in ovitraps of the Fay & Eliason (1966) design, 4·2% were colonised by *Limatus durhamii.* Lounibos & Machado-Allison (1986) successfully used split cocoa pods as oviposition traps for *Trichoprosopon digitatum.*

Snail ovitraps

Several species of *Eretmapodites* preferentially oviposit in the water-filled shells of *Achatina fulica*, and Lounibos (1980) used them as oviposition traps in Kenya. He half-filled clean shells with spring water and placed them on the ground in the shade. After a 6-day exposure the shells were collected, the larvae removed and reared to adulthood, and the water discarded. Two to 3 days later the dry shells were immersed for 24 hr in water containing liver powder to stimulate hatching of unhatched eggs. Finally, the snail shells were placed in boiling water for 5 min to kill any remaining eggs and to sterilise them before they were returned to the field as oviposition traps. In the Shimba hills a total of 539 *Eretmapodites silvestris conchobius*, 569 *Eretmapodites quinquevittatus* and 58 *Eretmapodites subsimplicipes* were identified from snail ovitraps and their seasonal incidence plotted. Other species occasionally found in the traps were *Aedes calceatus, Aedes aegypti, Aedes bromeliae, Aedes soleatus, Aedes heischi* and *Culex nebulosus.* In Kombeni forest 164 *Eretmapodites quinquevittatus* were collected from similar snail traps.

Presence–absence technique

Mogi *et al.* (1990*a*) applied for the first time presence–absence sampling, a technique previously used with agricultural pests (Wilson & Room, 1983), to *Aedes* ovitrap surveys in Thailand. They also combined it with sequential sampling procedures.

The model used for presence-absence sampling was as follows

$$\log_e \bar{x} = \log_e a + b \log_e \{-\log_e (1 - p)\}$$

where $\bar{x}$ = the mean, p = proportion of positive samples, and a and b are constants which can be determined by plotting the linear regression of log $\bar{x}$ against

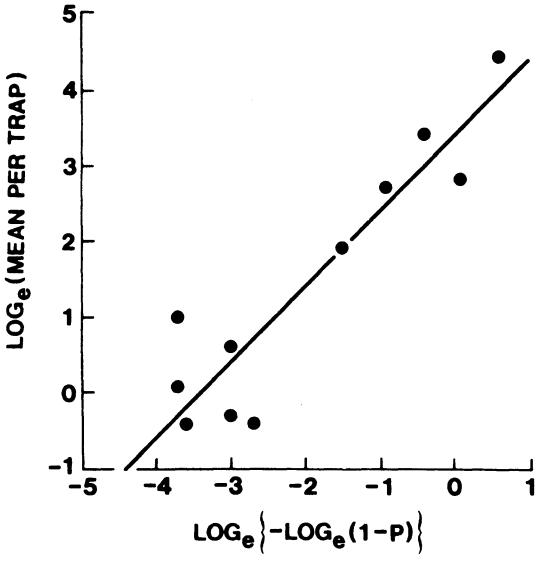

FIG. 1.13. Mean density ($\bar{x}$) as a function of the proportion of positive samples ($\hat{p}$). Regression equation: $\log_e \bar{x} = 3{\cdot}38 + 0{\cdot}99 \log_e \{-\log_e (1 - \hat{p})\}$ (Mogi et al. *1990a).*

$\log_e \{-\log_e (1 - p)\}$. From Fig. 1.13 presented by Mogi *et al.* (1990*a*) the regression equation is $\log \bar{x} = 3{\cdot}38 + 0{\cdot}99 \log_e \{-\log_e (1 - p)\}$, where $a = 29{\cdot}3$ and $b = 0{\cdot}99$. Now the number of samples (n_0) needed for a set and predetermined level of precision, D is given by

$$n_0 = \frac{p}{D^2 (1 - p)} \left\{ \frac{b}{-\log_e (1 - p)} \right\}^2$$

In their situation they calculated that 100 ovitraps could keep $D < 0{\cdot}3$ for $0{\cdot}11 < p < 0{\cdot}99$, and be sufficient to study *Aedes aegypti* populations in their area.

Mogi *et al.* (1990*a*) concluded that for the implementation of presence–absence sampling it may be necessary to proceed stepwise by making: (i) a preliminary survey to establish the $m - p$ relationship; and (ii) a trial survey to compare estimates and actual counts, and then if the agreement is good routine surveys based on presence–absence sampling can be undertaken.

They also combined presence–absence sampling with sequential sampling using computer simulations to decide when population levels of *Aedes aegypti*, as determined by their ovitraps, had reached a size that needed to be controlled to prevent potential dengue outbreaks. The practical difficulties of this approach are discussed by the authors. A brief account of presence–absence sampling is given by Kuno (1991).

Oviposition attractants

Several laboratory and field workers have identified a variety of substances ranging from cow manure to chemicals such as *n*-capric acid and acetoxyhexa-

decanolides as stimulating mosquito oviposition. For example, Hwang *et al.* (1978) reported that 1% chicken manure in water after fermentation for 7–15 days provided a good oviposition attractant for *Culex quinquefasciatus*, but was an oviposition repellent to *Culex tarsalis*. Purina Laboratory Chow was repellent to both species. The actual repellents were identified as lower aliphatic acids, namely butyric, isobutyric, propionic, acetic, isovaleric and caproic acids, the first forming about 8% of the total weight of the acidic fraction. Their repellency was directly related to their concentration. Mixtures of hay, dried brewer's yeast and lactalbumen powder have proved attractive in collecting gravid females of *Culex* species (Reiter, 1983; 1986; Reiter *et al.*, 1986), while others have found cow manure very attractive (Hoban *et al.*, 1980; Leiser & Beier, 1982). Both Dadd & Kleinjan (1974) and Nakamura (1978) found that water containing egg rafts of the *Culex pipiens* group was attractive to ovipositing females, while Bruno & Laurence (1979) traced the attractant to the apical droplets of the eggs of *Culex quinquefasciatus*. The substance was later shown to be a volatile chemical, *erythro*-6-acetoxy-5-hexadecanolide (approx. 0·3 μg/egg raft) (Laurence & Pickett, 1982), and Sakakibara *et al.* (1984) determined its exact configuration. When as little as 0·02 μg of synthetic acetoxyhexadecanolide was placed on polystyrene discs floated on the water it attracted gravid females from about 5·5 cm. It is worth noting that in the laboratory *Culex tarsalis* is attracted to both its own egg rafts and those of *Culex quinquefasciatus* (Bruno & Laurence, 1979). Later Laurence & Pickett (1985) reported that another strain of *Culex quinquefasciatus* maintained in their laboratories did not respond in the same way to its own egg rafts despite the apical droplets containing acetoxyhexadecanolide. Otieno *et al.* (1988*a*) showed that Kenyan populations of *Culex quinquefasciatus* were in the laboratory attracted to *erythro*-6-acetoxy-5-hexadecanolide. Clearly the addition of this chemical to oviposition traps might increase their efficiency at collecting egg rafts. Furthermore, when synthetic 6-acetoxy-5-hexadecanolide (Dawson *et al.*, 1990) formulated as a 20 mg effervescent tablet containing 5 mg of the active isomer was added to known breeding places of *Culex quinquefasciatus* significantly more eggs rafts were deposited in them, than in habitats without a tablet (Otieno *et al.*, 1988*b*). It remained an oviposition attractant for 4 days. However, even massive doses (up to 1280 mg) of pheromone failed to induce gravid females to oviposit in breeding places not already colonised by this species.

Wilmot *et al.* (1987) give a useful list of references on pheromones or other chemicals that might be oviposition attractants that are associated with the presence of mosquito larvae. They showed that females of *Culex pipiens, Culex quinquefasciatus* and *Culiseta incidens* oviposited preferentially in containers having conspecific larvae.

In laboratory experiments Ikeshoji *et al.* (1975) evaluated five fatty acids as mosquito oviposition attractants. They found that *n*-capric acid was the best attractant for *Culex pipiens* (*molestus* form), whereas *n*-pelargonic acid was the best *Aedes aegypti* attractant. They also discovered that bacteria, *Pseudomonas aeruginosa*, acted on these fatty acids to produce the actual oviposition attractant, which was later shown to be 7,11-dimethylocatadecane (Ikeshoji *et al.*,

1979). Further work on oviposition-stimulating proteins in the eggs of *Culex pipiens* form *molestus* was undertaken by Sakakibara & Ikeshoji (1989), who also found that various animal proteins, especially glycoprotein (bovine), at 0·1 ppm, stimulated oviposition.

Among the substances found to be attractive to ovipositing *Culex* mosquitoes are fatty acids, *Pseudomonas* and *Aerobacter* bacteria, *n*-capric acid and oviposition pheromones (Hazard *et al.* 1967; Ikeshoji *et al.*, 1975; Maw, 1970; Maw & Bracken, 1971; Osgood, 1971; Starratt & Osgood, 1972, 1973; and see p. 30). Methyl propionate added to water was reported to enhance *Aedes aegypti* oviposition (Fay & Perry, 1965; Klowden & Blackmer, 1987), but Reiter *et al.* (1991) failed to find this was a useful attractant in oviposition traps.

Bentley *et al.* (1976) found that water which had contained 4th instar larvae of *Aedes triseriatus* or *Aedes atropalpus* contained an oviposition attractant for *Aedes triseriatus*. Later Bentley *et al.* (1979) identified *p*-cresol, obtained from aqueous infusions of birch (*Betula papyrifera*) wood, as one of the attractant oviposition components for *Aedes triseriatus*. When such infusions were placed in ovitraps comprising 400-ml pyrex beakers covered with a grey fibreglass funnel having a top diameter of 8 cm and tapering to 1·5 cm diameter situated just 5 cm above the solution, they attracted many more females, and for some unexplained reason males, of *Aedes triseriatus* than beakers with just water. Later Bentley *et al.* (1981, 1982) identified three other related compounds including, the saturated analogue, 4-methylcyclohexanol, that were about as equally attractive to this species, but they were not field-tested. Holck *et al.* (1988) reported that *Aedes triseriatus* was attracted to ovipositing in water containing 10% fish oil emulsion, but Beehler & DeFoliart (1990) believe the addition of such oil actually repels oviposition. In contrast, increasing optical density by adding three drops of an odourless vegetable green dye and three drops of red dye increased oviposition up to fourfold.

Tyagi *et al.* (1981) reported that in laboratory experiments *Aedes aegypti* showed a very marked preference to oviposit in containers filled with water containing secretions and excrement of aquatic snails (*Lymnaea*) than in containers with just tap water. This observation needs further evaluation, but it seems unlikely that snail contaminated water will prove more attractive then water containing yeast, oats, leaf litter and other debris that are more usually placed in ovitraps. Other examples of experiments to demonstrate oviposition attractants or stimulants include those on *Aedes aegypti* (Benzon & Apperson, 1988; Roberts & Hsi, 1977; Soman & Reuben, 1970), *Aedes triseriatus* (McDaniel *et al.*, 1979), *Aedes togoi* (Trimble & Wellington, 1980), *Aedes atropalpus* (Kalpage & Brust, 1973; Maire, 1984, 1985; Roberts & Hsi, 1977), *Aedes communis* (Maire & Langis, 1985) and *Culex tarsalis* (Hudson & McLintock, 1967; Osgood, 1971). Knight & Corbet (1991) give useful references to studies that have identified various chemicals as oviposition attractants. In field trials with hexanoic acid and four derivatives they found that 5-methylhexanoic acid and 5-methyl-2-hexanone were the best at enhancing oviposition by *Aedes aegypti* ssp. *formosus*. They recorded, however, a marked dose-dependent reversal response with hexanoic acid, that is decreasing numbers of eggs were laid at increasing release rates. In marked contrast

to the above investigations Ahmadi & McClelland (1983) found no evidence of any egg-, larval- or pupal-originated attractant or stimulant with *Aedes sierrensis*. These authors provide a useful table of mosquito oviposition attractants found by other workers.

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