

Chapter 5

Sampling Adults by Animal Bait Catches and by Animal-Baited Traps

The most fundamental method for catching female mosquitoes is to use a suitable bait to attract hungry host-seeking individuals, and human bait catches, sometimes euphemistically called landing counts, have been used for many years to collect anthropophagic species. Variations on the simple direct bait catch have included enclosing human or bait animals in nets, cages or traps which, in theory at least, permit the entrance of mosquitoes but prevent their escape. Other attractants, the most widely used of which are light and carbon dioxide, have also been developed for catching mosquitoes. In some areas, especially in North America, light-traps, with or without carbon dioxide as a supplement, have more or less replaced human and animal baits as a routine sampling method for several species (Chapter 6). However, despite intensive studies on host-seeking behaviour no really effective attractant has been found to replace a natural host, and consequently human bait catches remain the most useful single method of collecting anthropophagic mosquitoes. Moreover, although bait catches are not completely free from sampling bias they are usually more so than most other collecting methods that employ an attractant. They are also easily performed and require no complicated or expensive equipment.

HUMAN BAIT CATCHES

Attraction to hosts

Compounds used by mosquitoes to locate their hosts are known as kairomones, that is substances from the emitters (hosts) are favourable to the receiver (mosquitoes) but not to themselves. Emanations from hosts include heat, water vapour, carbon dioxide and various host odours. Wright (1975) considered warmth and humidity were the main attractants of mosquitoes to humans, and doubted whether there was any skin odour involved in host attraction, but Khan (1977) believed that in addition to skin temperature and skin colour, body odour and other factors were involved. Price *et al.* (1979) concluded that female *Anopheles quadrimaculatus* were mainly attracted to humans by chemicals emanating from the skin, while studies by Schreck *et al.* (1981, 1990) showed that there were unidentified attractants to mosquitoes in the sweat from human

subjects. There are two types of sweat, eccrine sweat which comes from most body surfaces but especially from the palms of the hands and soles of the feet, and apocrine sweat from the axillary, perigenital and perianal regions. [S]-Lactic acid (formerly called L-lactic acid) is in fact produced by glycolysis in the eccrine sweat glands, and excess remains in the final secretion—sweat. Most mammals have apocrine-type sweat glands; birds lack sweat glands. Schreck *et al.* (1990) found that sweat from the face and hands generally elicited the greatest response from mosquitoes, and there were significant differences between the attractiveness of sweat from the hands of different people. However, of the 12 mosquito species tested four showed no response, while another four species were only weakly attracted to human sweat: the species most attracted was *Aedes aegypti*, followed by *Aedes albopictus* and *Anopheles albimanus*. It was pointed out by Schreck *et al.* (1990) that attractant substances from the skin (e.g. sweat) might contaminate equipment used in mosquito behaviour studies, and that traps frequently handled might catch a disproportionate share of mosquitoes.

Bar-Zeev *et al.* (1977) summarised the available information on the factors that appeared to attract *Aedes aegypti* to humans. They also carried out laboratory studies on responses to carbon dioxide, relative humidity, temperature and emanations from a human forearm, and confirmed the attractancy of [S]-lactic acid (Acree *et al.*, 1968; Smith *et al.*, 1970). More recently Kusakabe & Ikeshoji (1990) found that lactic acid, heat, black colour, movement and sound were all to some degree attractive to both sexes of *Aedes aegypti* but carbon dioxide was not attractive. In an interesting paper Gillett (1979) discussed possible mechanisms by which mosquitoes orientate upwind to hosts in the absence of visual cues; he also presents some pertinent physical characteristics of wind speed near the ground. Takken & Kline (1989), Takken (1991) and Lehane (1991) have briefly summarised what is known about substances that attract mosquitoes to baits and odour-baited traps, while the role of carbon dioxide in host attraction has been reviewed by Gillies (1980).

McIver (1982) listed five types of stimuli that have been shown to elicit host responses in mosquitoes, namely vision, heat, water vapour, carbon dioxide and host odours. A major activator in host location is the concentration of carbon dioxide emitted by hosts, which mosquitoes detect by capitate pegs on their palps. An increase of only 0.01% in carbon dioxide concentration may be detectable, and the response is almost logarithmic to a saturation level of 0.05–0.5%. As the biological range of carbon dioxide concentration emanating from animals is between 3–5%, it is not surprising that artificial concentrations as great as 10% from dry ice or gas cylinders elicit little additional response. Carbon dioxide from animals is therefore 100 or more times greater than the background concentration of 0.02–0.04%, but yet much less than the concentration of about 100% at the release point emitted from gas cylinders or dry ice. Although discharge rates can be altered, the mixing of the gas in the air at various distances from the trap—that is its concentration—will depend on local environmental conditions, which can be very variable in both time and space, and usually remain largely unknown in trapping experiments.

In addition to carbon dioxide expired breath contains several organic compounds (Teranishi *et al.*, 1972) including acetone (Crofford, 1976), some of which may be attractive to mosquitoes. Sutcliffe (1986) gives a good review of how blackflies locate their hosts, and much of this will be of interest to those concerned with host orientation by mosquitoes. Takken & Kline (1989) reported for the first time from field experiments that octenol had potential as a mosquito attractant. Later Kline *et al.* (1990) conducted field trials in Florida with unlit CDC-type light-traps baited with various combinations of attractants including: (1) CO₂; (2) octenol; (3) octenol + CO₂; (4) octenol + butanone + CO₂; (5) lactic acid + CO₂; (6) lactic acid + octenol + CO₂; (7) honey; (8) phenols; and (9) phenols + octenol. Not surprisingly different mosquito species sometimes responded differently to these chemicals. Basically very few species are attracted in any numbers to octenol alone, but when octenol and carbon dioxide were used together there appears to have been a synergistic effect and a twofold or greater catch was obtained with most species of *Aedes*, *Psorophora*, *Anopheles*, *Coquillettidia* and *Mansonia* encountered in the area. With *Culex* species, however, there was little attraction to either chemical alone or in combination. But in contrast to these generalisations *Aedes taeniorhynchus* and *Coquillettidia perturbans* seemed to respond to octenol alone. Honey (500 ml diluted with 300 ml 29% sodium chloride, then extracted overnight with 250 ml hexane in a liquid/liquid extractor, followed by concentration over a steam bath) was very attractive to *Aedes taeniorhynchus* (not *Coquillettidia perturbans* as stated in the paper's abstract). The presence of butanone seemed to decrease collections of all species.

Laboratory experiments with *Aedes aegypti* and other species have suggested that mosquitoes might selectively feed on hosts having a rise in temperature due to viral or other parasitic infections (Gillett & Connor, 1976; Mahon & Gibbs, 1982; Turell *et al.*, 1984). In laboratory experiments Day & Edman (1983) reported that mice were more susceptible to feeding mosquitoes when they were infected with malaria, but in later experiments hypothermia had no significant impact on numbers of mosquitoes feeding on mammals (Day & Edman, 1984a). If infected hosts are more susceptible to biting mosquitoes, and this applies to human malaria, then there could be epidemiological consequences. This topic and other aspects of blood-feeding and host location are reviewed by Edman & Spielman (1988), while Bowen (1991) provides a good review of host finding cues.

Arrival at bait

Species which normally feed at twilight or during the night will often bite during the day if a suitable host is present. In England species which were essentially crepuscular and nocturnal were caught in large numbers during the day whenever bait catches were performed in sheltered sites, where unfed females were resting among the vegetation (Service, 1969a, 1971b). In contrast few or no mosquitoes were caught during day-time catches in exposed areas such as in fields or on pathways. At night, however, baits in both sheltered and exposed areas were bitten (Service, 1971b). It was concluded that although during the day mosquitoes resting among vegetation were not actively orientated to host

feeding, they would nevertheless readily feed if a host was in the immediate area. At night under the influence of an endogenous biting rhythm adults actively flew in search of blood-meals and were consequently encountered in both sheltered and exposed areas. The same phenomenon has been described for *Aedes africanus* in West Africa (Germain *et al.*, 1973).

Several workers have reported a burst of biting activity during the first 15–20 min in daytime bait catches followed by a decline in numbers (Germain *et al.*, 1973; Mogi & Yamamura, 1981; Nishimura, 1982; Roberts & Scanlon, 1975; Service, 1969a, 1971b; Wellington, 1974), but McCrae *et al.* (1976) working on *Anopheles implexus* in Uganda undertook the best analyses of this type of behaviour. They pointed out that during passive (opportunistic) biting by mosquitoes formerly resting amongst vegetation, there would be two principal categories; namely a static component (*s*) representing mosquitoes already present within the host's area of attraction, and a component of arrivals (*a*) flying into the host's attractant area after the bait had arrived. But because different species exhibit very different catch curves when collected from the same hosts at the same time, then clearly there must also be different intrinsic behaviours in addition to environmental stimuli affecting their sequence of arrival at bait.

Roberts & Scanlon (1975) observed a depletion effect in their series of 15-min catches, that is high initial catches of *Aedes atlanticus*, *Aedes tormentor* and *Psorophora ferox* during the first 5 min followed by a decline during the next 10 min. The initial high biting rate was said to be caused by host movement, supporting the contention of Gillett (1972) that movement attracts diurnally active mosquitoes to their hosts. Roberts & Scanlon (1975), however, failed to observe any obvious depletion effect with nocturnal species such as *Culex salinarius* and *Aedes fulvus*. An initial high catch was also reported in *Aedes aegypti* in catches performed both outdoors and in huts in Kenya (Teesdale, 1955).

Colless (1956, 1957) considered that mosquitoes arrived at bait by a process of random wandering, or at least by a process not directly orientated to the bait. In Singapore he found that the numbers of *Culex annulus* caught each hour (1900–0600 hr) declined progressively with time, and fitted the following linear relationship:

$$\log (K - C) = a + bt$$

where *K* = the initial population, *C* = the cumulative catch, *t* = time, and *a* and *b* are constants. This is in fact an example of removal trapping (see Chapter 2), i.e. the numbers caught depends on the population still available for capture. Colless (1957) stated that in Singapore the biting cycles of most *Culex* and *Mansonia* species were characterised by a depletion of catch with time. This implies that there is no marked temporal cycle of attraction to the bait, a theory that does not really explain the arrival patterns of most mosquitoes to a host.

In Japan Nishimura (1982) obtained high initial catches of *Aedes albopictus*, and *Aedes japonicus* during the first 10 min of human bait catches, but not with *Tripteroides bambusa*. It seemed that this was because *Tripteroides bambusa* caught at bait consisted of only actively host-seeking females, and not those rest-

ing amongst vegetation. Often a shift of only a few metres can result in another high initial catch (Gillett, 1967; Service, 1969a, 1971b). By modifying the method of Service (1971b) Nishimura (1982) concluded that the range of attraction for female *Aedes albopictus* was 4.0 m while for *Aedes japonicus* it was 9.0 m. However, Mogi & Yamamura (1981) saw this paper by Nishimura (1982) before it was published and criticised some of the methodology. They also adopted Service's method, and after performing a 30-min human bait catch undertook a second catch at distances of 2, 4, 6, 8, 10 or 12 m. They analysed the results by applying a new type of removal method similar to that of Kono (1953), and concluded that the range of attraction for *Aedes albopictus* was 4–5 m.

Because of such opportunistic feeding it may be necessary to perform a preliminary bait catch for about 30 min, or even 1 hr, to clear an area of hungry unfed mosquitoes before diel biting cycles can be studied (Service, 1969a; Teesdale, 1955). In England, however, Renshaw (1991) believed the high initial catch of *Aedes cantans* was due to hungry females following her through a wood to the catch site. Another characteristic of some bait catches is that adults may arrive in waves (Haddow, 1954; Service, 1969a), that is the sudden appearance of several individuals followed by short periods when few or no individuals arrive. This is possibly explained by slight changes in the drift of host odours causing the simultaneous stimulation of small groups of resting mosquitoes, which then arrive at the bait more or less together.

Alekseev *et al.* (1977) were the first to demonstrate that there was an 'invitational effect', whereby female mosquitoes (*Aedes communis*) were attracted to a host (human) in proportion to the numbers of other females already feeding on the host. Later Ahmadi & McClelland (1985) using *Aedes sierrensis* and guinea pigs in laboratory feeding experiments confirmed this invitational effect, and concluded that it was caused by a chemical stimulus (pheromone) emanating from the feeding mosquitoes. This has implications in trap design and interpretation of results. For instance, it might be expected that an animal bait-trap which prevented mosquitoes feeding on the host would attract fewer mosquitoes than one in which blood-feeding was allowed. In fact Emord & Morris (1982) reported that with bird-baited traps double-screening to prevent mosquitoes feeding on the birds was accompanied by a considerable reduction in numbers of mosquitoes caught. They considered that the extra screening diminished host odours escaping from the trap, but it is possible that the reduction in mosquitoes caught was due, at least in part, to the prevention of host feeding.

There may be a marked interval between the appearance of adults on nearby vegetation and their alighting on the bait. Such pre-biting resting behaviour has been reported in *Mansonia* species (Haddow, 1961a; Service, 1969a; Wharton, 1962) and *Anopheles* (Colless, 1956; Hudson, 1984; Lee *et al.*, 1980; Moorhouse & Wharton, 1965; Ribbands, 1946; Senior White, 1953; Smith, 1958).

A delay of several weeks between adult emergence to blood-feeding has been recorded independently several times in *Aedes cantans* (Renshaw, 1991; Service, 1977; Sulaiman, 1982), in *Culiseta morsitans* (Service, 1969a), in *Aedes sierrensis* (Bennett, 1978; Garcia *et al.*, 1975; Lee, 1971), and in *Aedes thibaulti* (Shields & Lackey, 1938). The reasons for such a delay remain unexplained.

Composition of the catch

Unfed females invariably predominate in human bait catches and in most they are the only category caught. The capture of unfed females that have either alighted on, or are hovering around, the bait is commonly taken as showing that they have been attracted for the purpose of taking a blood-meal, but this may not always be true. *Uranotaenia*, *Ficalbia* and *Hodgesia* species are sometimes caught at bait (Haddow *et al.*, 1951; Macdonald, 1957; Mattingly, 1949b), but there is often no clear evidence that they would bite if given the opportunity. In Canada, Hocking *et al.* (1950) and Haufe (1952) recorded separately the landing and biting rates of *Aedes* species attracted to man, and Haddow & Ssenkubuge (1963) emphasised the importance of distinguishing between these two phenomena, i.e. arrival at bait and the intention of feeding. Even with a highly anthropophilic species such as *Aedes aegypti* collection in bait catches does not prove that they are orientated to blood feeding. As much as 18% of the female *Aedes aegypti* collected in a series of catches in Tanzania refused to take a blood-meal (McClelland & Conway, 1971). In addition to unfed females, blood-fed, partially and fully gravid females (Gould *et al.*, 1970) and also unfed females with fat reserves are occasionally caught at bait (Service, 1969a). In Kenya Wijers & Kiilu (1977) found that 20.0% of the *Anopheles funestus* and 3.1% of *Culex quinquefasciatus* arriving at human bait were half-gravid or gravid. In Sweden Andersson (1990) found that many mosquitoes caught at bait, especially *Aedes communis*, had fed on nectar (fructose). It was concluded that nulliparous females commenced nectar-feeding earlier than parous ones, but parous individuals contained most fructose. Fructose, an indicator of nectar-feeding, was found in females in all gonotrophic conditions, and moreover, Andersson (1990) observed blood-engorged mosquitoes feeding on flowers. Van Handel & Day (1990) caught *Aedes taeniorhynchus* attracted to humans and by quantitatively testing them for fructose concluded that it appeared that nectar-feeding occurred mostly after the onset of darkness, with very little or any feeding during the daytime. Both these papers contain some useful references to nectar-feeding in mosquitoes.

Generally, investigations in Africa (Corbet, 1961, 1962; Corbet & Smith, 1974; Germain *et al.*, 1973; Gillett, 1957; Gillies, 1957; Gillies & Wilkes, 1963, 1965; Hamon *et al.*, 1959, 1961; McCrae, 1972), Trinidad (Nathan, 1981), Malaysia (Chiang *et al.*, 1984a) and in Myanmar (De Meillon & Sebastian, 1967) have failed to show any real difference between the age composition of mosquitoes biting at different times. However, in West Africa, Coz (1964) reported a small difference between the proportions of parous and nulliparous *Anopheles gambiae* biting at different times of the night, and Hamon (1963a) found a slight tendency for older *Anopheles* to bite more in the middle of the night and in the early morning than in the early evening. In Sri Lanka Samarawickrema (1967, 1968) found small differences between the age composition of *Culex quinquefasciatus* and *Mansonia uniformis* biting at different times, while in Trinidad, Senior White (1953) found slight differences between the biting times and age of *Anopheles aquasalis*. In England there was a small but significant decrease between the proportion of parous *Aedes detritus* biting between 0300–0500 hr than at other hours (Service, 1969a). Yajima *et al.* (1971) found a higher parous rate in *Culex*

tritaeniorhynchus caught in the latter half of the night from pig-baited traps. Furthermore, older uniparous females with contracted dilatations were commoner in the early part of the night, whereas adults with uncontracted or partly contracted sacs were commoner later in the night. In Brazil Charlwood & Wilkes (1979) found that based on 755 female *Anopheles darlingi* caught at human bait there was a preponderance of nulliparous individuals biting at dusk (64.4%) and again at dawn (71.0%). Apart from these examples no convincing large differences have been found between the biting times of parous and nulliparous mosquitoes, as have been found in some species of *Simulium* (Davies, 1963; Le Berre, 1966; McCrae *et al.*, 1969) and *Chrysops* (Duke, 1960).

Klowden *et al.* (1988) showed that host-seeking avidity was greater in large *Aedes aegypti* than in smaller adults, while Nasci (1991) showed that large females were significantly more persistent biters than smaller ones. In England Renshaw (1991) found an increase in the size of *Aedes cantans* at human bait later in the season, and that those that were still nulliparous many weeks after emergence were small individuals. These results support the idea that larger adults are more successful in getting a blood-meal.

In India the mean numbers of *Culex vishnui* and other vectors of Japanese encephalitis biting a man per night were multiplied by the proportion parous to give a Parous Man Biting Index, because nullipars are epidemiologically unimportant. The Parous Dusk Index represented the mean numbers biting per man-hour at dusk \times the proportions parous (Anon, ? 1989).

Working in the Congo Carnevale & Molinier (1980) generated a general formula for determining the average number of times a parous anopheline bites in one day (L) based on its gonotrophic cycle and its behaviour before and after oviposition, the formula for *Anopheles gambiae* for example is

$$L = \frac{1}{4 - A - \alpha}$$

where A = the proportion of females which bite on the night that eggs are laid, and α = the proportion of females which oviposit 2 days after their blood-meal. The value of 4 is derived from these patterns of behaviour, and for *Anopheles nili* for example which has an extended gonotrophic cycle the figure is 5. When calculated values of L are multiplied by the anthropagic index the parameter a of Macdonald (1957) is obtained. Their paper gives graphical illustrations of the biting and oviposition rhythms of both these malaria vectors.

In addition to females, males of a few species are not infrequently encountered at bait (Cordellier & Geoffroy, 1974; Hamon, 1963*b*). Substantial numbers of male *Aedes aegypti* have been collected in human bait catches (Boorman, 1960; Corbet & Smith, 1974; Hartberg, 1971; Lumsden, 1957*a*; McClelland, 1960; Pillai & Rakai, 1976; Soman, 1978), and both De Meillon & Sebastian (1967) and Lumsden (1957*a*) caught males of *Culex quinquefasciatus* on man. Bates (1944*a*) reported that male *Aedes aegypti* settle on a bait and await the opportunity to pounce on females coming to feed. It seems that the host can be a focal point for mating in *Aedes aegypti* (Hartberg, 1971) as has been shown for *Aedes variipalpus* and *Aedes sierrensis* (Lee, 1971; Peyton, 1956). Other examples of male

mosquitoes being attracted to hosts for sexual encounters are *Mansonia uniformis* (McIver *et al.*, 1980), *Aedes vittatus* (Cordellier & Geoffroy, 1974), *Aedes triseriatus* and *Aedes albopictus* (Reeves, 1951), *Aedes furcifer/taylori* (Jupp, 1978; McIntosh *et al.*, 1977), *Eretmapodites chrysogaster* (Gillett, 1971) and *Armigeres subalbatus* (Das *et al.*, 1983).

In most catches the periodicity of males arriving at bait is similar to that of the females. Trpis *et al.* (1973) thought that the arrival at bait of males might better indicate the underlying endogenous activity rhythm of a species than the arrival of females. They argued that an abundance of suitable hosts in the morning might result in a smaller percentage of unfed *Aedes aegypti* remaining in the local population to bite in the afternoon, thus causing a depression in the late afternoon biting peak.

Environmental conditions

Wind and rain, though not necessarily light drizzle, usually drastically reduce the numbers of mosquitoes caught biting, and catches have sometimes to be abandoned because of bad weather. It is important to know whether adverse weather conditions have prevailed during any part of a bait catch, but have not been reported, or perhaps not even noticed, by the collectors, so that the entire catch, or perhaps only part of it, can be excluded from the results. Snow (1980) reviewed the flight speed of mosquitoes, and in original observations in The Gambia recorded that above a wind speed of 1.2 m/s biting by *Anopheles melas* and *Culex thalassius* virtually ceased. But in field experiments using a wind tunnel Gillies & Wilkes (1981) found that maximum flight speed of *Mansonia uniformis/africana*, *Anopheles ziemanni* and other *Anopheles* was 1.4–1.8 m/s. Flight speed seemed to be unrelated to size, thus not substantiating the general rule of Lewis & Taylor (1967) that insect flight speed is directly correlated with body size. Failure to correlate wing size with flight speed in The Gambian mosquitoes might, however, have been because there were only small variations in wing lengths of the species they caught.

In South Africa Sharp (1983a) investigated the effect of environmental factors, such as temperature, wind speed and rain on the biting cycle of *Anopheles merus*. Not surprisingly both an increase in wind speed or rain decreased, or sometimes stopped, biting activity. Although wind and low temperature can inhibit biting, it must be remembered that some temperate and subarctic species may continue to fly in winds of 2–8 m/s and temperatures as low as around 4°C (Jaenson, 1988). Temperature changes may also cause shifts in peak biting times. For example, in Pakistan Reisen & Aslamkhan (1978) found that *Anopheles* bit mainly during the evening in the cool season, but later at night in the warm season. In East Africa *Anopheles merus* bites mainly after midnight, whereas in South Africa Sharp (1983a) found that females may bite earlier in the night when minimum temperatures drop to 16°C. In Bangladesh there were large fluctuations in the nightly biting pattern and numbers of *Anopheles dirus* caught, but none of the recorded climatic parameters, rainfall, wind velocity, cloud cover, wet and dry bulb temperatures could explain these variations. This lack of correlation between biting behaviours and climatic variables emphasised the need for large

numbers of collections when studying biting and other behavioural patterns (Rosenberg & Maheswary, 1982).

An analysis of mosquitoes, and other haematophagous insects, biting man in Panamanian forests demonstrated that biting activity is largely dependent on temperature and vapour pressure, and that most activity is concentrated in quite a narrow range of these two environmental factors (Read *et al.*, 1978). For example, the greatest numbers of *Haemagogus lucifer* were caught biting both at ground level and in the canopy when temperatures were 26.5–28.1°C, and the vapour pressure (millibars) was 31.4–32.3 (forest floor) and 29.4–30.3 (canopy). In fact, during 1973 and 1974 86–96% of both *Haemagogus lucifer* and *Haemagogus equinus* were caught biting when temperatures were in excess of 24.7°C. Both species are day-biters, and during the day temperatures are usually higher and relative humidities lower than at night, but it must be remembered that both are influenced by rainfall and wind. For instance, heavy rain will tend to lower ground temperature for hours or even days, which in turn may reduce evaporation and result in air near the ground or even in the canopy, being near saturation. Later Read & Adames (1980) investigated the relationship of air temperature, dew point temperature and evaporation on numbers (>435 000) of *Mansonia dyari* biting human baits; the following empirical regression equation was derived

$$Y = -522 + 1035 X$$

where Y = the number of *Mansonia dyari* caught biting man/24 hr and X = evaporation (mm)/48 hr. The minimum value limiting the applicability of this equation is 0.5 mm/48 hr of evaporation. It was calculated that 66% of all variations in the numbers biting is accounted for by the evaporation rate.

Read & Adames (1980) believed that the above equation could be used to predict times when biting densities should be high, but because of the complexity of factors affecting biting activity they cautioned it was best to regard predictions in terms of probabilities.

Charlwood & Galgal (1985) calculated polynomial regressions of the percentage of the total catch of *Armigeres milnensis* caught every 5 min in human bait catches against both light intensity and time. The multiple r^2 for a polynomial regression of degree 2 was 0.383 for percentage biting against light (log lux), and 0.510 for percentage biting against time, thus showing that time was a better predictor of biting activity than light intensity. Subra (1972) gives a useful account of how weather conditions affect outdoor biting by *Culex quinquefasciatus*, while Service (1980) briefly discusses the effect of wind on suppressing biting behaviour, pointing out that whereas winds of about 8 km/hr or less usually prevent host-seeking activities, arctic species seem to continue biting in such winds. In Canada for example, Haufe (1966), reported that only speeds of about 29 km/hr or more deterred mosquito flight.

Light intensity is often the most important environmental factor influencing mosquito activity, and moonlight has a biological effect on the behaviour of many animals, including mosquitoes. Bowden (1973a) has shown that at Kampala, Uganda, light from the full moon at zenith (0.2 lux) is about the same as that experienced 30 min after sunset (about civil twilight) on a clear moonless evening.

At full moon the decrease in illumination in the hour following sunset is much less than on a moonless night. On nights immediately following a full moon illumination may decrease to starlight (0.009 lux) before moonrise, but thereafter increase sharply. Muirhead-Thomson (1940) gives some interesting light readings of moonlight measured in India, while Davies (1975) and Callahan (1964) both describe the construction of inexpensive light-meters for recording moonlight and other low intensity illumination.

Many nocturnal mosquitoes are more numerous in bait catches on nights of full moon (Bidlingmayer, 1964; Charlwood *et al.*, 1986c; Pandian & Chandrashekar, 1980), it being suggested that this is due to moonlight enhancing the mosquito's ability to locate hosts, and also oviposition sites (Allan *et al.*, 1987; Charlwood *et al.*, 1988). In Senegal Hervy *et al.* (1986) found that *Aedes taylori* increased in numbers at human bait during moonlit nights, and in China Wang & Chang (1957) found that biting and flight activities of *Anopheles sinensis* were greater during moonlit nights.

It might be thought that the illumination from the moon on nights just before and after full moon might change the shape of the crepuscular biting profile of mosquitoes on these nights. However, Haddow (1964) failed to find that a full moon affected the timing of the crepuscular biting peaks of mosquitoes inhabiting the forest canopy near the equator. Furthermore, although Corbet (1964) found that the numbers of mosquitoes caught in light-traps above the forest canopy may be *less* on nights with a full moon, there was no evidence that their times of appearance significantly differed from those recorded on nights with little moonlight. In human bait collections in India the biting cycle was exactly the same whether catches were performed in houses or out of doors (Pandian & Chandrashekar, 1980). Similarly Krafur (1977) reported that endophagic and exophagic *Anopheles* in Ethiopia had similar biting cycles. McClelland (1960) found that in coastal Kenya the indoor biting cycle of *Aedes aegypti* exhibited no pronounced peaks, although in Uganda an outdoor biting population showed pronounced peaks in biting (McClelland, 1959).

In contrast to many of the foregoing examples van Someren & Furlong (1964) showed that on Pate Island, just off the Kenyan coast, moonlight had a pronounced effect on the biting times of *Aedes pembaensis* and *Aedes mombasaensis*. With *Aedes pembaensis* biting appeared to be enhanced by moonlight and inhibited by darkness and so biting was most intense in the early evening at new moon and during the first quarter, times when the moon rose before sunset. When the moon rose after sunset, such as at full moon and in the last quarter, the early evening wave of biting was depressed, but the early morning peak was bigger. With *Aedes fryeri* moonlight appeared to modify times of biting at spring tides, whereas at neap tides the phases of the moon did not appreciably alter the biting pattern. Also more females of both *Aedes pembaensis* and *Aedes mombasaensis* were collected at bait during spring tides. Gillies & Furlong (1964) found that there was a tendency for a higher proportion of *Anopheles parensis* to bite later in the night when the moon rose late, such as during the last quarter. At new moon there appeared to be a slight increase in biting just after 1800 hr, and just before 0600 hr. In other words there was a slight, but significant, tendency for

increased biting activity during periods of moonlight, compared with hours of greater darkness. In Papua New Guinea *Anopheles farauti* bites mainly in the middle and latter parts of the night during full moon periods, but on moonless nights most activity is in the early evenings (Charlwood *et al.*, 1986c). In Colombia there were earlier peaks of biting by *Anopheles punctimacula* and *Anopheles nuneztovari* during light moon phases (crescent and full), whereas by contrast *Anopheles darlingi* showed the same degree of biting during the new and crescent phases (Elliott, 1972). Davies (1975) found that at new moon in Trinidad there were biting peaks of *Culex portesi* and *Culex taeniopus* on mice during twilight of the evenings and mornings, although the dawn peak was not very pronounced with *Culex taeniopus*. At full moon, however, the evening and dawn peaks were replaced with increased activity at moon rise and during the middle of the night.

In Bangladesh the most concentrated biting by *Anopheles dirus* with respect to time (i.e. sharpest peak) occurred during the first quarter of the moon, when about half of the moon was above the horizon as early as sunset (approx. 1842 hr) (Rosenberg & Maheswary, 1982). As the quarters advanced moonrise was progressively later, until during the last quarter moonrise was near midnight (Fig. 5.1b) and biting peaked at midnight (Fig. 5.1a). During new moon when there was no moonlight, biting peaked at 2200 hr and remained high until 0145 hr, and this activity was believed to represent their intrinsic biting pattern. In Borneo, Colless (1957) found that in his catches (2000–2300 hr) more *Anopheles balabacensis* were caught out of doors between half and full moons than during other moon phases. During the first half of the night in Uganda biting catches of *Anopheles implexus* were similar at all phases of the moon, but in the latter half of the night, greater numbers were caught at full moon and activity was prolonged (McCrae *et al.*, 1976).

Provost (1958) found that swarming in *Psorophora confinnis* was extended for at least an extra hour at full moon because illumination had not dropped to the critical level (0.02 lux). In studying the diel and seasonal flying activities of *Culicoides* species in Florida Lillie *et al.* (1987) divided the 24-hr day into 20 periods based on times of sunset, sunrise, and twilight so that catches at different times of the year could be compared when there were changes in the duration of photophase and scotophase. Photophase was represented by 10 equal periods (which ranged from 62 to 84 min depending on the time of year). Period 1 started at sunrise, while period 10 ended at sunset (Fig. 5.1c). Evening twilight comprising the time from sunset to the end of civil twilight was period 11 (52–60 min). Scotophase comprised 8 equal periods (60–90 min) starting with period 12 at the end of twilight and ending with period 19 at the beginning of morning twilight. The duration of these periods will of course not just vary seasonally but in different parts of the world.

Further information concerning moonlight and its possible effects on insect behaviour is to be found in the publications of Brown & Taylor (1971), Bowden (1973a,b), Bowden & Church (1973), Beck (1968) and Bidlingmayer (1967, 1985), see also the effect of moonlight on light-trap catches in Chapter 6 (pp. 538–40).

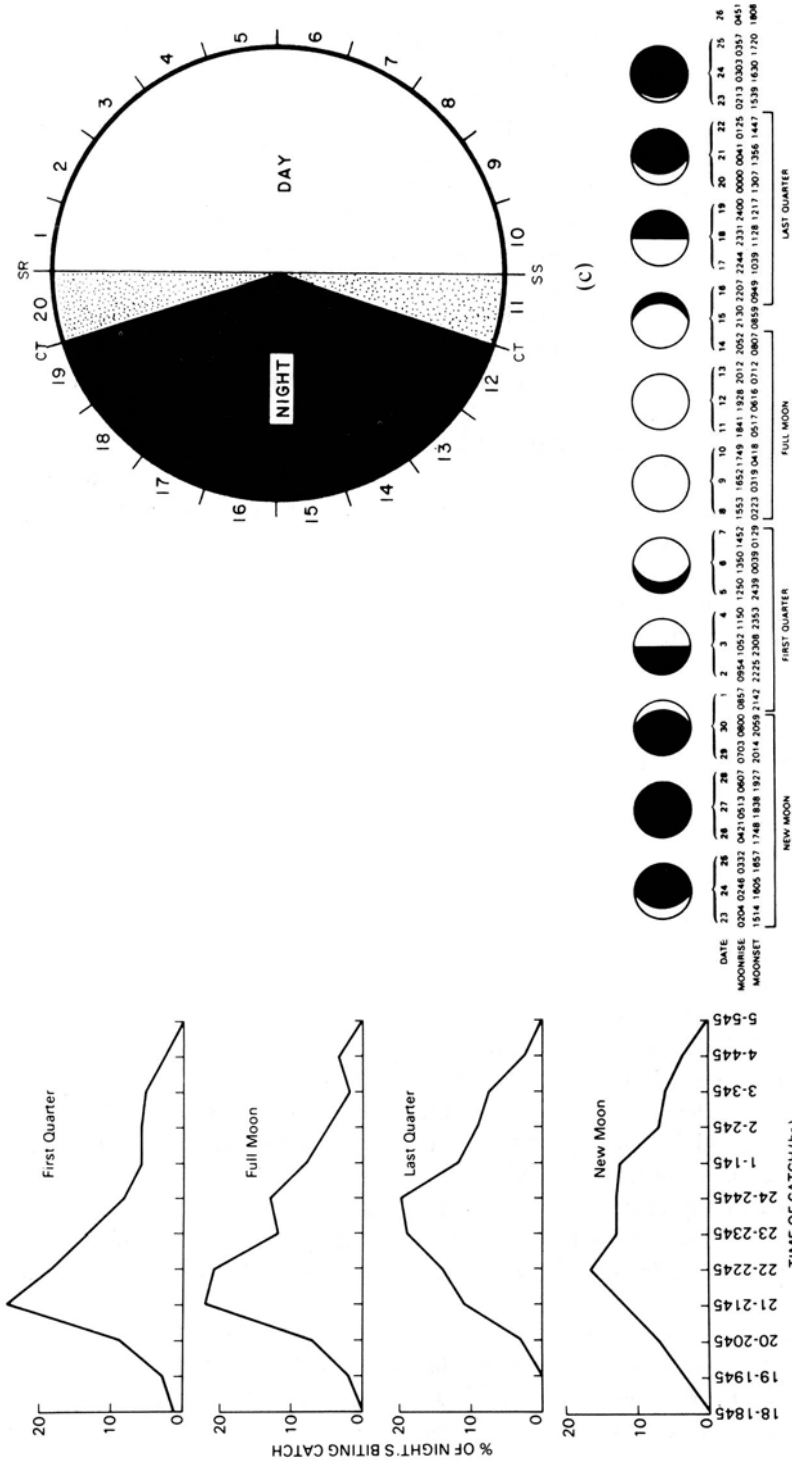


Fig. 5.1. (a) Influence of moon phase on *Anopheles dirus* biting man outdoors (Rosenberg & Matheswary, 1982); (b) phases of moon and times of moonrise and moonset at 20° N latitude, 23 June to 26 July 1976. Time of sunset was 1943 hr on 23 June and 1840 hr on 26 July (Rosenberg & Matheswary, 1982); (c) division of diel cycle into 20 periods, based upon times of sunrise (SR), sunset (SS), and civil twilight (CT). (Lillie et al., 1987).

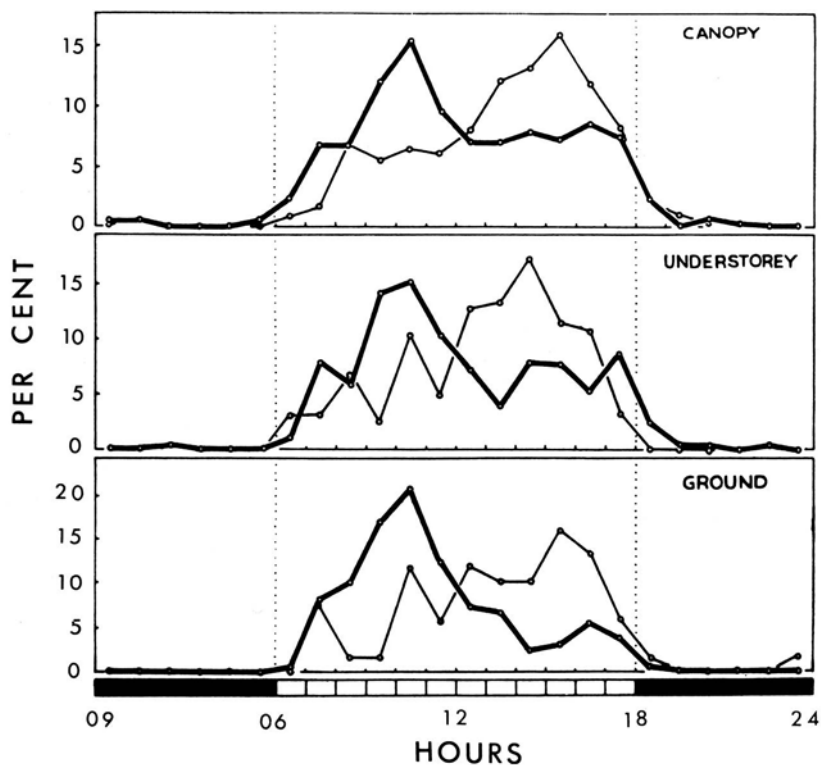


FIG. 5.2. Biting cycle of *Aedes apicoargenteus* by hour and level in Zika forest (thick line) and Bwamba (thin line), Uganda (after Haddow, 1961a).

Twilight and crep units

Frequently the numbers of mosquitoes caught biting each hour, or more usually the transformed counts obtained from a series of similar catches, are expressed as percentages of the total 24-hr catch to give diel biting profiles of the species (Fig. 5.2). Sometimes the numbers caught in smaller time intervals, such as every 5, 10 or 15 min, or even each minute (Haddow, 1956*b*, 1964) are recorded (Fig. 5.3). The beginning or cessation of biting in many species appears to be initiated by changes in illumination, and biting profiles are often correlated with times of sunset and sunrise. Such correlations may be difficult because these times alter according to both locality and season. The importance of adjusting catch times in relation to exact times of sunset and sunrise was stressed by Lumsden (1952, 1957*a*) and Haddow (1954). Even at the equator a variation of 31 min can occur between times of sunset at different times of the year. Much greater variations in sunset and sunrise times are encountered further from the equator. During a series of bait catches in May to September in England the difference between times of sunset and sunrise was about 2.5 hr. It was shown that in August the peak biting times of *Aedes detritus* and *Coquillettidia*

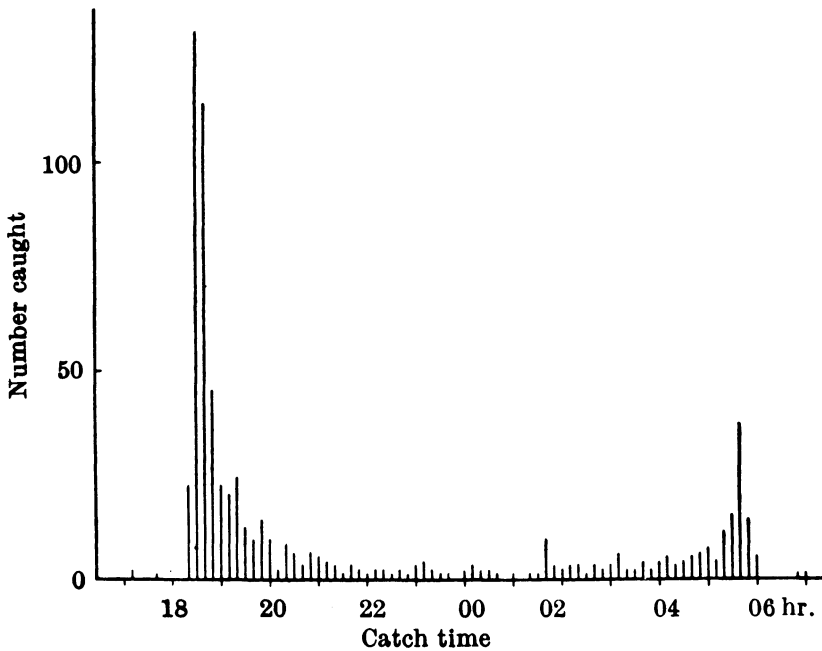


FIG. 5.3. Females of *Coquillettidia fuscopennata* caught in Uganda in a series of 24-hr human bait catches expressed as numbers caught each 10-min period. Sunset at 1800 hr (from Haddow, 1956b)

richiardii were an hour earlier and later than during July (Service, 1969a), corresponding to the new times respectively of sunset and sunrise.

Another difficulty is that away from the equator day length varies and consequently it is difficult to adjust the catch clock to times of both sunset and sunrise. One method, at least near the equator, is to use sun times, that is to start each continuous 12-, 18- or 24-hr catch at the time of sunset, which in fact will entail catches beginning at different clock times (Lumsden, 1952).

Nielsen (1963) pointed out that illumination differs in two important respects from environmental measurements, such as temperature, humidity and wind speed, in that the daily variations are enormous. Secondly, under standard conditions, that is excluding the effects of cloud cover, haze, etc., the level of illumination at any locality at any time can be precisely calculated. This is because illumination depends only on the sun's altitude which is completely predictable. The sun's altitude is measured as the angle (As) between a line from the centre of the sun to the observer and from the observer to the horizon. The angle of elevation depends on latitude (f), the date and hour, and is calculated as follows:

$$\sin (As) = (\cos t \times \cos f \times \cos d) + (\sin f \times \sin d)$$

where t = the hour angle and d = the declination of the sun. Values for each day of the year and for each hour of local time are found in navigational tables (e.g. The American Ephemeris, The Nautical Almanac, The Air Almanac, and Ex-

planatory supplement, 1961). Local time is derived from standard time (e.g. Middle European time, Eastern standard time) by adding 4 min for each degree of longitude west on which standard time is calculated. Similarly 4 min are subtracted for each degree east of the longitude on which standard time is based.

Nielsen (1963) defined civil twilight as the period when the sun passes from 0°.50' to 6°00' below the horizon, and on this basis nautical and astronomical twilight would end when the sun was 12° and 18° respectively below the horizon. The Smithsonian Physical Tables also define sunset (astronomical) as lasting until the sun is about 18° below the horizon, but some authorities (Nautical Almanac) give twilight not as a duration but as the moment when the sun is 6°, 12° or 18° below the horizon. Nielsen's definition is retained here as it is convenient to talk about a twilight period. Using it in this sense, the duration of twilight varies considerably according to locality and season. At the equator the sun sets at 1800 hr and twilight lasts only 20–23 min, but its duration increases progressively further from the equator. At latitudes greater than 50° twilight lasts until midnight, above a latitude of 61° it lasts all night at midsummer and at latitudes above 67° there is no twilight as the sun never sets. It is important to realise that the same changes in light intensity are experienced during twilight irrespective of locality. At the moment of sunset light intensity is 395 lux but by the end of civil twilight it has been reduced to 3.55 lux. The only difference is that nearer the equator the changes in illumination are faster. It is therefore clear that it is not meaningful to compare biological phenomena, such as biting cycles, with clock times in localities having widely different latitudes. To facilitate comparisons at different latitudes and also in different seasons, Nielsen (1961) introduced the crep unit, which is defined as the interval between sunset and the end of civil twilight, i.e. the period when light is decreasing to the level of 3.55 lux. Crep values are calculated as follows:

$$\text{crep value} = \frac{\text{time of day} - \text{time of sunset}}{\text{duration of civil twilight}}$$

or

$$\frac{\text{time of sunrise} - \text{time of day}}{\text{duration of civil twilight}}$$

Positive crep values refer to periods when the sun is below the horizon and negative values when it is above the horizon. A value of 0 corresponds to times of sunset and sunrise, and + 1 indicates the end of twilight in the evening or the beginning at dawn. Figure 5.4a shows the crepuscular biting cycles of *Coquillettidia richiardii* and *Aedes detritus* in England plotted against minutes before and after sunset and sunrise, and also against corresponding crep values (Service, 1969a). Some of the relatively few studies that have used crep units are those by Forattini & Gomes (1988) on biting cycles of *Culex ribeirensis* (Fig. 5.4b) and *Aedes scapularis* in Brazil, and by Forattini *et al.* (1981) on various Brazilian culicine mosquitoes.

Useful tables of illumination in log lux + 10 (this does away with the use of a negative index) corresponding to both positive and negative crep values, of the relationship between crep and the sun's altitude and also the correction in log

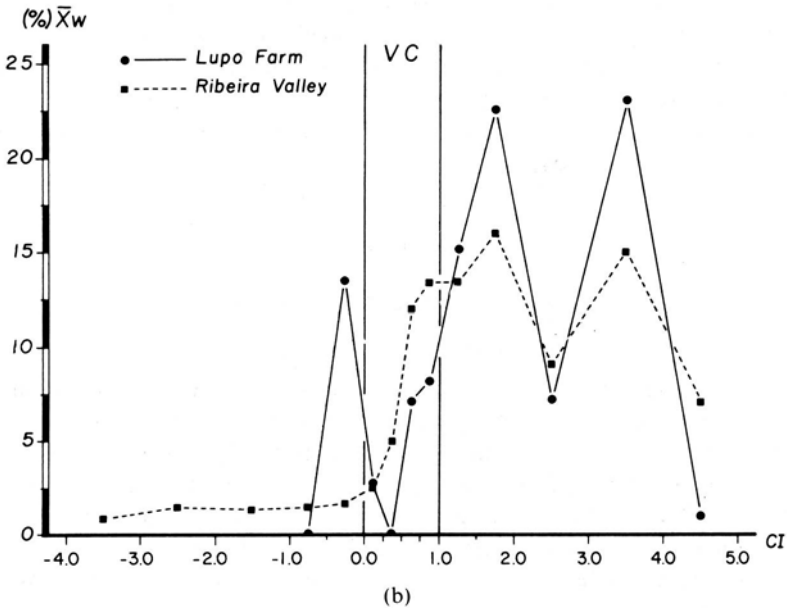
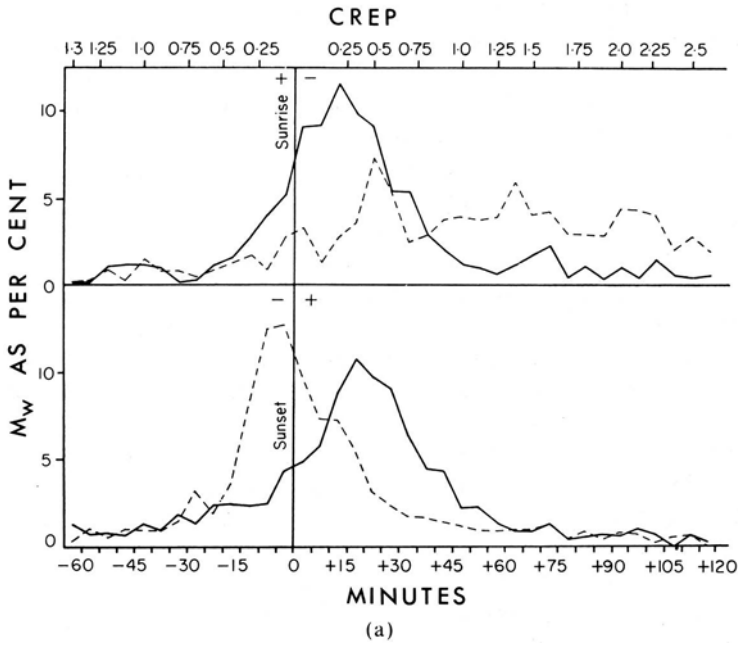


FIG. 5.4. (a) Crepuscular biting cycle of *Coquillettidia richiardii* (solid line) and *Aedes detritus* (broken line) in relation to times before (–) and after (+) sunset and sunrise, and also crep units (from Service, 1969a); (b) vespertine crepuscular and pericrepuscular biting by *Culex ribeirensis* at human bait at two localities (Lupo farm, Ribeira valley) in Brazil, CI — crep intervals corresponding to 1700–2000 hr; VC — vespertine crepuscular period (Forattini & Gomes, 1988).

lux that must be added in different months for latitudes greater than 40°, are presented by Nielsen (1963). Haddow (1964) gives some minute to minute zenith light readings taken near the equator in Uganda and discusses these in relation to mosquito biting activity and the readings of illumination calculated by Nielsen (1963). Haddow *et al.* (1968) have shown that even at, or near, the equator when twilight is of such a constant duration the conversion of catch times to crep values is still well worthwhile, as it shows better than clock times the forms of waves of biting activity. One of the more interesting facts to emerge from this study was that peak biting activities were governed more by the sun's altitude than clock times. This important paper should be consulted for an account of a detailed analysis of catch data with reference to crep units, and also for data on light values at sunset, including the rate of decay of illumination. In the USA Wright & Knight (1966) studied the effect of decreasing light intensity (and also changes in temperature and relative humidity) around sunset on the biting density of *Aedes vexans* and *Aedes trivittatus*.

The effect of polarised light on mosquitoes arriving at bait was studied in North America by Wellington (1974). He found that nearby resting adults of various *Aedes* and *Culex* species arrived at human bait irrespective of whether polarised light was present, but mosquitoes arrived at bait from further afield only when there was natural polarised light present. Polarisation is most intense near sunset and sunrise. Passing clouds reduced polarisation and this was associated with a reduction in the numbers of mosquitoes arriving at bait.

Location of bait catches

Human bait collections can be performed in a variety of environments, such as in houses, animal shelters, caves, on verandahs and in buildings and natural shelters, or outdoors in village compounds (Fig. 5.10a), cleared bush, banana plantations, and farms and forests. On Samoa Samarawickrema *et al.* (1987) used two two-men teams to collect *Aedes polynesiensis* in a series of 10-min human bait catches at different sites from 0830–1030 hr and from 1600–1800 hr, and for *Aedes samoanus* from 1930–2130 hr. One team, consisting of bait and collector, caught indoors while the other team worked out of doors. More rarely catches were performed indoors and outdoors from 0600–1800 hr, and just indoors from 1800–0600 hr. In Jakarta *Aedes aegypti* were caught by a team of three people catching adults inside houses for half-hour periods every hour, from 0600–1800 hr. At the end of every hour one of the collectors was replaced by another, and by employing a team of six people each collector worked 3 hr then had 3 hr rest (Nelson *et al.*, 1978). From biting collections in 72 houses during both the wet and dry seasons the largest catches were obtained in houses where breeding was occurring. It was tentatively suggested that adults may be attracted to human bait in the same houses from where they had emerged, and/or that after feeding adults may tend to oviposit in the same houses.

Gass *et al.* (1982) pointed out that with mosquitoes such as *Mansonia* a comparison of only indoor and outdoor biting did not always identify the true degree of vector-man contact. For example, in Thailand they found that the ratios of the numbers caught biting in forests, on verandahs and indoors was 7.2:2.2:1

for *Mansonia annulata*, and 9.5:3.9:1 for *Mansonia uniformis*, demonstrating that although both species were reluctant to enter houses a substantial number bit people on verandahs. Moreover, peak biting occurred just after sunset, a time when people relax on their verandahs. Das *et al.* (1983) studied daytime biting by *Armigeres subalbatus* in India by having collectors catch mosquitoes from a man lying on a bed inside a house, and from another person on a bed outside a house.

In Australia Kay (1985) compared the numbers of mosquitoes biting human baits indoors in darkness, indoors with a 75-W bulb, and out of doors in natural darkness. Catches were made from two humans simultaneously in two paired situations from 1900–1945 hr on 12 nights on each of four visits, in a randomised block design so that each paired situation (e.g. bait 1 indoors in darkness and bait 2 indoors in light) was replicated. To minimise collector bias each situation evaluated contained an equal number of collections by each collector. For example, for the 12 nights, each situation (indoors/dark; indoors/light; outdoors/dark) was analysed on the basis of eight collections each, i.e. four each by collectors 1 and 2. Catches were transformed to $\log(n + 1)$ and results analysed by 2-way ANOVA to detect any differences between situations (2 degrees of freedom (d.f.)), changes in abundance during the four trips (3 d.f.) and their interactions (6 d.f.). This useful paper on the statistical design of experiments to compare vector-man contact and the risk of being infected with pathogens in different situations should be read.

In Mexico in an evaluation of the effect of insecticidal residual house-spraying Bown *et al.* (1984) organised simultaneous indoor and outdoor human bait collections from 1800–0600 hr. The indoor and outdoor collectors changed places every 3 hr and each collected for 6 hr before being relieved. In addition *Anopheles albimanus* engorging on a person seated near the door of a house were dusted with fluorescent powders and their movements into the house followed with the aid of an ultraviolet lamp. Their activities, such as number of landings, types of resting surfaces in the house and duration of resting times, were recorded for 1 hr. At the end of this period the mosquitoes were collected and kept for 24 hr to determine their survival. In Brazil a series of human bait collections were made 10, 20 and 40 m from a house. Only *Anopheles darlingi* was caught in collections made nearest the house, but six *Anopheles* species, *Aedes fulvus* and *Psorophora cingulata* were caught 20 m from the house, and six *Anopheles* species, *Aedes fulvus*, *Psorophora cingulata* and *Culex spissipes* were caught biting 40 m distance (Roberts *et al.*, 1987).

In Ethiopia Krafur (1977) employed two people to collect mosquitoes attempting to bite a sleeping person inside a house from 1800–0700 hr. He compared the numbers of *Anopheles* caught biting per man-hour inside and outside houses, and also estimated the biting rate deduced from pyrethrum spray-sheet collections in houses. For example, the mean number of bites per man per night estimated by the total catch over a year of *Anopheles wellcomei* biting indoors divided by the number (in this instance 276) of man-nights was 1.95. But the mean number biting a man per night obtained by dividing the mean number caught in a house by the proportion of those that were blood-fed, and then dividing this by the numbers of people in the house was 0.005. The ratio of

1.95:0.005 shows that the estimated average man-biting rate inside houses was in fact 390 greater than suggested by collections from space-spray collections made in the early morning. This clearly shows that while *Anopheles wellcomei* may be partially endophagic, it is strongly exophilic.

In studying malaria transmission in Bangladesh Rosenberg & Maheswary (1982) undertook human bait catches inside a house and simultaneously had two men seated outside 15 ft away under a 4-m² tarpaulin sheet. Each night's catch consisted of hourly 45-min collections from 1800–0600 hr. The 15 'free' minutes each hour were used to record rainfall, wind velocity, cloud cover, and wet and dry bulb temperatures; and also to collect mosquitoes from exit traps and light-traps. One team of two collectors worked from 1800–2400 hr, and the other from 2400–0600 hr, and indoor and outdoor teams exchanged positions hourly. From 198 collections over 21 months 6098 *Anopheles* of 15 species or species groups were caught. By far the commonest species was *Anopheles dirus*, of which in 1975 81.4% were caught biting outdoors, whereas the following year only 57.5% were caught outdoors. Other anophelines included *Anopheles philippinensis*, *Anopheles maculatus*, *Anopheles karwari*, *Anopheles annularis*, *Anopheles vagus* and the *Anopheles hyrcanus* group.

In China using a so-called special human bait hut (HBH) to sample anthropophilic mosquitoes was considered much more realistic than bait collections on mosquito workers. In 1982 and 1983 the mean number of bites per night by *Anopheles sinensis* in the human bait hut was 1.7 and 1.0, whereas in more artificial human bait collections the mean numbers were 14.6 and 20.9 (Guan *et al.*, 1986).

Catches can be performed at ground level or at various heights up to the tree canopy by fixing wooden ladders and platforms to forest trees (Aitken *et al.*, 1968b; Bates, 1944a; Bugher *et al.*, 1944; Deane *et al.*, 1948; Galindo *et al.*, 1950; Garnham *et al.*, 1946; Haddow *et al.*, 1947; Happold, 1965; Mattingly, 1949a,b; Novak *et al.*, 1981; Trapido & Galindo, 1955, 1957). Alternatively, steel or wooden towers can be erected which allow catches to be performed not only at various heights up to the forest canopy but also beyond it (Haddow, 1964; Haddow *et al.*, 1961; Haddow & Ssenkubuge, 1965; Rickenbach *et al.*, 1971). To enable catches to be made at different sites, but at the same height a small bridge, or walk, has been suspended between trees in a Panamanian forest. Some species, e.g. *Aedes ingrami*, *Aedes africanus*, *Coquillettidia aurites*, *Coquillettidia pseudoconopas*, have been shown to exhibit pronounced daily vertical migrations within the forest (Germain *et al.*, 1972, 1973; Haddow, 1954, 1961a,b; Haddow & Ssenkubuge, 1965; Mattingly, 1949a). The percentage biting at different levels can vary according to time. The species composition and biting cycles of the same species may differ according to locality, habitat and height (Galindo *et al.*, 1950; Germain *et al.*, 1972; Haddow, 1945b, 1961a,b; Haddow & Ssenkubuge, 1965; Happold, 1965; Lumsden, 1958a; Rickenbach *et al.*, 1971; van Someren & Furlong, 1964). In Zika forest, Uganda McCrae *et al.* (1976) showed by 24-hr human bait catches that biting times of *Anopheles implexus* varied greatly according to different ecological zones. For example, within the forest about 66% of the mosquitoes were caught during the day whereas at the exposed forest edge only 3% bit during the daytime.

In common with many other sampling techniques, bait collections in certain localised sites may result in larger catches than similar catches made only a short distance away (Service, 1969*a*, 1971*b*). For example, in Papua New Guinea Charlwood *et al.* (1984) found that the numbers of *Anopheles farauti* caught in human bait catches in nearby areas in a village differed considerably, emphasising that the number of bites people receive depends on their whereabouts in the village. Again this stresses the difficulties of realistically estimating biting rates (pp. 384–93). Furthermore, species composition may also differ over relatively short distances (Service, 1971*b*). As with other sampling procedures it is therefore important that collecting procedures are strictly standardised.

Duration of catches

Bait catches are frequently performed for about 1–3 hr, but sometimes for much shorter periods lasting only a few minutes (Thompson & Dicke, 1965). In Florida Freier & Francy (1991) had a collector attract *Aedes albopictus* for 2 min, but did not allow any to land on clothing or exposed lower legs, then for the next 5 min those mosquitoes that landed on the body were collected. When biting activity is mainly crepuscular bait catches may be restricted to about 1–2 hr before sunset and 1–2 hr after sunrise. If biting starts around dusk and continues during the night, it is usually more productive to continue collecting throughout the night than just for a part of it, but in El Salvador although *Anopheles albimanus* could be caught biting throughout the night a 2-hr bait catch in the early evening was sufficient for monitoring changes in biting density (Rachou *et al.*, 1965). In Tanzania, Corbet & Smith (1974) concluded that because of the consistency of diel landing rates of *Aedes aegypti* it was unnecessary to catch this species over its entire biting cycle (about 0500–1900 hr) but for only a part of this time, 0600–0900 and 1500–1900 hr, to obtain reliable measurements of density. Many diurnal species can be adequately sampled by short daytime bait catches. For example, in studying the dispersal of domestic and peridomestic *Aedes aegypti* by mark–recapture methods Trpis & Häusermann (1986) caught adults during 15-min biting collections in 16 houses in a Kenyan village.

Haddow (1945*a*) appears to have been the first to have introduced and emphasised the importance of a continuous 24-hr bait catch for collecting representative samples of all anthropophilic mosquitoes in an area. A detailed appraisal of the 24-hr catch technique, which has been widely adopted though sometimes with modification, is given by Haddow (1954). Cheong *et al.* (1988) provide a good example of 24-hr bait catches undertaken in Malaysia, in which at least 13 culicine species were collected. Because an unusually high biting rate may be experienced at the start of any catch (Haddow, 1954; Service, 1969*a*, 1971*b*; pp. 352–3) 25-hr catches are sometimes performed and the numbers caught during the first hour excluded from the results (de Kruijff, 1972). In a Trinidadian forest for example, bait catches were undertaken for 25 hr because it was noticed that at the start of each catch abnormally large counts were encountered (Aitken *et al.*, 1968*b*). In addition, the arrival, every 2 hr, of the relief team of catchers also resulted in an increased catch. This was probably caused by mosquitoes being attracted to the movements of the catchers and following them

as they walked to the catch site. To overcome this, catching commenced at even and odd hours on alternative working days.

Although bait catches may be made for 12 or 24 hr, mosquitoes may not be collected continually throughout this period. For instance, in California Cope *et al.* (1986) collected an average of 828 mosquitoes/night, mainly *Culex erythrorhax*, by two people catching mosquitoes at human bait for 15 min in each hour, from 1900–0600 hr. In Texas in 14-hr human bait collections one person exposed his bare legs and arms to mosquitoes while the rest of his body was protected by mosquito netting, heavy clothing and boots. Collections were made for only 15-min periods each hour (Roberts & Scanlon, 1975). The biting behaviour of *Anopheles darlingi* in Brazil was investigated by having two human baits collecting together for 30 min every hour throughout the night in a house, while at the same time a similar collection was performed outside 20 m away. Sometimes, however, because of high biting densities just a single person caught mosquitoes, inside or outside, for 10–15 min of each hour (Roberts *et al.*, 1987). In the Dominican Republic Mekuria *et al.* (1990) performed human bait catches for 45-min periods each hour from 1800–0600 hr.

In Sweden Andersson (1990) used hand-nets for 5–10 mins at 30-min intervals to collect 16 species of mosquitoes arriving at bait, either throughout the 24-hr day or from 1600–0300 hr; the shorter time being used when biting densities were high. Biting activity was recorded as the numbers of mosquitoes, mainly *Aedes communis* (63%), caught per minute. In Sri Lanka Amerasinge & Munasingha (1988) compared the numbers of species collected in diurnal (1400–1700 hr) and nocturnal (sunset plus 6 hr) human bait collections, in CDC light-traps placed outside houses, and from searches inside thatched temporary huts. A total of 1172 male and 14071 female mosquitoes belonging to 71 species were identified, and although most (50%) were caught in the light-traps they trapped large numbers of only a few species (*Mimomyia hybrida*, *Culex pseudovishnui*). Thirty-two species were collected resting indoors, the most common being *Mansonia annulifera* and *Culex quinquefasciatus*. In human bait catches 38 species were collected biting during the afternoons, the most common being *Aedes albopictus*, *Aedes w-albus*, *Aedes novalbopictus* and *Aedes jamesi*, whereas 48 species were caught in night-time collections, the most common being *Mansonia annulifera*, *Culex gelidus*, *Culex fuscocephala*, *Mansonia uniformis* and *Mansonia indiana*.

Continuous catches of longer duration than 25 hr, may be lasting several days, are sometimes performed, such as the 96-hr bait catches in Kenya performed by Linthicum *et al.* (1984).

In studying outdoor biting by *Anopheles gambiae* Haddow & Ssenkubuge (1973) found that although there were general trends, it was nevertheless 'dangerous' to sum nightly or daily bait catches to establish biting patterns, because important but subtle variations could easily be missed.

Stationary direct bait catches

In these man acts both as bait and collector. Kerr (1933) working in West Africa was largely responsible for developing the collection of mosquitoes from man as a routine sampling method, the technique being later modified and standardised

by Kumm & Novis (1938) in South America. A common procedure for conducting a bait catch is for a person to sit on the ground, or stool, and allow hungry unfed mosquitoes to alight on his clothing or exposed skin. After a mosquito has landed it may 'freeze' for a few seconds and during this period it is readily disturbed by the host's movements. Following this short initial period of apparent inactivity there is usually a short exploratory period before the mosquito actually probes the skin (Gillett, 1967). Service (1971a) studied the feeding behaviour of several British species, while Yates (1979) investigated the feeding behaviour of *Aedes geniculatus*. They recorded the total time (116–240 s) spent on the host but also divided this into three behavioural pauses, the exploratory period (7–31 s), penetration period (25–92 s), and the feeding period (82–150 s). Mosquitoes generally spent at least 1 min hovering in the vicinity (1–2 m away) of the host prior to alighting. Clearly the behaviour of host-seeking mosquitoes will vary among different species, but whenever possible they should be caught before they have had a chance to insert their mouthparts. Not only does this avoid unpleasant irritations due to the act of biting, but in some areas will be important in eliminating the risk of acquiring mosquito-borne infections. In practice, however, it may prove impossible to collect all adults before they have bitten and it may therefore be advisable that collectors take prophylactic antimalarial drugs and are immunised against yellow fever. Shirt sleeves or trouser legs may be rolled up or shirts removed, but frequently there is no need to expose bare skin to attract mosquitoes. In fact, it is sometimes advantageous to wear 'protective' clothing (Roberts & Scanlon, 1975). If the weather is not too hot, an anorak, or similar water-proof hooded coat, with hood pulled over the head can be worn by the collector who sits on the ground with outstretched legs. Mosquitoes being unable to bite the back of the bait because of the close weave of the anorak are forced round to the front, where once they have settled can be caught by carefully placing a test tube, or some other suitable transparent vial, over them. Some four to five mosquitoes separated by cotton wool plugs can be conveniently caught in a 13-cm long test tube, but if several species are caught at bait and more than one individual confined in the space between the plugs of cotton wool, it may be subsequently difficult to sort out the catch without killing the mosquitoes. In Ohio Haramis & Foster (1990) supplemented afternoon human bait catches for *Aedes triseriatus* with 1 kg dry ice placed 0.5 m from the collectors. For other examples of adding carbon dioxide to bait catches see pp. 504–5. Both Loor & DeFoliart (1970) and Clark *et al.* (1985) reported they caught more mosquitoes, such as *Aedes triseriatus*, if the hand was placed on top of the head thus exposing the forearm to mosquitoes. In Britain Packer & Corbet (1989) studied seasonal abundance of host-seeking *Aedes punctor* with human baits always facing downwind of any breeze and collecting was restricted to 1.5 hr before to 0.5 hr after sunset.

Occasionally mosquitoes are caught in chloroform killing tubes (Beadle, 1959; Galindo *et al.*, 1950). They can also be collected with small battery operated aspirators, or in oral aspirators and then blown into suitable containers. These consist of small Barraud cages (Fig. 5.5a), cardboard cartons with one or both ends removed and replaced with netting, or glass cylinders or lantern globes

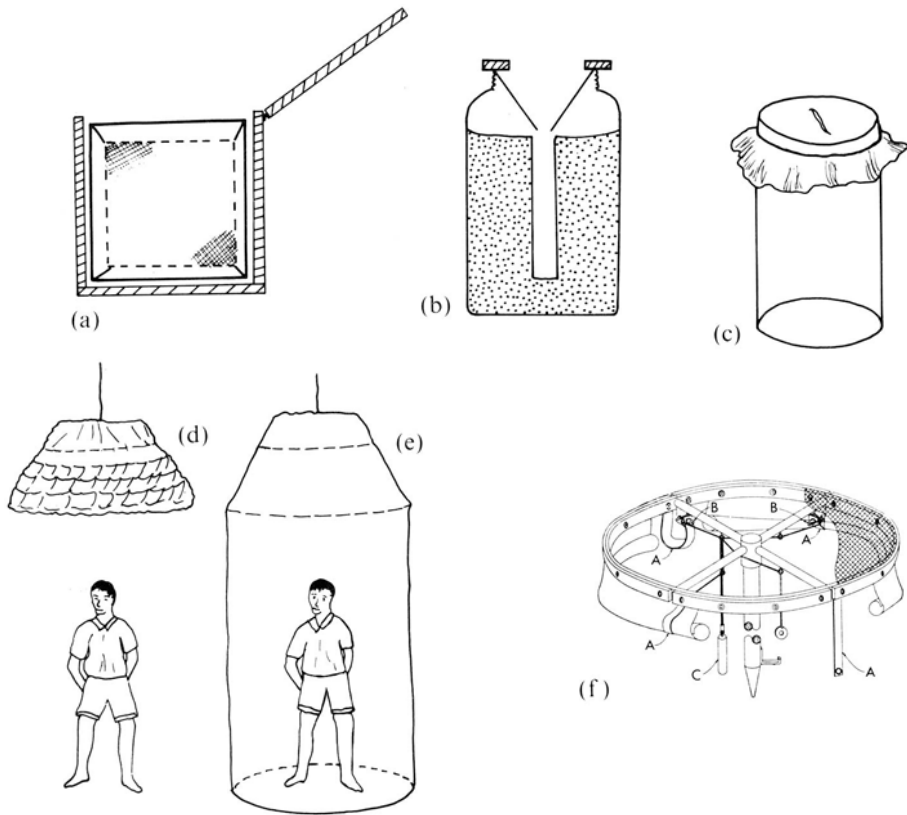


FIG. 5.5. (a) Small Barraud cage placed in wooden box for transportation; (b) Kilner (Mason) jar lined with plaster of Paris; (c) container with self-closing slit in rubber top; (d) drop-net rolled up; (e) drop-net descended to enclose bait; (f) 'umbrella-type' drop-net showing A — four cloth straps, B — pins, C — handle (after Klock & Bidlingmayer, 1953).

which have a piece of rubber sheeting with a 1-in slit stretched over one end (Fig. 5.5c). This slit allows the end of the aspirator to be inserted to discharge the catch, but conveniently closes on withdrawal. Another useful container consists of a quart-sized Kilner or Mason jar having a screw cap lid. One or two 1-in wide vertical strips of adhesive tape are fixed to the inside walls, and then the bottom and walls coated with plaster of Paris. When this is dry the strips are removed to reveal windows through which the catch can be observed (Fig. 5.5b). Mosquitoes are blown from an aspirator into the jar through a plastic funnel which is inserted into its mouth (Aitken *et al.*, 1968a). If the plaster is dampened before use mosquitoes can be held in these jars with very little mortality for a considerable time. They are also useful for keeping blood-fed mosquitoes alive in the laboratory. In Colombia Bates (1944a) abandoned the use of oral aspirators in bait catches because of the possibility of getting the tongue infected with larvae of *Dermatobia* through sucking up mosquitoes harbouring their eggs.

Whether a single person or a group of people participate simultaneously in bait catches depends on the resources available, the type of bait catch and information required. Much useful information can be obtained from bait catches performed under standardised conditions by a single person (Service, 1969*a*; 1971*a,b*). When more than one person participates it is essential to minimise individual bias in both the skill and collecting efficiency of the catchers and in their attractiveness to mosquitoes, which is likely to vary from person to person (Freyvogel, 1961; Khan *et al.*, 1965, 1971; Shidrawi *et al.*, 1974; von Rahm, 1956, 1958; Woke, 1962). No one person or group of people should always catch mosquitoes during the same specific time interval in a continuous bait catch, or from one locality when catches from more than one are being compared. In other words there should be a 'rotation of the catchers'. Haddow (1954) gives an explicit account of how he reduced this type of sampling bias. One difficulty in performing continuous bait catches is that in order to minimise bias a large number of collectors usually has to be recruited. The sources of variation in catch size that may be encountered in human bait collections have been well described by Kettle & Linley (1967, 1969*a,b*) for *Ceratopogonidae*. These important papers should be read as they describe how the actual catches from individuals had to be adjusted to take into consideration differences between individual attractiveness of the collectors, location of catches, time of catches and catches from different limbs. A correction factor in logs was computed for each species from all these sources of variation and was added to the individual transformed catches ($\log(x+1)$) whenever $x > 0$.

Many haematophagous Diptera have well-defined preferred biting regions. As long ago as 1921 Wesenberg-Lund reported that in Europe *Aedes cinereus* normally bit the legs, but also the hands if they were placed down amongst the grassy vegetation harbouring the insects. Haddow (1954, 1956*a*) found that 97–98% of the adults of *Eretmapodites chrysogaster* bit a standing man below the knees, almost entirely from the ankles upwards, i.e. in a well-defined band of 6–18 in from the forest floor. A man lying horizontally on the ground was rarely bitten, but if he was raised some 6 in all parts of the body were attacked. *Aedes simpsoni* feeds mainly on the head (Haddow, 1945*a*), and Aitken has observed that *Sabethes belisarioi* bites almost exclusively on the nose (Gillett, 1971). Self *et al.* (1969) reported on the preferred biting sites of *Culex quinquefasciatus* on adult Myanmar males. In India Das *et al.* (1983) collected 58.3% of *Armigeres subalbatus* from people's lower extremities while only 10.07% and 7.48% bit the abdomen and face respectively. In Malaysia Moorhouse & Wharton (1965) conducted human bait catches by having one man standing, another sitting down and a third lying down. Every hour these three baits changed their positions.

When catches are performed at night, or inside dark houses or shelters, subdued light from torches or hurricane lamps is used to locate mosquitoes settling on the body. In Kenya head-mounted torches have been employed during all night bait collections (Chandler *et al.*, 1975*b*). Murphey & Darsie (1962) used a red lens over their torches which only transmitted light of 6720–6869 Å, which they considered did not disturb feeding mosquitoes. Red cellophane was placed over a torch by Aitken (1967) for catching mosquitoes at night in Trinidadian

rain forests, and used by Grimstad & DeFoliart (1974) in Wisconsin so that the light had a wavelength of about 6800 Å and was thus invisible to the mosquitoes. In Surinam during bait catches Hudson (1984) covered torches with red plastic to make the light less visible to *Anopheles darlingi*. To overcome the high consumption of torch batteries in routine night collections in Kenya Pearson (1971) adapted his torches to operate from car batteries. The normal torch bulb was replaced with a 12-V, 0.2-A bulb, as used in many car instrument panels, and about 10 m, of wire was soldered to the torch case and to its switch. Wire from two torches can be connected to a junction box where a suitable length of wire, say 30 m, is connected by crocodile clips to a car battery. Alternatively this wire lead can be fitted with a plug and inserted into the socket for an inspection lamp or the cigarette lighter found on the instrument panel of many cars. A normal 12-V battery should provide light for two torches for about 100 hr.

Hand-net collections

Mosquitoes, especially those which have not landed on the collectors but are hovering around, can be caught in small nets. When mosquitoes are overwhelmingly numerous the best procedure may be to make a number of figure of 8 sweeps with a small hand-net around the head of the collector, or his colleague, and thus standardise sampling in this manner (Gjullin *et al.*, 1961). In fact in the sub-arctic mosquitoes may be so numerous that it is impossible to perform conventional stationary bait catches. In these situations it may only be possible to sample mosquitoes, which are attracted in clouds, by sweeping the air around the head and body and then retreating to the safety of a car, tent or building to sort out the catch. Mosquito repellents may be useful in reducing the numbers attracted. Alternatively, the collector can wear protective clothing, including a mosquito net over, but away from, the face, and restrict the collection of adults to only a few minutes from a specific site on the body, such as below the knee. However, biting catches under these conditions are frequently unsatisfactory. One reason is that collections over very short periods may give unrepresentative samples of the very large mosquito populations that are present.

A combination of sweeping the air around the host and aspirating settled adults was the method employed by Ho *et al.* (1971) to collect *Aedes albopictus*. In Canada Hocking *et al.* (1950) caught mosquitoes 'on the wing' by 20 sweeps through the air with a net directly they arrived at a catch site, in addition to those landing on them to feed. Also in Canada Lewis & Bennett (1979, 1980) collected mosquitoes attracted to humans by performing once during the day and again at dusk, or later during darkness, 40 standardised figure of 8 sweeps with a 30-cm diameter net. Similarly Taylor *et al.* (1979) collected host-seeking Canadian *Aedes* and *Mansonia* by having a collector make 40 figure of 8 sweeps about himself, this took about 1 min and comprised a single sample. To collect *Culex salinarius* in New Jersey Slaff & Crans (1981) walked to a specific catching station, waited for 1 min, then swept the air around the body with a sweep-net for 5 min. This routine was repeated every 30 min for 3 hr after sunset (i.e. time of maximum host-seeking activity), and then hourly until 1 hr after sunrise. Most adults were caught during the first 30–60 min after sunset.

In a biting study of Canadian mosquitoes Lewis & Webber (1985) collected adults by three methods. Namely, catching mosquitoes that landed on a 0.09-m² blue cloth placed on the person's lap during 2-min intervals; those biting or attempting to bite the left forearm during 2-min intervals; and thirdly by 40 figure of 8 sweeps made around the collector with a 30-cm diameter net. Consecutive counts and collections were made by a single person. Most mosquitoes, of which *Aedes punctor* and *Aedes communis* were the commonest species, were obtained by sweep-netting, followed by bait catches, and then landing counts on the blue cloth. All three collecting methods showed the same seasonal trends in abundance.

In Bolivia because some *Sabethes* were difficult to catch even while feeding, Roberts *et al.* (1984) found it necessary to use small hand-nets in addition to aspirators to collect adults arriving at human bait. But they pointed out that this may have meant that some other mosquitoes were caught in the net, such as a few *Uranotaenia*, that were not actually attempting to feed on people. A common procedure in Russia is that after a predetermined number of sweeps (10–100) over a period of 15 min or longer—depending on the density of mosquitoes—the sack of a butterfly net containing the catch is removed and replaced with a new sack. Rasnitsyn & Kosovskikh (1979) described and figured an improved butterfly net which had the upper 20 cm made of cloth and the lower 40 cm of mosquito netting, to the end of which is attached a removable small (12-cm long, 6-cm diameter) bag, into which the mosquitoes are collected. Masalkina & Kachalova (1989) found that they need make only 50, not 100, sweeps to get reliable data. In Britain Packer & Corbet (1989) caught mosquitoes arriving at bait with a 40-cm diameter butterfly net.

Drop-net catches

Although mosquitoes may not be so excessively numerous as to preclude the performance of bait catches, they may sometimes, nevertheless be too many for the collector to catch in test tubes, aspirators or even with hand-nets. In other situations 'nervous' or 'shy' ('dilettante') species may be encountered. These are species which although attracted to the collector hover around for a considerable time before settling, and even then may be very easily disturbed. In ordinary bait catches such species are more difficult to collect than those which readily settle on bait and consequently they may be underestimated. One method of partly overcoming these difficulties is to use a drop-net. This commonly takes the form of a cylindrical or bell-shaped tube of cloth weighted along the bottom edge with a metal hoop and rolled up and suspended just above the head of the collector (Fig. 5.5*d*). A cord which is pulled at intervals causes the net to descend rapidly to enclose the bait and mosquitoes (Fig. 5.5*e*) in the immediate vicinity (Blagoveshenskii *et al.*, 1943; Dyce & Lee, 1962; Minar, 1959; Mohrig, 1969; Monchadskiy & Radzivilovskaya, 1947; Rasnitsyn & Kosovskikh, 1979, 1983).

The dark bell of Monchadskiy or Berezantev, described and figured by Monchadskiy & Radzivilovskaya (1947), has been commonly used in human bait collections in Russia since about 1937. There are several variations, but basically

the trap consists of a bell-shaped net made of dark cloth that is suspended about 2 m above the bait. After an exposure time, ranging from 2–15 min or longer, a cord is pulled and the collector becomes enclosed within the drop-net. In some designs the trapped mosquitoes accumulate in a small transparent dome-shaped chamber at the top of the dark bell. Mosquitoes are killed with chloroform or aspirated alive from this apical chamber. In comparative trials Rasnitsyn & Kosovskikh (1979) reported that there was no real difference between the mosquitoes caught with the dark bell and those flying around a human bait which were collected in 25- or 30-cm diameter butterfly nets. Their dark bell caught 1153 mosquitoes belonging to 20 species, while 1214 mosquitoes belonging to 15 species were collected with the butterfly net having removable sacks (see p. 374). With both methods *Aedes vexans nipponii* (38.5 and 49.5%) and *Aedes punctor* (14.6 and 11.9%) were the two most common species.

In four different localities in Russia Masalkina (1979) found there were no differences between the coefficients of similarity (Jaccard, 1912; Shorygin, 1939) between the numbers of mosquitoes, and also species, caught by the dark bell net, butterfly nets with removable sacks, and catches from the forearm. Nine species were identified, the most common being *Aedes vexans*. Collections from the dark bell and the forearm were considered generally to give the most complete picture of the species present. These two methods, together with catches with a butterfly net, gave similar results as regards the percentage species composition. In contrast light-traps collected almost only *Aedes vexans*, but they were tested in only two of the four areas. In later evaluations Masalkina (1981) found that there was a linear relationship between the numbers of mosquitoes, mainly *Aedes vexans*, caught with the dark bell and on a forearm, and with a butterfly net with removable sacks and those biting the forearm, but only up to a density when it became impossible to collect all those biting the forearm. At densities up to 300 per sample with the dark bell, and up to 150 per sample with the net, the ratio was constant and a linear regression could be obtained between the numbers caught by the two methods

$$\log y = \log 47.76 + 0.34 \log x$$

$$\log x = 2.94 \log y - 4.94$$

where x and y = the numbers caught by the net (10 sweeps) and bell, respectively. From this it was concluded that the numbers caught by the net (x) could be converted to the numbers that would have been collected by the dark bell ($y = 2.64x$), and *vice versa* ($x = 0.38y$), a relationship confirmed by Masalkina & Kachalova (1989) in later studies ($y = 2.6x \pm 43$, and $x = 0.4y \pm 17$). They recorded a total of 13 species, the most common being *Aedes communis*, *Aedes punctor*, *Culiseta alaskaensis*, *Aedes pullatus* and *Aedes cantans*. *Culiseta bergrothi* was caught by sweep-netting and not in the dark bell net, while *Aedes hexodontus* was caught only in the dark bell net. To save time they suggested that the exposure period in their net could be reduced from 5 to 2 min, and the number of sweeps from 100 to 50. Their sweep-net had interchangeable bags. The numbers of mosquitoes caught by either method could be converted to the other by simple regression, as was done by Masalkina (1981).

In Kenya a drop-net having at the top an 82-cm diameter ring of wire covered in canvas was supported on four 2.5-m right-angled poles which held the net in the 'up' position (Chandler *et al.*, 1976b). At 30 min past each hour throughout the night a man stood under the raised net for 3 min before allowing it to descend and enclose him and the mosquitoes. The lower half of the net was held in position by a 120-cm diameter steel ring which rested on the ground. From 35 12-hr night collections 3571 mosquitoes belonging to 12 species were trapped, the commonest being *Mansonia uniformis*, *Mansonia africana*, *Anopheles arabiensis*, *Anopheles ziemanni* and *Aedes circumluteolus*.

Klock & Bidlingmayer (1953) described an umbrella-type drop-net. It consists of a 4-ft diameter wooden or metal circular frame covered with mosquito netting and supported on a 7½-ft centre pole. The netting extends down over the outside of the frame to reach the ground, but is initially held in a rolled-up position by four equally spaced cloth straps (Fig. 5.5f). The free ends of these straps are loosely held in position over four steel pins, behind each of which is a metal washer tied to a cord, which in turn is connected to a handle. When mosquitoes have been attracted to a man standing on a canvas or plastic sheet underneath the trap, the handle is pulled and the net rapidly drops to enclose the bait. Mosquitoes are collected from within the drop-net by aspirators, or are knocked down onto the floor sheet with a pyrethrum space-spray. In another arrangement that has been used in Florida (W. L. Bidlingmayer, pers. comm., 1973), the bait stands on a raised circular wooden platform having a metal rim around the perimeter and another platform and rim situated above the head. After a short exposure period two curtains, suspended from the upper rim and weighted at the bottom, are suddenly drawn round the bait, much as in a bathroom shower.

Tent traps

The orange tent method of Trpis has been used in both Czechoslovakia (Trpis, 1962a,b, 1971) and Tanzania (Tonn *et al.*, 1973; Trpis *et al.*, 1973) to collect mosquitoes. The technique consists of one or more people sitting in an orange coloured canvas gable-type tent (about 2 m × 2 m × 2 m) which has one end wide open and catching all mosquitoes that enter. In Czechoslovakia the procedure was to open the tent entrance for 15 min every 2 hr over the period of a 24-hr catch. At the end of each 15-min catching period the tent was closed and the mosquitoes caught in a small hand-net. During darkness a small 3-W bulb, operated from a 6-V battery, was suspended inside the tent, not to attract mosquitoes, but to facilitate catching. In South Africa Sharp *et al.* (in press) converted a frame tent (3.65 m long, 2.75 m high, 1.90 m min. height) with a built-in ground sheet into a bait trap. Two 1-m long horizontal and 7.5-cm wide entrance slits tapering to a 1-cm opening inside the tent were made on two opposite sides at the top just underneath the roof, which overhung and prevented light being visible from inside the tent. Muirhead-Thomson-type 1-ft cube exit traps were fitted with Velcro into all four sides of the tent (Fig. 5.6). Two people acting as bait stayed in the tent overnight and were protected by mosquito nets. In the morning (0530 hr) mosquitoes were collected from both the tent and exit traps. From 17 nights trapping 564 *Anopheles gambiae* complex, mostly *Anopheles*



FIG. 5.6. A side view of an experimental tent showing three of the four window traps and the roof overhang covering the entry slits (photograph courtesy of B. Sharp).

arabiensis, were caught, this being considerably more than in human-baited bed-nets. Only a few exophilic species, such as *Anopheles ziemanni*, *Anopheles pharoensis*, *Anopheles tenebrosus* and *Anopheles merus*, were caught (Sharp, 1983b; Sharp *et al.*, in press).

Bed-nets

One of the earlier references to the use of a trap to catch host-seeking mosquitoes is by Ross (1902) who considered that the number of mosquitoes found in a room during the day was not necessarily a reliable guide to the number that had fed on the occupants during the night. To overcome this disparity, and to measure the numbers seeking a blood-meal, Drs H. E. Annett and J. E. Dutton suggested that a servant slept under a mosquito-net with holes in it, as this would retain many of the mosquitoes that fed on him during the night. Much more recently Bryan (1983) considered that as many people in The Gambia used mosquito nets these could provide a useful method for collecting female *Anopheles gambiae* and *Anopheles melas*. It is usually difficult to compare the relative abundance of these two species in houses from house-resting collections, because *Anopheles melas* is more exophilic than *Anopheles gambiae*, but it is likely that biting a person under a net prevents many from leaving after feeding.

In Panama Le Prince & Orenstein (1916) caught large numbers of *Anopheles* in mosquito nets which were placed over a man, dog or chickens and which had the lower edges pulled up and pinned back. However, it was Gater (1935) who was responsible for developing and popularising the bed-net technique. The fol-

lowing account is mainly concerned with human baits under mosquito nets, their use with animal baits is described under a separate section (pp. 407–15) as are the general limitations of bed-nets (pp. 415–17), whether enclosing people or animals.

Starting in 1928 Manalang (1931) devised and experimented with several types of human-baited bed-nets in the Philippines, but it was Gater (1935) who introduced the method to Malaysia where it has now been used for many years (Colless, 1959; Hodgkin, 1956; Reid, 1961; Wharton, 1953; Zairi, 1990). The original trap consisted of a large net 10 ft long and both 7 ft wide and high with a 3-ft wide flap on each longer side which was rolled up to leave two entrances. A person entered the net around sunset and slept enclosed within a smaller protective net. At about sunrise he was able by pulling on cords to unroll the two flaps of the outer net without leaving his own net. With both entrances closed the entrapped unfed hungry mosquitoes were collected. The original design has undergone various modifications to suit specific purposes. For example, smaller nets are frequently used, especially when they are baited with birds or small mammals, and the inner protective net is often omitted to allow trapped mosquitoes to engorge on the bait, which if an animal is usually tied to a stake or placed in a cage. Removal of the inner net usually results in a larger catch of mosquitoes. Mosquitoes also tend to escape too easily if the original type of net is used; consequently the entrances by which mosquitoes enter, have frequently been modified. Very often a single door-like opening is used (Colless, 1959) (Fig. 5.7a). Sometimes one or more sides of the net are partly rolled up and pinned or tucked into place (Sasa & Sabin, 1950; Service, 1963), or a horizontal slit made (Davidson, 1949). Alternatively, the entire net can be raised a few inches from the ground (Akiyama, 1973; Hamon, 1964; Laarman, 1958) to give access to hungry mosquitoes (Fig. 5.7b).

Baited bed-nets have been used by a number of Japanese entomologists (e.g. Omori, 1942; Takeda *et al.*, 1962; Wada *et al.*, 1967, 1970). Those used by Wada and his colleagues consist of a double net (400 × 240 × 240 cm) having a single opening (240 cm long and 120 cm high) usually with one, but occasionally two, men enclosed within an inner net (200 × 110 × 150 cm).

In Nigeria Bown & Bang (1980) compared the numbers of *Aedes* species caught biting a man underneath a mosquito net to a man outside the net. In the net collections 162 *Aedes africanus*, 38 *Aedes aegypti* and 17 other *Aedes* mosquitoes were trapped, whereas outside the net 234 *Aedes africanus*, 10 *Aedes aegypti* and 23 other *Aedes* were caught. This emphasises that there may be differences in the relative proportions of species caught by direct bait catches and by bed-net collections.

Charlwood *et al.* (1986b) reported that in Papua New Guinea bed-nets raised about 8 cm from the ground allowed a considerable number of unfed *Anopheles punctulatus* to escape, irrespective of whether the human bait was enclosed within an inner protective net. To overcome this problem they placed a person within an inner protective net and surrounded him with a larger bed-net raised approximately 8 cm from the ground, but which had an inverted CDC light-trap suspended from the top of it (Fig. 5.7c). Bait catches caught significantly more female *Anopheles farauti* (593 ± 228/night) than bed-nets having CDC traps with (348 ± 138) or without a light (39 ± 22). Differences between numbers collected from

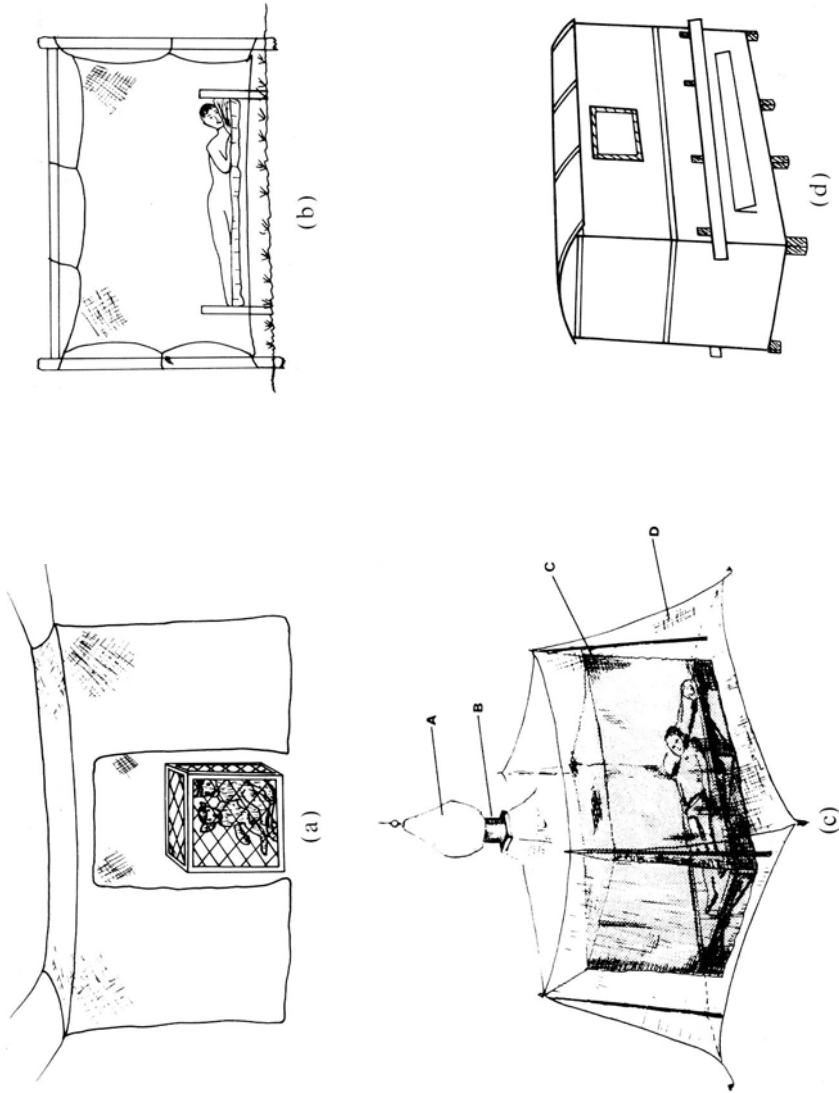


FIG. 5.7. (a) Bait-net with cage for small mammals; (b) bed-net raised from ground; (c) light-trap of bed-net, A — cage for collecting mosquitoes, B — updraft CDC suction trap, C — inner bed-net protecting sleeping bait, D — outer bed-net with edges raised (Charlwood et al., 1986b); (d) trap hut of Bertram & McGregor (1956).

the two net traps were considered to be due to the light attracting mosquitoes within the net into the light-trap, whereas in the unlit trap many mosquitoes escaped. A rather similar method was employed in Kenya by Mutero & Birley (1987). They placed an updraft trap without any light about 40 cm above the head of a person sleeping under a bed-net. Their trap was made from an ordinary plastic bucket, 30-cm tall with diameters of 30 and 24 cm at the top and bottom, respectively. A 10-cm length of plastic, or metal, tubing was thrust halfway through a hole cut from the centre of the bottom of the bucket. A 12-V, 0.17-A d.c. electric motor, powered by a 12-V, 5.7-A d.c. sealed lead-acetate battery, and carrying a small 3-bladed plastic propeller was placed inside the tubing. Mosquitoes hovering around a host in bed were sucked up into the bucket which had the top covered with netting. Moist cotton wool placed inside the bucket prevented mosquitoes from desiccation. The trap was operated from 1800–0600 hr and caught mainly hungry unfed female *Anopheles gambiae*, *Anopheles arabiensis*, *Anopheles merus* and *Anopheles funestus*. The battery was recharged daily from a solar panel (12-V, 1-A), which was often placed on the roof of the house.

Lu Bao Lin (pers. comm., 1990) informs me that in China a standard method of collecting anthropophagic mosquitoes is to erect a pyramid-shaped net having a 150-cm square base over a human bait. A gap of 20 cm is left between the ground and the net's lower edges for hungry mosquitoes to enter.

Parsons (1977) described a bait trap for collecting anthropophagic mosquitoes in which a person is protected from bites by being enclosed in an inner compartment; non-human hosts could also be placed in the trap. Basically his trap consisted of a plastic mesh rectangular cage (76 × 76, 52-in high) supported at the four corners by vertical poles connected at the top of the trap by horizontal ones. A zippered flap in the middle of the trap provides the entrance for a person (bait) to enter. The trap is divided into two 76-in long, 52-in high but just 12-in wide outer chambers, each having a 2-in wide entrance slit. Mosquitoes attracted to the bait in the inner compartment pass through the two horizontal slits into the outer compartments and are collected by the bait with an aspirator inserted through six circular openings fitted with sleeves arranged in two rows on the two walls separating the inner and outer compartments. The trap can apparently be dismantled by two men in 10 min, and is easily transported. It is somewhat reminiscent of a Shannon net trap, except that hungry mosquitoes enter through slits and not through a gap separating the two outer compartments from the ground. No details are presented of the numbers caught, except that in Panama it attracted *Anopheles albimanus*.

Moving bait catches

Most human bait catches are performed with a stationary bait, but occasionally collections are made by the person slowly walking through vegetation and periodically stopping to catch mosquitoes that have alighted on himself or his companions. Catches of this sort, termed roving catches, have been made in forests of Trinidad (Aitken *et al.*, 1968*a,b*). The same type of catch but called a walking-landing method was used in Tanzania (Tonn *et al.*, 1973). The method

consists of slowly walking through the bush for 1 min then sitting on the ground and collecting mosquitoes as they land for 5 min, or until no more land. The collector then moves on and repeats the process. To sample *Aedes cantator* in Maryland a collector upon arrival at a site stood still for 1 min and counted the mosquitoes landing below the waist, then he moved around the area for 5 min aspirating host-seeking mosquitoes. Another 1-min count was then taken before proceeding to the next catching station (Weaver & Fashing, 1981). In Uganda, Henderson *et al.* (1972) employed mosquito catchers to walk at least 25 yd then stop and catch for 10 min, after which they proceeded for another 25 yd or more. In Kenya more adults of *Aedes aegypti* and other *Aedes* species were caught by catchers slowly walking through the bush than in stationary catches (Teesdale, 1955, 1959).

The success of this type of collecting method depends largely on the mosquito fauna and the manner in which they are attracted to baits. In England, for example, I have found that mosquitoes (including *Aedes cantans*) are not usually caught by slowly walking through vegetation during the day, although it may be harbouring very large mosquito populations. The explanation is that a moving bait does not stimulate the resting population in time for them to fly out, locate and settle on the collector. On the other hand, Renshaw (1991) believed that *Aedes cantans* followed her when she walked to a catch site, and this was one of the reasons for a high initial catch (p. 353).

Analyses of results

It is usually better to transform the numbers of mosquitoes caught in bait catches to $\log(n + 1)$ to allow more realistic means to be calculated, and the application of parametric statistical tests. Results are often plotted as total catches, means or percentages against unit time, e.g. hour, week or month. Downing (1976) emphasised the need to transform data to logarithms to calculate geometric (William's) means, and also pointed out how daily fluctuations in numbers caught could be smoothed out by taking moving (running) means, such as over 5-day periods. Both procedures are explained and illustrated step-wise in his paper. Hawley (1985) used 11-day running means on daily catches of *Aedes sierrensis* at human bait.

In analysing the relationship between the numbers of individuals and the numbers of mosquito species caught in a series of bait catches in Tanzania, Nagasawa (1973) found that the results fitted both the logarithmic series of Fisher (1943) and the truncated lognormal distribution introduced by Preston (1948). There was, however, a slightly better fit to the log-normal model. An advantage of having data fit this distribution is that it enables the number of uncaptured species to be estimated. In this instance Nagasawa (1973) predicted that about 11 species (29.7%) were missed in the catches, but should eventually have been caught if catching had been continued. The accuracy of such predictions is attested for by the good agreement between the number of tabanid species estimated to be available for capture by this method and the actual results obtained from field collections (Nagasawa, 1967).

Ability to predict relative abundance of insect pests would be an enormous benefit in control programmes, and in fact various attempts have been made to

forecast insect abundance. For example, time-series analyses (Hacker *et al.*, 1973) and stochastic probability models (Moon, 1976) have been tried with mosquitoes, while thermal summation models (Ring & Harris, 1983; Toscano *et al.*, 1979) have been used in attempts to predict outbreaks of agricultural pests. Milby (1985) used logarithmic transformed mean monthly numbers of female *Culex tarsalis* caught in a previous month in New Jersey light-traps, and meteorological variables such as: (i) monthly rainfall; (ii) cumulative rainfall since January 1; (iii) mean temperature in °F; (iv) number of day-degrees above 65°F; and (v) cumulative day-degrees since January 1, to develop regression equations for predicting *Culex tarsalis* numbers in future months. Only limited success was achieved. She found (predictably!) that the more recent the data of the variables were to the month that she wished to predict, the better the prediction. Often the only variables needed were (i), (iii) and (vi), and for some months the only variable that mattered in predicting the size of *Culex tarsalis* populations was its population size the previous month.

Strickman & Hagan (1986) analysed the results of human bait collections of *Chrysops variegatus* for seasonal distribution, and effect of meteorological conditions on the numbers caught. A modified form of time-series analyses as used by Hayes & Downs (1980) on catches of *Culex quinquefasciatus* was employed to determine any seasonal periodicity of biting. In this approach the numbers of flies caught (Y) is given as

$$Y = A + \{B \cdot \cos [(C \cdot X) - D]\}$$

where A = mean number of flies throughout the study period; B = degree of amplitude of the periodic waves; C = length of periods, where the period in weeks is equal to $(2\pi)/C$; X = week number; and D = shift in phase. The Kolmogorov–Smirnov test of difference was used to test the significance of the results from ‘white noise’, together with the asymptotic 95% confidence limits of the calculated period.

The effect of meteorological variables on biting activity was analysed using multiple linear regression, as follows:

$$Y = A + (B \cdot X_1) + (C \cdot X_2) + (D \cdot X_3)$$

where Y and A as already defined, and X_1 = dry bulb temperature in °C; X_2 = wind speed on a scale of 0–4; and X_3 = % relative humidity.

These statistical procedures are relevant to mosquito ecology, and the paper by Strickman & Hagan (1986) should be consulted by those interested in applying this approach.

Polynomial regressions of the percentages of the total catch of *Armigeres milnensis* caught at 5-min intervals were used to detect any relationship between certain environmental factors by Charlwood & Galgal (1985).

The relative abundances of mosquitoes, such as in bait catches, are often shown by just ranking the species by their absolute numbers, but problems arise when this approach is used with data from several distinct collection sites, because no weight is given to the within site spatial distribution of populations. The alternative approach of scoring the presence or absence of species in differ-

ent sites ignores among site variability in numbers of the species present. Because of these limitations Roberts & Hsi (1979) proposed a new Index of Species Abundance (ISA) that is calculated for individual species, and which takes into account numerical abundance and spatial distribution. Their paper describes the application of this method to the collection of 47 mosquito species at human bait from 12 collection sites in Brazil.

The first step is to tabulate in a column all species caught and then to fill in the numbers of each species caught in the different collection sites in rows. Then keeping the species in the column, another table is made ranking each species in order of abundance at each collecting site, (the most common species being ranked 1), calculating mean ranks for tied scores, then the statistic C is calculated, where $C = \text{single largest assigned rank} + 1$, in other words the highest number in the table. This value of C is then multiplied by the number of zero cells in all columns for each row (i.e. species) to derive the statistic a . In other words the number of collecting sites without a particular species $\times C$. Then R_j is calculated for each row (species) by adding all the rank numbers in that row (Table 5.1). Then for each species the ISA is

$$\text{ISA} = \frac{a + R_j}{K}$$

where $K = \text{number of collection sites}$.

Clearly the range of ISA will be determined by the largest rank number (i.e. number assigned to rarest species), and will differ for different sets of data. To

TABLE 5.1
SCHEMATIC ARRANGEMENT SHOWING DATA FOR CALCULATING ISA (INDEX OF SPECIES ABUNDANCE) (AFTER ROBERTS & HSI, 1979)

Species	Collection site				(Number of zero cells in K columns times c) a	(Sum of assigned ranks) R_j	ISA (Index of species abundance)
	1	2	3 K			
1							
2							
3							
.							
.							
.							
.							
N							

assigned ranks (R_{1j})
(R_{2j})
(R_{3j})
(R_{kj})

overcome this and to be able to compare the index on a scale of 0–1 the standardised ISA can be calculated as follows

$$\text{standardised ISA} = \frac{C - \text{ISA}}{C - 1}$$

where as already defined C = largest assigned rank number + 1. The most common abundant species at all sites will have the smallest ISA values, whereas they will have the largest standardised ISA values. The variance of the ISA is derived as follows

$$\text{VAR}_{\text{ISA}} = 1/12K [N^2p^3(4-3p) + 6Np^2q(2+q-2C) + 3pq(1+q-2C)^2 - p^2(6-5p)]$$

where p = the overall proportion of cells in the table of rankings which have non-zero counts at all sites, where $q = 1 - p$ and N = total numbers of species.

Roberts *et al.* (1984), in studying mosquitoes caught biting human baits and resting inside houses in Bolivia, used this index.

In studying population fluctuations of mosquitoes caught in bait catches in Panama (Wolda & Galindo, 1981) seasonal fluctuations were distinguished from yearly fluctuations by calculating the Annual Variability parameter (AV) of Wolda (1978). For example, the numbers of mosquitoes of species i caught in year 1 is termed N_1 ; and in the second year N_2 ; so change in abundance from year 1 to year 2 is the ratio of $N_2/N_1 = R_i$. It is better to use logarithms and obtain $\log R_i$. In a catch of n species a total of n values of $\log R_i$ are obtained and can be plotted as a frequency distribution which will approximate normality. The mean of $\log R_i$ gives information on whether all species together tend to increase (positive mean) or decrease (negative mean) in abundance from one year to another. The variance of $\log R_i$, termed Annual Variability (AV), reflects differences between species. If all species change more or less the same way, then AV will be small, but if some species increase greatly in abundance while others decrease, AV will be large. Consequently AV can be used as a measure of stability of the species being collected. Only those species that are represented by at least five individuals each year should be used in determining R_i .

Wolda & Galindo (1981) analysed data for seven mosquito species and found that AV varied from 0.105–0.525, denoting large variations in abundance.

In studying changes in abundance of different species at bait in Sri Lanka Amerasinghe & Ariyasena (1991) applied the Index of Change (IC), previously devised to study changes in abundance of larval populations. Amerasinghe & Ariyasena (1990) also applied the procedure for collections of mosquitoes in light-traps and those resting indoors.

Biting rates and inoculation estimates

Seasonal shifts in feeding behaviour

Human bait catches are made for several reasons, including the estimation of biting rates and infection rates, the assessment of the effectiveness of control operations, and the monitoring of temporal changes of relative population size.

In studying seasonal changes in population size the attraction of man as bait should not change over the sampling period. But such changes do occur and can be due to genetic and behavioural (Boreham & Garrett-Jones, 1973; Gillies, 1964), or environmental (Edman, 1974), factors or to changes in vector or host abundance (Chandler *et al.*, 1977; Reeves, 1971). In Florida, for example *Culex nigripalpus* feeds more on birds than mammals during the cooler months of the year, but the reverse occurs in the warmer months (Edman & Taylor, 1968). Also in Florida *Culex tarsalis* feeds increasingly on mammals as opposed to birds during the summer (Tempelis *et al.*, 1967), while in India *Culex quinquefasciatus* feeds more on man in the hotter months than on cattle (Kaul & Wattal, 1968). In Jamaica Muirhead-Thomson & Mercier (1952) noted a marked increase in the proportion of *Anopheles albimanus* biting indoors in the rainy season. Consequently an increase in the numbers caught biting indoors reflected this change in behaviour as well as a general increase in population size. A similar shift to indoor biting during the monsoon, and increased proportions biting out of doors during the cooler months, has been observed in *Culex quinquefasciatus* in India (Gubler & Bhattacharya, 1974). In Colombia, Elliott (1968) found that during maximum population densities outdoor biting by *Anopheles darlingi* could be less important than indoor biting, whereas at other times outdoor biting was often more important. There may also be seasonal shifts in biting times. For example, in Pakistan Reisen & Aslamkhan (1978) recorded that in warm weather *Anopheles culicifacies*, *Anopheles stephensi* and *Anopheles subpictus* fed on cattle mainly late at night, but with the onset of cooler months they became crepuscular feeders. However, no such seasonal shifts in biting activity were recorded in *Anopheles nigerrimus*, *Culex tritaeniorhynchus* and *Culex pseudovishnui*.

People's behaviour

Elliott (1972) presented a very cogent paper on the realities of measuring mosquito-man contact, in which he stressed the importance of taking into account people's behaviour when performing bait catches. For example, he firstly undertook out of door biting catches, then at bedtime switched the bait to inside houses. Although now about 20 years old this paper is still worth reading. Because people in El Salvador usually remained outdoors until 2000 hr Austin (1973) arranged that human bait catches were performed outdoors from 1800–2000 hr then inside huts from 2000–2200 hr. This short 4-hr catch gave us reliable results on the numbers of *Anopheles albimanus* biting man per night as did more tedious 12-hr catches.

Trying to take into consideration people's behaviour Mosha *et al.* (1981) working in Tanzania performed human bait catches for the *Anopheles gambiae* complex, *Anopheles funestus* and *Culex quinquefasciatus*, both indoors and out of doors from 1815–2215 hr, then just indoors until 0615 hr.

The human blood index of *Anopheles farauti* in Maraga village, Papua New Guinea, was only about 5% (Charlwood *et al.*, 1986a; and P. M. Graves & T. Burkot quoted by Saul, 1987), because most feeding was on pigs. Mark-recapture studies estimated the biting population per night to be about 46000, so some 2300 feeds should be on humans. As the human population in the area was 125, the mean number biting a person per night would be about 20. However, in

human bait collections some 500 *Anopheles farauti* were caught per person per night. If this capture rate at bait is used to calculate vectorial capacity, or malaria inoculation rates, estimates 25 times greater than those indicated by the human blood index and population estimates of both mosquitoes and man are obtained. This emphasises the danger of uncritical use of man-biting rates. In bait catches the collector is trying to collect as many mosquitoes as possible and is therefore likely to catch more than would have bitten him under natural conditions. Furthermore, a proportion of people may be using mosquito nets, which even if torn will nevertheless lessen the chance of getting bitten. On the other hand, there may be a number of interrupted feeds on a hut occupant—not engaged with catching mosquitoes—which will increase the number of bites received, but this will not be accounted for in routine bait collections. Multiple feeding by mosquitoes on humans can be investigated experimentally by having people with different blood chemistry offered as hosts (Boreham *et al.*, 1978; Port *et al.*, 1980; see p. 392). Relatively simple methods can detect ABO blood-group substances (up to 24–30 hr after feeding), but the method is limited by cross reactions which develop as digestion proceeds. An alternative and more reliable system uses differences in serum protein haptoglobins (up to 16–20 hr after feeding), but both methods have their limitations (Boreham & Lenahan, 1976).

In epidemiological studies man-biting rates, based on 12- or 24-hr catches during different seasons, are often estimated to obtain a theoretical number of bites a person could receive in a year, and to calculate the inoculation risk with malarial sporozoites, microfilariae or arboviruses. A common procedure involves the collection of mosquitoes throughout the night that are attracted to a human bait sitting outdoors in a village compound. But the numbers caught may have little bearing on the average number of bites a person receives if he normally sleeps indoors. Even if catches are made indoors there may still be differences between the numbers caught and those normally biting a hut occupant. In India, in trying to get realistic estimates of the biting density of *Culex quinquefasciatus*, Gubler & Bhattacharya (1974) employed two people working for 2-hr shifts both inside and outside houses throughout most of the night to catch mosquitoes from a person who slept, acted and dressed normally. They appreciated that estimating biting densities was difficult, but calculated that in a Calcutta suburb a person would receive over 115 000 bites a year from *Culex quinquefasciatus*, and moreover that this was probably an underestimate! From their estimated biting densities and from filarial infection rates they calculated the number of infective bites a person receives over a year, and also the numbers of larvae of *Wuchereria bancrofti* deposited on such a person.

In Liberia Kuhlow & Zielke (1978) had two people sitting during the night in a partitioned part of a village hut that was otherwise used normally by the occupants. From the numbers of vectors caught per man-night in different months they estimated by simple proportions: (i) the numbers of bites a person would receive in a year; (ii) the number of bites with mosquitoes infective with *Wuchereria bancrofti*; and (iii) the number of infective larvae deposited on a person during a year. They were careful to use these values as comparative indices and not as absolute estimates of biting and transmission potential.

Nathan (1981) studied the intensity of bancroftian filariasis transmission in Trinidad by weekly collections over a year of indoor biting (1900–0600 hr) *Culex quinquefasciatus*. Because very few people were active outdoors during darkness, catches were made only indoors. To try and avoid sampling bias houses were selected from a list of 200 random non-replacement numbers. If a house was unoccupied, or could not be used for some other reason, then the nearest house was used as a catching station. Residents of houses were asked to carry out their normal domestic duties while the collector sat on the floor and caught mosquitoes from his exposed legs and feet. Torches were used for only about 2-min durations to minimise any affect torchlight might have on the normal behaviour of the mosquitoes. To prevent ovarian development before the mosquitoes could be dissected they were placed in glass jars lined with damp plaster of Paris and kept in an ice chest. Nathan (1981) estimated that a person was exposed to 17 948 bites a year from *Culex quinquefasciatus*, including 14 infective bites. This transmission potential is very low when compared with estimates of 1850 infective bites for Calcutta (Gubler & Bhattacharya, 1974) and 1106 for Pondicherry (Rajagopalan *et al.*, 1977), although in Tanzania (White, 1971) and in Kenya (Wijers & Kiilu, 1977) estimates of numbers of infective bites a person received per year were only 23 and 46, respectively.

In Malaysia Chiang *et al.* (1984b) estimated the monthly transmission potential of *Brugia malayi* by *Mansonia bonnea* from the biting rate, proportion of infective mosquitoes and the mean number of mature larvae per infective mosquito.

Kuhlow & Zielke (1978) pointed out there are many factors that can introduce bias in calculating transmission potential. For example, infection rates are sometimes based on biting catches, other times on sampling resting populations. Nathan (1981) advocated the standardisation of collecting methods and analyses to allow more realistic comparisons of transmission risks from different areas, such as the procedure practiced in West Africa in the evaluation of the effectiveness of onchocerciasis control (Walsh *et al.*, 1978).

Vectorial capacity

A useful concept in the epidemiology of disease is the basic reproduction rate (R_0), which is the average number of secondary cases of a disease (e.g. malaria) arising from each primary infection in a defined population of susceptible individual hosts. In other words R_0 represents the maximum reproductive rate per generation, leaving aside complications such as host immunity and superinfection. If $R_0 > 1$ the disease is maintained, the level of transmission depending on the size of R_0 but if $R_0 < 1$ the disease decreases and will eventually disappear from the population. Vectorial capacity is the entomological component of the basic reproduction rate of malaria. It is the average number of inoculations from a single case of malaria in unit time, usually a day, that the vector population transmits to man, where all vectors biting an infected person become infective. Reducing vectorial capacity reduces R_0 . The usual formula for vectorial capacity (C), in terms of a daily rate, as derived by Garrett-Jones (1964) is

$$C = \frac{ma^2 P^n}{-\log_e P}$$

but a parameter V (sometimes written as c or b) can be inserted to describe the inability of all mosquitoes that become infected to become infective, in other words V is the proportion of mosquitoes with sporozoites actually infective to man, thus

$$C = \frac{ma^2 V P^n}{-\log_e P}$$

where C = new infections disseminated per person per day by each mosquito, ma = the number of bites/man/day, a = the proportion of females feeding on man divided by the duration of the gonotrophic cycle in days. a is multiplied by ma because refeeding is necessary for transmission. P = probability of daily survival, estimated vertically (sometimes called cross-sectional as it is based on acquisition of infection with age) from the population age structure if the duration of the gonotrophic cycle is known, or horizontally (longitudinal, based on time) from the daily loss rate of identified cohorts over time, and n = time from infection to infectivity in days and is usually estimated from the ambient temperature using a degree-day relationship. Thus, P^n = probability of a mosquito surviving to become infective, and the expected duration of life in days = $1/(-\log_e P)$.

Molineaux *et al.* (1978) rewrote the definition of vectorial capacity as

$$C = ma (P/F) e^{-n/E} E$$

where ma = number of mosquito bites/man/day, i.e. the biting rate, P = the proportion of blood-meals taken on man, F = the interval between feeding and refeeding in days, n = duration of extrinsic incubation period (e.g. of malaria) in the mosquito, and E = life expectancy of the mosquito calculated from $1/(-\log_e P)$. The term $ma (P/F)$, like ma^2 defines mosquito-man contact, while $e^{-n/E} E$ or its equivalent $P^n/(-\log_e P)$ is the expectation of infective life, which is compounded in terms of probability of survival to a later age ($x + n$) and the life expectancy of survivors at that age.

In Pakistan Reisen & Boreham (1982) estimated malaria vectorial capacity by this modified formula of Molineaux *et al.* (1978) where ma was calculated as $(P_t a)/(gc/H)$; where P_t = daily population size of female vectors—estimated in this instance by the Lincoln Index with Bailey's (1952) correction, a = proportion of blood-meals positive for human blood amongst those tested from representative collection sites (note, confusion can arise here because a is being used to mean something different to a in the Garrett-Jones' formula), gc = duration of gonotrophic cycle in days; H = human population—determined in this instance by a household census, n = duration of sporogony of *Plasmodium vivax* and *Plasmodium falciparum*—calculated by heat summation as described by Detinova (1962), and E = life expectancy of the female vector, that is where P = daily female vector survivorship—estimated in this instance by the regression of numbers of ovarian dilatations against age in days.

Because of a very low biting rate on humans during the monsoon season and relatively low life expectancy, the pooled vectorial capacity for *Anopheles culicifacies* and *Anopheles stephensi* was less than 1.34×10^{-2} for *Plasmodium vivax* and 8.58×10^{-3} for *Plasmodium falciparum*, very low figures when compared to estimates (0.006–22.25) for the *Anopheles gambiae* complex and *Anopheles funestus* in Africa (Reisen & Boreham, 1982).

In Sichian Province, China entomological surveys showed that the vectorial capacity of *Anopheles lesteri anthropophagus* was 0.654, and for *Anopheles sinensis* 0.019, similarly the entomological inoculation rate (see p. 390) calculated as the product of the man-biting rate and the sporozoite rate was 0.003367 for *Anopheles lesteri anthropophagus* and 0.000185 for *Anopheles sinensis*. Using Krafusur & Armstrong's (1978) formula for estimating risk of infection (R) defined as the probability of receiving one or more sporozoite inoculations per unit time, then $R = 1 - e^{-sn/t}$, where s = sporozoite rate, n = number of bites in t days, so sn/t is the entomological inoculation rate, the probability (risk = R) of inoculation with sporozoites was 0.1829 and 0.0110, respectively for both vectors, that is 94.3% of local malaria transmission is by *Anopheles lesteri anthropophagus* and 5.7% by *Anopheles sinensis* (Liu *et al.*, 1986).

Calculations of vectorial capacity are usually based on random biting and the man-biting rate ($\epsilon^2 V/B$) is usually based on the average biting rate per person among a team of B bait collectors. The resulting estimate of vectorial capacity ($\epsilon^2 V/\delta H$) is at best proportional to the true vectorial capacity. When, however, there is non-random biting by mosquitoes on hosts, then, as shown by Dye & Hasibeder (1986), the vectorial capacity is likely greater than when calculated on the assumption of random biting. Taking into consideration the heterogeneity factor (summation part of equation below), the definition of vectorial capacity in their model can be written as

$$C = \frac{\epsilon^2 V}{\delta H} \sum_i \frac{\gamma_i^2}{h_i}$$

where V and H = the numbers of vectors and hosts (man) in the area, of which a proportion of hosts (h_i) reside in area i and are bitten by a proportion (γ_i) of all mosquitoes, ϵ = number of bites taken on man by one mosquito per day, and $1/\delta$ = expectation of infective mosquito life. In reality, however, it is usually impossible to estimate h_i and γ_i , and so the cruder estimate of vectorial capacity (Garrett-Jones, 1964) has to be used, which, however, is likely to change proportionally with the true but unknown vectorial capacity. Dye & Hasibeder (1986) showed that when this crude estimate of vectorial capacity is reduced (e.g. by vector control or chemotherapy) then calculations based on random mixing of biting on people will at first produce a conservative estimate of the success of any control programme, but when transmission is much reduced, predictions on reduced transmission rates will be over optimistic. For further explanations see Dye & Hasibeder (1986) and Hasibeder & Dye (1988).

Despite the relative sophistication of models for estimating vectorial capacity, it appears that this measure is only sometimes marginally better correlated with parasitological data on malaria transmission than the very much simpler measure

of challenge based on the man-biting rate (ma) (Dye, 1986). It therefore seems questionable whether the extra work involved in calculating vectorial capacity is justified, and as Dye (1986) has pointed out 'methods based on untested assumptions are used to estimate parameters with unknown errors'. An interesting paper on measuring the vectorial capacity of simuliid blackflies as vectors of onchocerciasis concluded, that if there was little variation in the proportion of infective flies, then estimating their survival rate for computing the vectorial capacity was redundant, and that the easiest parameter to obtain, namely the biting rate, could account for variations in inoculation rate recorded in different areas and at different times (Dye & Baker, 1986). This paper is of interest to those concerned with the epidemiology of vector-borne diseases.

It should be realised that vectorial capacity is an indirect method of estimating transmission rate by a vector, a more direct way is to use the entomological inoculation rate (EIR) or infective biting rate (IBR), or as it is often called the inoculation rate (h), which is simply the product of the (man) biting rate and the infection rate. For example, in malaria the man biting rate (ma) is multiplied by the sporozoite rate s to give

$$h = mabs$$

where b = is the proportion of mosquitoes containing sporozoites that are actually infective. This is not an easy parameter to measure, but b is little if at all affected by changes in the indirect factors. However, when transmission is at a low level the sporozoite rate is usually low, and the confidence intervals at the 95% probability level vary considerably according to the numbers of mosquitoes dissected. For example, if 2000 mosquitoes are dissected and the sporozoite rate is 0.10% the sporozoite rate could be 0.01–0.36%, and even if 8000 mosquitoes were dissected the true rate could be as low as 0.04% or double the calculated value (0.20%) (Onori & Grab, 1980). But as the sporozoite rate is a function of the mosquito survival rate, the sporogonic cycle and the gametocyte rate, then the inoculation rate can be estimated without recourse to the sporozoite rate as follows

$$h = \frac{ma^2bgxP^n}{agx - \log_e P}$$

where gx = the gametocyte rate, n = the duration of the sporogonic cycle, m = vector density in relation to man, a = the man-biting rate, b = proportion of vectors with sporozoites actually infective, and P = the daily survival rate. The inoculation rate is very sensitive to changes in P and n .

The paper by Krafsur (1977) on the calculation of sporozoite inoculation rate and the probabilities of a person receiving one or more inoculations per year is worth reading.

Birley & Boorman (1982) showed that the expected infective life of a mosquito (V) may be estimated as

$$V = P^{du} / (1 - P)$$

where P = survival rate per oviposition cycle, d = duration of the extrinsic incubation period of the parasite in the mosquito and u = estimated length of the

interval between blood-feeding and oviposition, i.e. the oviposition cycle. Clearly V is extremely sensitive to small changes in P , the survival rate. In this approach survival rate is calculated over discrete time-intervals, the oviposition cycles, and does not represent the daily survival rate which is a more continuous measurement.

Smith (1987) presented a modification of the malaria reproduction rate formula of Macdonald (1952) to estimate the reproductive rate of an arbovirus (R), such as western equine encephalomyelitis, where R is defined as the average number of vertebrate maintenance hosts infected by mosquitoes infected from a single vertebrate maintenance host, thus

$$R = \frac{mbhs_m V s_v P^i}{-\log_e P}$$

where m = bites/bird/night, b = number of feeds by a mosquito each day (if gonotrophic cycle is 4 days then the value is 0.25), h = proportion of blood-meals taken from birds (say 0.85), s_m = vector competence for WEE (say 0.67), V = duration of infective viraemia in birds (3 days), s_v = proportion of birds susceptible to infection (say 0.67), P = mosquito daily survival (say 0.8) and i = intrinsic incubation period of WEE (say 6 days). Using these values $R = m (0.25 \times 0.85) 0.67 \times 3 \times 0.67 (0.8^6) (1/-\log_e 0.8)$ which equals $m \times 0.336$ (Reisen, 1989). So if $R = 1$, which is necessary for WEE maintenance then, $m = 2.98$ bites/bird/night. Now when m is greater than this then R is > 1 , and represents the numbers of new infections/infected bird. However, this approach, based on so many untested assumptions, has been criticised by Dye (1992).

There is a series of six interesting papers on the estimation of vectorial capacity, mainly orientated to arbovirus infections, published in the *Bulletin of the Society of Vector Ecology* (1989) 14, 39–70, and an excellent and readable account of vectorial capacity is presented by Dye (1992).

Preferential biting, age and sex

Another practical difficulty in epidemiological studies is preferential biting, in which biting is biased in favour of, certain individuals, age-classes, host size, sex or health, and other factors (Day & Edman, 1983; Elliott, 1968; Port *et al.*, 1980; Smith, 1961; Spencer, 1967). Experiments with *Aedes aegypti* have also shown that a person's attractiveness can vary over short periods (Khan *et al.*, 1971). In Nigeria Shidrawi *et al.* (1974) found a four-fold difference between the numbers of *Anopheles gambiae* and *Anopheles funestus* caught by different men aged 16–25. Carnevale *et al.* (1978) in the Congo compared biting rates in teams of different ages (0–2, 2–10, 10–20, >20 years) and sex, and discovered that the number of bites received from *Anopheles gambiae* s.s. increased proportionally as 1:2:2.5:3 for the four age-groups. Males and females were bitten indiscriminately. In Sierra Leone Thomas (1951) reported that 59.3 and 79.2% of the variation in numbers of *Anopheles gambiae* s.l. biting people in two families was apparently due to age, fewer bites being on younger people. In Jamaica Muirhead-Thomson (1951) concluded that the large variations (65.4, 81.7 and 91.3%) in biting rates of *Anopheles albimanus* in three families were also due to age, most

biting being on adults. In Kenya Boreham *et al.* (1978) showed that *Anopheles gambiae* s.l. and *Culex quinquefasciatus* fed more frequently on mothers than babies. In The Gambia by typing human blood in engorged mosquitoes into A, B and O blood groups, and by identifying different haptoglobins, Port *et al.* (1980) attributed the larger number of bites on adults than young children to their greater size, i.e. both weight and estimated surface area of skin. In fact with both infants (less than 18 months) and adults they obtained significant regressions of the numbers of *Anopheles gambiae*, and other mosquitoes, biting with increasing weight of the baits. Gass *et al.* (1982) showed that adolescents and adults were more attractive to *Mansonia annulata* than children, although this bias was not found in the other three *Mansonia* species they collected. Port *et al.* (1980) have briefly reviewed earlier papers on the selective biting by mosquitoes on different age-groups of people.

Other problems of estimating man-biting rates

Non-random biting on people violates the assumption made in nearly all mathematical models on disease transmission, that is that everybody is at equal risk from mosquito bites (e.g. Bailey, 1975, 1982). Dye & Hasibeder (1986) found that when mosquitoes selectively feed on certain people this results in the vectorial capacity and the basic reproductive rate of malaria being larger than, or equal to, their estimated values under homogeneous mixing; a result anticipated by Dietz (1980). In fact, the results of Muirhead-Thomson (1951) indicate that non-random host-biting by *Anopheles albimanus* can result in a basic reproduction rate more than 2.5 times than would occur with uniform exposure. Dye & Hasibeder (1986) emphasise the limitations of estimating vectorial capacity from field collected entomological data. Burkot (1988) and Dye (in Burkot, 1988) present a mini-review of non-random host selection and its epidemiological implications in malaria transmission.

Clearly the assessment of man-biting rates is based on an artificial system. With some species an alternative approach is to base man-biting rates on the numbers of freshly blood-fed mosquitoes resting in, and leaving, a house (Garrett-Jones, 1968, 1970; Garrett-Jones & Shidrawi, 1969). However, it is unlikely that all the mosquitoes that have fed on hut occupants will be collected the following morning. Gubler & Bhattacharya (1974) for example believed that basing biting rates on the numbers of blood-fed female *Culex quinquefasciatus* found resting indoors during early morning collections (about 0700–0830 hr) seriously underestimated biting rates, because they had observed, by using exit traps, that a substantial number of adults left houses at 0400–0600 hr. Furthermore, the problem of interrupted feeding remains. Theoretically a better approach to determine the degree of man-mosquito contact would be to collect all the outdoor and indoor resting mosquitoes in a small area (Ungureanu, 1947), but this is very rarely possible. However, Brady (1974) attempted to estimate the biting rate of *Anopheles* on man from the numbers of blood-fed and gravid mosquitoes found in houses in early morning pyrethrum spray-sheet collections and the proportions of blood-fed and gravid mosquitoes found resting outside. But the formula he derived is applicable only if the gonotrophic cycle lasts 48 hr (in which case there should

be no half-gravids in early morning pyrethrum catches), also relatively high outdoor resting densities must be discovered, and confirmation that blood-engorged mosquitoes have in fact fed on man. Because of these, and other limitations, his approach has rarely been used.

A useful review of some of the difficulties of correctly assessing man-biting rates and the epidemiological problems involved is given by Garrett-Jones (1970). Nájera (1974), also discusses some of these problems and emphasises the importance of obtaining reliable estimates of biting rate for use in models of malaria transmission.

Despite all the above limitations human bait catches remain of paramount importance in both epidemiological and ecological studies, and in assessing nuisance biting. For example, Morris & Clanton (1988) using a questionnaire undertook surveys in Florida as to the numbers of mosquitoes biting a person a minute, or lesser period, that would be rated as a problem on a scale of 1–5. Results showed that one bite every 12 min was generally regarded as causing a moderate problem to residents, an attack rate of one mosquito every 5 min was considered to be a bad problem. Later using correlation and multiple regression techniques Morris & Clanton (1989) found a significant association between the numbers of telephone complaints and mosquito population densities.

TRAP HUTS

During the 1950s and 1960s various so-called trap huts were developed to catch the types of mosquitoes that enter houses to feed. Although they are now rarely used, I nevertheless believe a description of them is still merited.

Bertram & McGregor huts

This trap was successfully used in The Gambia to catch adults of *Anopheles gambiae* and *Anopheles melas* attracted to man (Bertram & McGregor, 1956). The main difference between this and a stable trap (pp. 417–28) is that mosquitoes attracted to the bait are caught in entry traps, and are thus prevented from feeding on the occupants.

The original hut was 6 ft 8 in long, 4 ft wide and 7 ft high and was made of plywood fixed to a wooden framework (Fig. 5.7*d*). A curved roof extended about 6 in beyond all four walls of the hut, and a close fitting door was placed at one end of the hut. Both roof and walls were covered with tarred felt fixed in position by battens. A 14-in square window space was cut out 4 ft from the ground along each of the two longer sides. Three interchangeable fittings were made for these window spaces; (1) a panel of wood which completely covered the window; (2) a wooden frame with wire gauze mosquito netting in the middle; and (3) a wooden panel fitted with a 9-in cube framework covered with white mosquito netting and having an inverted funnel of dark metal gauze. A 4½ ft long, 1-in wide slit opening covered with netting and protected by an overlapping wooden flange was constructed in each long side of the hut 14 in from the ground to provide additional ventilation. The trap was raised from the ground on 6-in legs.

Finally, a wooden batten extending about 3 ft beyond each end of the trap was screwed on to each side of the hut 18 in above the base to enable four men to lift the trap and carry it short distances. It can be transported over longer distances by pick-up vehicles or lorries. Bertram & McGregor (1956) recommended that the hut could be improved by placing an entry window trap in all four walls, and providing them with a cover to help keep out the rain.

Bertram & McGregor baited their trap with a man sleeping on a bed. Mosquitoes were either removed from the window cages periodically throughout the night or just after dawn. Because it proved difficult to collect mosquitoes from the cages with an aspirator they were sprayed with pyrethrum, containing piperonyl butoxide, prior to placing them in position. As a result mosquitoes were knocked down within a few minutes after entering the trap; no repellency was noted.

When the huts were positioned along an east-west axis with the two window cages facing south and north 5901 females, but no males, of *Anopheles gambiae* and *Anopheles melas* were collected within 18 nights. If the wind persistently blew from one direction then nearly all the catch was caught in the window trap on the leeward side, but when wind direction was variable or when nights were mostly windless mosquitoes were collected about equally in both traps. There are several other records of larger catches of mosquitoes in leeward rather than windward traps. In Panama for example, Le Prince & Orenstein (1916) found that *Anopheles*, but curiously not *Culex*, mosquitoes were much commoner in entry traps fitted on the leeward side than those on the windward side of houses. In South Africa 60 females of *Anopheles funestus* were caught on the leeward side but none from the windward side of a baited, screened tent (De Meillon, 1935). Because of this upwind approach Bertram & McGregor (1956) considered that if entry traps were fitted in only one wall of an experimental hut, this could lead to confusing results concerning mosquitoes attracted to the enclosed bait. This criticism also applies to the use of entry traps in village huts.

In studying the dispersal of *Anopheles melas* from their breeding sites into a Gambian village to feed, Giglioli (1965) used a large number of hut traps similar to the model of Bertram & McGregor (1956), except that entry cages were fixed in all four walls. Larger traps were baited with a man and smaller ones with a goat.

Reuben's hut

Reubens (1966) constructed a trap hut in India differing in a few minor details from the original Gambian model, but mainly by being water-proofed with shiny black plastic sheeting, and having a window trap in three walls and the door. Although in The Gambia the hut trap worked successfully on its first night (Bertram & McGregor, 1956), in India it did not catch many mosquitoes until after much of the plastic covering had been removed by several months of weathering. It appears that the plastic had a slight oily smell and it was thought that this might possibly have been a deterrent to host-seeking mosquitoes. However, after this initial weathering period the trap proved to be about as efficient as a stable trap placed 100 ft away, when both were baited with a bullock. Adults of *Anopheles culicifacies*, *Culex quinquefasciatus* and *Culex fuscocephala*

were commoner in the hut trap whereas *Anopheles tessellatus*, *Anopheles subpictus* and *Anopheles vagus* were caught in greater numbers in the stable trap. Reuben (1966) thought that these differences most likely reflected real differences in behaviour of the mosquitoes towards the type of trap. The trap hut was especially useful in sampling *Culex quinquefasciatus* and *Anopheles culicifacies*, two important vector species. An advantage of the hut trap over the stable trap was that mosquitoes caught in it were prevented from feeding on the bait animal, and could therefore be used in virus isolation studies.

Burton's portable human bait hut

A portable hut was developed by Burton (1963) for the study of mosquito biting rates and the pick-up of blood parasites from a sleeping man. Basically the hut is made of ¼-in thick plywood sheets measuring 4 × 8 ft mounted in a frame of right-angled metal beams such as 'Dexion'. The hut measures about 8 × 8 × 8 ft. Although not essential, a sloping roof is useful in allowing rain to run off; the hut floor is raised about 6 in from the ground. Three identical screened windows, 22 × 26 in, which have louvred openings with ½-in gaps to allow mosquitoes to enter, are recessed in three sides. The door is bolted from the inside. To facilitate rapid erection all panels are numbered and bolts with wing nuts are used. According to Burton (1963) it takes three men 4 hr to erect the hut, but only 2 hr to dismantle it. No indication is given as to the numbers or type of mosquitoes caught in the hut, except that blood-fed individuals occasionally entered.

EXIT AND ENTRY TRAPS FITTED TO HUTS

Experimental huts fitted with various exit traps have been widely used since the 1950s to assess the impact of insecticides on endophilic mosquitoes. A description of the use of these huts is given in Chapter 8. The present account is concerned only with the use of exit and entry traps fitted to huts to catch mosquitoes attracted to human or animal occupants. The idea is that natural cracks and crevices, open doorways, windows and eave gaps allow mosquitoes to both enter and leave houses, but when one or more traps are inserted into the walls, windows or door of a house a sample of the mosquitoes leaving, or entering, are caught.

Muirhead-Thomson exit trap

The most widely used exit trap is probably that developed by Muirhead-Thomson (1947, 1948), or one of its modifications. The original trap consisted of a cage made from a 1-ft cube framework of wire covered with white mosquito netting. One side was inverted to form an entrance funnel narrowing to about a ¼-in diameter opening. The funnel was supported within the cage by string tied from its narrow end to the four corners of the trap (Figs 5.8a, 5.10c). One or two small cloth sleeves incorporated in the sides of the cage enabled aspirators to be inserted to remove the catch. The trap was usually placed in the middle of a piece of black cloth which was secured over a hut window. A large proportion

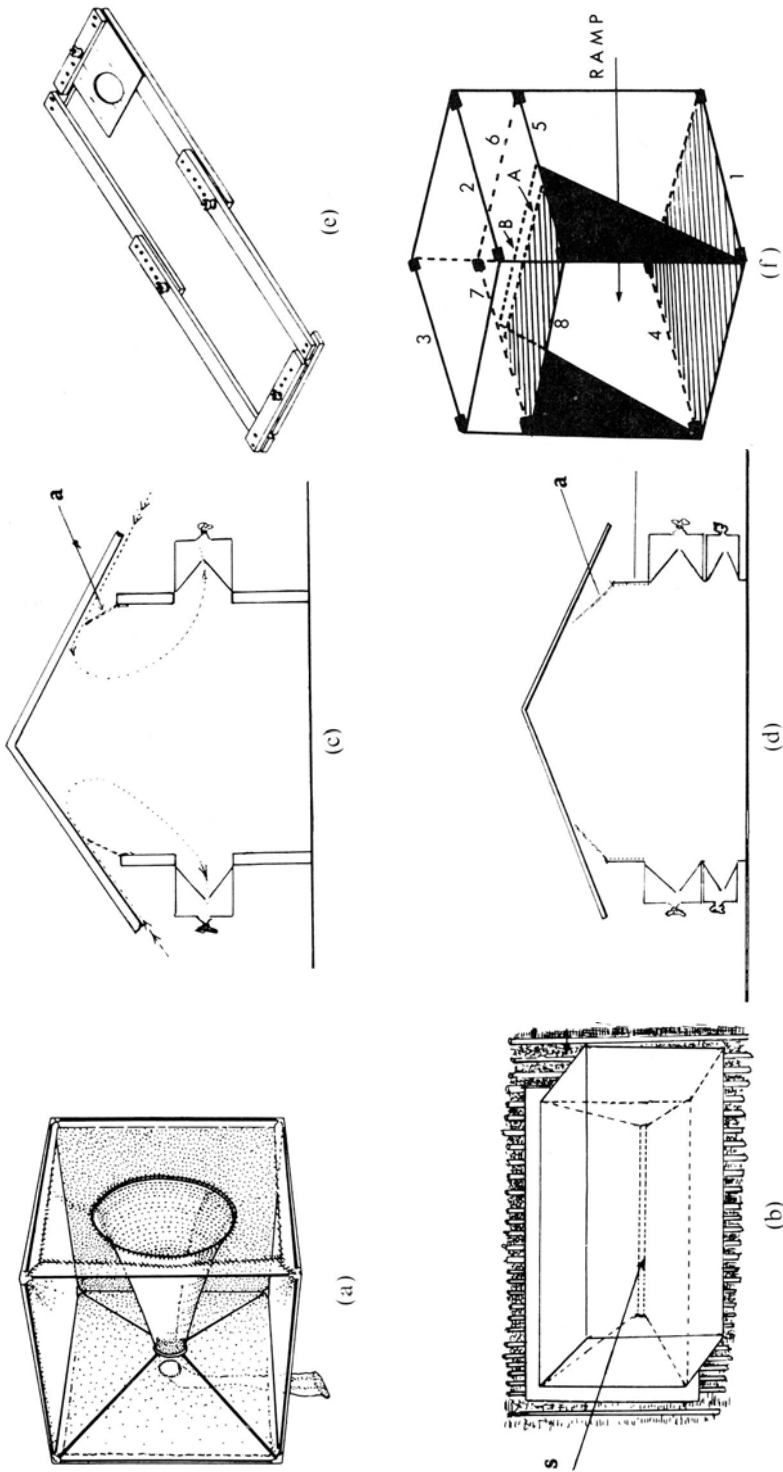


Fig. 5.8. Exit traps: (a) Muirhead-Thomson type; (b) wall-type trap showing entry S — entry slit (after World Health Organization, 1975b); (c) and (d) exit traps fitted to houses showing a — partially blocked eaves (World Health Organization, 1975b); (e) retractable door frame for fitting exit trap (Mpofu et al., 1988); (f) collapsible type of exit trap, 1–8 — rods, A and B — rods forming a 2-cm gap (Shidrawi, 1965).

of the mosquitoes which sought to leave the hut at dawn were attracted by light entering the window, and were consequently caught in window traps as they tried to escape. It is sometimes possible, or even necessary, to partially block the eaves and various cracks and crevices (Mpofu *et al.*, 1988) to allow mosquitoes to enter, but to discourage them from leaving by the eaves (Fig. 5.8c,d).

Various modifications have been made to this original design to take into account local building materials and variations in hut construction as well as the behaviour of the mosquitoes entering and leaving houses. Githeko (1992) found that in Kenya if the small circular opening was too close to the facing posterior wall, many *Anopheles* failed to fly through and enter the trap, but instead turned round and flew out. He found the best distance was 10.2 cm. Instead of using cones, traps having horizontal entry slits can be used. For example, WHO (1975b) recommended a rectangular prism-shaped trap about 1–2 m long, 35 cm deep and 40–50 cm high, with a long horizontal slit (Fig. 5.8b). One or more such traps can be inserted into house walls. Brian Sharp (pers. comm., 1991) considered that it was easier to make a square 'cone' terminating in a 10 × 1.6-cm horizontal slit than a typical cone ending with a circular opening. Such traps have been very useful in South Africa, and I think might prove better than those with circular openings.

In Nigeria a 1-ft cube cloth-covered window trap was used as both an entry and exit trap when fitted to ordinary village huts (Fig. 5.8a). Because normal window openings in the Kaduna area were too small to accommodate the traps larger windows, or well fitting doors, were constructed. They were provided with a 1-ft square flap-like door hinged along the top edge, which when not bolted in place to close the opening was pushed upward to rest on the top of the exit trap. This afforded some protection from rain, but the main purpose of the hinged door was to provide the hut owner with a means of closing the exit trap space when a trap was not fitted, thus giving security to his hut (Service, 1963). Any spaces between the cage and opening in the doors or window were filled with cotton wool, foam rubber or leaves. When the cages were collected a plug of cotton wool was inserted into the narrow opening of the entrance funnel. In some areas most, if not all, huts have no suitable door over their entrances in which exit traps can be inserted. To overcome this, mosquito netting exit traps are sewn into a large piece of dark coloured cloth which is nailed in position over the outside of the doorway (Pant *et al.*, 1969). A disadvantage, however, is that this prevents the occupants having free access to their huts; they have to retire early and stay inside. Although nobody should be entering or leaving any hut with an exit trap after it has been placed in position, there is invariably some degree of movement. This can, and must often, be tolerated if close fitting doors are fitted and these are shut every time a person enters or leaves.

In Zimbabwe lobster-type (Muirhead-Thomson) traps were used to monitor the exodus of mosquitoes from houses (Mpofu *et al.*, 1988). But before they could be fitted, doors had to be removed and replaced by an adjustable retractable door frame (Fig. 5.8e), having black calico sheeting pinned to it to block the doorway. A plywood insert with a hole was fixed into each frame to allow a window-type exit trap to be fitted. All eaves and crevices were effectively

plugged with cotton waste to maximise the catch of *Anopheles arabiensis* in the exit traps.

Up to four window traps, one on each wall, have occasionally been used (Hadjinicolaou in Muirhead-Thomson, 1968). In Malaysia Wharton (1951a) constructed wooden huts with thatched roofs which were raised a foot or two from the ground. They resembled typical village huts. The ceilings and inside walls were lined with smooth brown opaque paper to prevent light entering the huts and also to make the collection of mosquitoes easier. In Malaysia *Anopheles maculatus* is not so markedly orientated to feeding on man and entering houses as is *Anopheles gambiae* in Africa, and the natural cracks and openings in these experimental huts were insufficient for the entry of adults into the huts. Consequently, special louvre openings were constructed in the two opposite longer walls of the huts for the access of *Anopheles maculatus*. The louvres consisted of a series of black ½-in thick wooden slats 2.5 ft long, fitted one above the other at an angle of 30° with the vertical so as to leave eighteen 1¼-in wide longitudinal entrance gaps. Mosquitoes leaving these experimental huts were caught in a Muirhead-Thomson-type mosquito netting exit trap fitted to a window (Reid & Wharton, 1956; Wharton, 1951a,b,c). To check on the proportion of mosquitoes that escaped via the louvres and not through the window trap, Wharton (1951a,b) placed an exit trap over about a quarter of each louvre surface. In unsprayed huts he estimated that about 44% of the *Anopheles maculatus* that entered the hut left via the louvres.

In comparing the relative attractiveness of *Anopheles* (Wharton, 1951b) and culicines (Wharton, 1951c) to different hosts the routine procedure was to bait one hut with a man and another some 30 ft away with a calf, goats or dogs. In the early evening, prior to introducing the bait into the huts and inserting the window traps, blinds which covered the louvres during the day were rolled up. Before sunrise these blinds were pulled down to prevent mosquitoes escaping, and the bait taken out, then about 1–1.5 hr after sunrise the exit cages which contained a representative sample of the mosquitoes leaving the huts were removed. Wharton (1951b) also attempted to determine the times that mosquitoes left the huts by counting the numbers in the exit traps every hour at around dawn. This, however, was not very successful because it was difficult to count mosquitoes in the traps due to their movements.

Specially designed huts built in Tanzania and baited with two men and provided with Muirhead-Thomson-type window traps were used to study the exodus of *Anopheles gambiae* and *Anopheles funestus* (Gillies, 1954). Mosquitoes entered the huts through a line of 2½-in high slit shutters placed in all hut walls just below the eaves. They were closed before dawn so that the only available exits for mosquitoes were the window traps. However, when the eave shutters were fitted with mosquito netting cages it was found that of the small numbers (4–8%) that left the hut as blood-feds, a high proportion escaped through the eaves before the shutters were closed just before dawn.

In certain areas much larger numbers of *Anopheles* are sometimes caught when huts are baited with large mammals instead of man. In Java, for example, insufficient adults were attracted to experimental huts baited with men for an

assessment of the effect of insecticides on the population of *Anopheles aconitus*. More attractive hosts, such as bovids, were needed. Existing thatched-roofed cattle sheds were therefore completely surrounded with bamboo canes and walls of finely woven bamboo. These incorporated horizontal 2-cm wide slits to allow mosquitoes to enter. Mosquitoes leaving the cattle sheds were caught in two window traps placed in the walls (Soerono *et al.*, 1965). When the sheds contained Zebu cattle a mean of 35 *Anopheles aconitus* was collected from the traps after 5 days, but when water buffalo were kept in the sheds 586 mosquitoes were caught in the traps. In Indonesia Barodji *et al.* (1986) compared catches from exit traps fitted to cattle sheds with collections of indoor resting adults. Although more *Anopheles aconitus* were caught by the latter method, exit traps proved useful in catching blood-fed females and thus confirmed that the vector was basically exophilic. In Japan Karoji *et al.* (1980) fitted the exit trap of Katô *et al.* (1966), usually used as part of a dry-ice trap (pp. 510–12), into two windows of two pig sties to trap *Culex tritaeniorhynchus* adults.

In Jamaica experimental huts were baited with a donkey because with human bait relatively few *Anopheles albimanus* were caught (Muirhead-Thomson & Mercier, 1952). In Nigeria cloth exit traps were fitted to horse and cow stables and also to chicken huts. Although few mosquitoes (28) were caught in traps placed in five chicken huts, larger catches of mosquitoes belonging to about 33 species entered traps fitted to both a cow (952) and two horse (3943) stables (Service, 1964). Precipitin tests on 53 blood-fed mosquitoes caught in the traps showed that only five females had fed on animals other than those in the stables.

Rachou exit traps

Working in El Salvador, Rachou *et al.* (1965) considered that any kind of restrictive entrance to an exit trap, whether a slit-like baffle or lobster-type funnel, probably hindered the entry of mosquitoes. Consequently, an exit cage without any kind of one-way entrance was used. At short intervals a partition was slid across the window cage to enclose the catch and enable the cage to be removed and another inserted. It was thought that if the cage was frequently changed very few mosquitoes would escape by flying back into the hut. Although such an exit trap providing unimpeded entry *may* catch more mosquitoes than one with a restrictive entrance, the necessity of regularly replacing the cages and removing the catch involves considerable manpower.

Collapsible window trap

Because of their bulkiness it is sometimes difficult to transport a lot of window traps to the field. A partial solution is to place them on the roof rack of a vehicle, a procedure that also prevents them becoming contaminated with pyrethrum solution and floor sheets which are often carried in the same vehicle. To try to overcome these difficulties Shidrawi (1965) described a collapsible window trap (Fig. 5.8f). This consists of two lengths of 3-mm thick wire rods bent and soldered to form two rectangular frames (55 × 40 cm). These are joined together at the corners by four lengths (Nos. 1–4) of 40-cm wire which are fixed into bushes

welded or screwed on to the rectangular frames. Four similar rods (Nos. 5–8) are fixed between the framework 15 cm from the top to divide the trap into a small upper (40 × 15 cm) and a large lower (40 × 40 cm) section. The ‘floor’ of the upper section is divided about 24 cm from its front by two horizontal rods (A, B) having about a 2-cm gap between them. A mosquito netting and cloth cage made to fit the shape of the trap is suspended within the frame-work by tapes. A ramp of mosquito netting is made to stretch from the bottom edge of the cage to the posterior wire rod (B). A rectangular piece of dark cloth covers the floor of the upper section from the front to the first rod (A). Mosquitoes entering the lower half of the trap are guided up the ramp through the narrow slit between rods A and B into the upper section and lower section behind the ramp. Triangular pieces of dark cloth are used for the sides of the entrance. Shidrawi (1965) claimed that these traps could be dismantled and reconstructed within 10 min, and that 10 such traps when collapsed occupy less space than a conventional cloth window trap.

Verandah traps of Smith

Although window traps are very useful in catching mosquitoes which are attracted by light entering windows at dawn, they trap only a relatively small proportion of such mosquitoes, and moreover not all species are attracted by early morning sunlight. Also, when huts are sprayed with certain insecticides a large proportion of the mosquitoes that would normally be caught in window traps may be stimulated to leave before sunrise, and many of these escape via the eaves. To sample such mosquitoes Smith (1965) developed a verandah trap. This is made by extending the thatch roof beyond the four walls of a square or rectangular hut to form a roof over a verandah, which is enclosed on two sides by mesh screening. Mosquitoes enter the hut through the eaves of the two walls having unscreened verandahs. These huts are discussed and figured in Chapter 8.

In preliminary trials in Tanzania 51% of the *Anopheles gambiae* population left a man-occupied hut each night and of these 85% left via the windows and 15% via the eaves. In marked contrast 90% of *Mansonia uniformis* left each night, of which 69% left via the eaves and 31% through the windows. In huts with iron roofs the exodus of *Anopheles gambiae* increased to 63% and most escaped through the eaves, but there was no significant increase in the percentage of *Mansonia uniformis* that left (Smith *et al.*, 1967). Although Smith fitted the verandah cages to specially constructed huts, normal village huts can sometimes be adapted for fitting these, or simpler, verandah cages.

In Burkina Faso Coz *et al.* (1965) used both verandah-type exit traps and window traps with horizontal entrance slits to catch mosquitoes leaving huts sprayed with insecticides. They also placed boxes having Bates-type entrance slits (p. 423) in the mud walls of huts, so that mosquitoes could enter but not leave. These boxes could be closed by lowering a hinged lid over their openings. In an evaluation of permethrin and fenitrothion residual spraying in houses in Kenya Taylor *et al.* (1981) used verandah-type exit traps (WHO, 1975a), but caught relatively few mosquitoes in them.

Coz (1971) in comparing the efficiency of window and verandah traps concluded that although the former were more easily managed and could be changed several times a day they tended to delay mosquitoes leaving huts when compared with catches in verandah traps. With sprayed huts this would mean increased insecticidal contact and higher mortalities.

In Brazil Roberts *et al.* (1987) fitted window-type traps to window spaces and to the gables of houses to sample *Anopheles darlingi* both leaving and entering houses. In addition two modified verandah traps were constructed. One trap enclosed a relatively large area of wall (1.8×2.95 m) which included an exit trap in one window, whereas the other verandah trap enclosed just the corner of a house ($1.8 \times 0.2 \times 0.2 \times 1.8$ m) at the meeting of two walls. These traps were quite successful in catching *Anopheles darlingi*, which were collected at 2-hr intervals so as to measure times of entry and exodus. The numbers of *Anopheles darlingi* caught in entry traps fitted to windows were greater than in similar traps fitted to the gables.

Curtain traps

Because many houses in Latin America have walls made of loose-fitting bamboo or matting, the collection of exodging mosquitoes by exit traps fitted to windows or doors, or even by verandah traps, is inefficient. To overcome these problems Elliott (1972), devised a technique by which a house was completely encircled from lower edge of the roof to ground with a nylon mosquito netting curtain (Fig. 5.9). The curtain was raised for the first 30 min of each hour to allow ingress of mosquitoes and then mosquitoes resting on the inside and outside surfaces of the netting collected. Interpretation of the data was complicated because the numbers caught leaving were 2–3 times greater than those caught entering. Elliott (1972) concluded that the net curtain trap was better at trapping exiting rather than entering mosquitoes. Another problem was that when the curtain was raised it was impossible to evaluate mosquito movements because they were able to enter and leave freely.

In Mexico Bown *et al.* (1985, 1986) used Elliott's technique, and a modification of it, to study the behaviour and mortality of *Anopheles albimanus*. Later only the modified approach was used, and this was as follows. The curtain was made by sewing several pieces of mosquito netting to form a rectangle, 3×40 m, which was attached to the eaves of the roof and dropped down to reach the ground. The bottom edges were bordered with calico to afford protection against wear. The ends of the wrap-round curtain overlapped considerably to allow house occupiers to enter without having to raise the curtain. Firstly all mosquitoes were removed from the house in the late afternoon, then again after dusk (1800 hr), and then once every hour (say on the hour) any mosquitoes resting on the outside of the curtain were collected, and unfed ones released into the house. This process of collection and release continued hourly until 0600 hr. In addition mosquitoes resting on the inside surface of the curtains were collected hourly at half past each hour and sorted into unfed and blood-fed females. Alternatively mosquitoes collected at human or animal baits were sometimes released into the house at the start of the night and since they were



FIG. 5.9. Colombian curtain in place and enclosing a house in Kenya (M. W. Service).

released together, the time they stayed in the house before being collected on the inside of the curtain was determined. Such collections gave useful information on mosquitoes entering and leaving houses.

Dead and moribund mosquitoes were also collected from inside the house and in the space between the wall and the curtain. To facilitate this a 1-m wide strip of white sheeting was placed on the floor inside the house around the walls, and in the ground space between the curtain and outside of the walls.

Bown *et al.* (1987) using this modified method collected *Anopheles albimanus* from the inside and outside of curtains surrounding unsprayed and sprayed houses (bendiocarb and deltamethrin) on alternate hours from 1800–0600 hr. For an hour all mosquitoes caught on the outside of the curtain were collected, but only the unfed females were released into the house. During the subsequent hour mosquitoes resting inside were collected and classified according to their physiological condition. Later Bown *et al.* (1991) used basically the same method, that is with the curtain lowered to the ground the inside of a house was searched for 45 min at 1715 hr to remove all live and dead mosquitoes. From 1830–2100 hr four people collected a minimum of 150 unfed female *Anopheles albimanus* from other houses in the village, and released them in the curtained house which had five to seven people in it. At hourly intervals from 2200–0600 hr mosquitoes resting between the interior of the curtain and outside house wall were recaptured and their gonotrophic conditions recorded. Collections of dead

and moribund mosquitoes were removed from the floor sheets placed between the walls, and at 0600 hr a final collection of alive indoor resting mosquitoes as well as dead ones was made.

Recently the 'Colombian curtain' has been used in Kenya in an evaluation of permethrin-impregnated bed-nets (A. Githeko, pers. comm., 1991), with a slight modification in that the curtain was placed on only two opposite walls, the other two walls were uncovered to allow entry of anophelines.

Further details on the use of the Colombian curtain in insecticide sprayed houses are found in Chapter 8.

Muirhead-Thomson type entry traps

Window traps have occasionally been used as entry traps to sample mosquitoes entering a hut to feed in distinction from catching those leaving a hut. The much smaller numbers usually caught in entry, as compared to exit, traps clearly show that they are not very efficient in sampling mosquitoes entering huts.

In Kenya large window traps, more than 1-ft cube and having an inverted funnel entrance of mosquito netting, caught over 14 000 mosquitoes belonging to 30 species when they functioned as exit traps, but when used as entry traps only 289 mosquitoes belonging to six species were caught (van Someren *et al.*, 1958). Similarly Teesdale (1955) caught considerably fewer mosquitoes in window traps used as entry rather than exit traps, but in Nigeria entry traps fitted to village huts caught substantial numbers of *Anopheles gambiae*, *Anopheles funestus* and even *Anopheles nili* (Service, 1963). They were very useful in showing that about 24, 16 and 52% respectively of these three species entered huts as blood-fed individuals. They also demonstrated that there was some movement of half-gravid and gravid females of *Anopheles gambiae* and *Anopheles funestus* into huts.

In Korea entry window traps fitted to pig-baited portable sheds have caught large numbers of *Culex tritaeniorhynchus* (Ree *et al.*, 1969). A common procedure in Japan is to place a bed-net, either with one side partially rolled up or with a section cut out, over the entrance of stables and cattle sheds to catch mosquitoes entering these animal quarters. By removing the catch at hourly intervals the biting times can be studied (Katô & Toriumi, 1950).

ANIMAL BAIT CATCHES

General considerations

There are several general considerations that are applicable to the use of animal baits. For example, as with humans, increase in host size, or numbers, seems to increase the numbers of mosquitoes attracted (Edman & Webber, 1975). If the conditions conducive to interrupted blood-feeding are also conducive to multiple feeding, then the dynamics of multiple feeding may be influenced by the interaction of many factors such as host density, host species, host behaviour, mosquito density, and infection of host and/or mosquitoes with disease organisms (Day & Edman, 1983, 1984*b*; Edman & Scott, 1987; Klowden & Lea, 1979; Walker & Edman, 1985*a,b*, 1986).

Several workers have recorded seasonal shifts in feeding patterns from birds to mammals and *vice versa* (Bertsch & Norment, 1983; Hayes *et al.*, 1973; Reeves, 1971; Suyemoto *et al.*, 1973; Tempelis, 1975). In Iowa Ritchie & Rowley (1981), reported an apparent midsummer increase in the proportions of *Culex pipiens*, *Culex restuans* and *Culex salinarius* feeding on mammals in preference to birds. In Massachusetts Nasci & Edman (1981a) found a seasonal feeding shift in *Culiseta melanura*, from almost exclusively feeding on passerines at the beginning of the year (June) to feeding on non-passerines, and to a much lesser extent mammals, reptiles and amphibia, later in the summer (August–September). In Kenya Chandler *et al.* (1977) recorded seasonal changes in feeding in the *Culex univittatus* group, which was believed to be due to changes in the availability of hosts. This shift involved predominantly feeding on ciconiform birds early in the year to feeding exclusively on passerines at the end of the year. In addition to changes in feeding on different types of birds, *Culex univittatus* also tended to shift towards feeding more on mammals, mainly cattle, at the end of the year. Nasci (1984) gives several references of seasonal changes in the feeding patterns of *Culex nigripalpus*, *Culex tarsalis*, *Culex univittatus* and *Culiseta melanura*. He found that in Indiana *Aedes vexans* and *Aedes trivittatus* exhibited considerable daily variability in the types of host fed upon both within and between different types of habitats.

Such switching in feeding behaviour can be due to changes in numbers and availability of hosts, or switches to feeding on more passive hosts when mosquito biting populations are very large and elicit host defensive reactions (see below).

Edman pioneered the study of host-defensive behaviour and feeding success of mosquitoes over 22 years ago (Edman & Kale, 1971), and since then there have been several interesting papers on host responses, such as defensive reactions to being fed upon by mosquitoes (e.g. Culley *et al.*, 1991; Day & Edman, 1984b; Downes *et al.*, 1986; Edman *et al.*, 1972, 1985; Edman & Scott, 1987; Kale *et al.*, 1972; Klowden, 1983; Klowden & Lea, 1979; Molyneux & Jefferies, 1986; Scott *et al.*, 1988, 1990; Walker & Edman, 1985a,b, 1986). Host-defensive reactions and other aspects of host location are reviewed by Edman & Spielman (1988). There are several references to biting insects, including mosquitoes, causing animals to seasonally migrate away from areas of intense biting. Downes *et al.* (1986) review the effects of insects, including mosquitoes, on caribou. Those interested in the protective measures adopted by animals against biting flies should also read the paper by Waage (1981) on how the zebra got its stripes, and his earlier paper (1979) on the evolution on insect-vertebrate associations—although mosquitoes are not referred to in either publication. Other interesting papers on host attraction have been published by tsetse entomologists. For example, Vale (1981) found that an ox's diet can greatly influence the numbers of tsetse flies it attracts, while Hargrove (1976) discovered that the presence of man near an ox reduces the catch of *Glossina morsitans* that would normally feed on the animal. But in The Ivory Coast the presence of a man collecting mosquitoes from an anaesthetised monkey had negligible effect on mosquitoes attracted (Cordellier *et al.*, 1983).

Generally there has been better critical work and evaluation of host attractants with respect to tsetse flies than mosquitoes, and a more scientific approach to sampling procedures, especially by workers such as Vale and Rogers. For example, tsetse workers, have identified carbon dioxide, acetone and octenol (1-octen-3-ol) as attractant components in ox odours, but there is considerable evidence that other ingredients such as various aldehydes, fatty acids and ketones are also attractive. In fact one ketone, namely butanone, which is found with acetone in the blood, milk and urine of cattle has proved to be a powerful tsetse attractant (Vale & Hall, 1985a; Vale *et al.*, 1986). That some of these and other chemicals can be repellent at high doses does not preclude them from being attractive at low dosages. It also seems that some components are not attractive alone but synergise others. For instance when Vale & Hall (1985b) incorporated acetone (5–5000 mg/hr), octenol (0.05–50 mg/hr) and carbon dioxide (0.02–20 litres/min) in visual attraction traps catches of *Glossina morsitans* and *Glossina pallidipes* were greatly increased, a mixture of all three chemicals increased catches up to 60 times. Catches of Stomoxinae and other muscoids were increased when carbon dioxide was used, but the other two chemicals had no effect on catches. In trials in Brazil octenol did not attract *Lutzomyia* sandflies (R. D. Ward, pers. comm., 1989), but Kline *et al.* (1990) have recently shown that when combined with carbon dioxide octenol appears to be attractive to mosquitoes (see pp. 350–1).

Although electric grids have been used to stun and/or kill tsetse in studies on their host seeking behaviour they are rarely used in sampling mosquitoes. However, the electrocuting device described by Rogers & Smith (1977) which operates from a small 2-V accumulator-type battery that can be recharged by solar cells or a car battery, might prove useful, especially as it weights only 1 kg. Battery drain is just 17 mA, and the battery lasts for 300 hr before needing to be recharged. However, note that the high voltage capacitor works at 3000 V d.c. not at 300 as given in the paper.

One method of determining the range of insect attraction to animals is to arrange non-attractive interception traps at various distances and direction from a host-baited trap (e.g. Gillies & Wilkes, 1970, 1972). In Florida Edman (1979) used ramp traps similar to those of Gillies (1969) to study host orientation to animal baits and carbon dioxide. Contrary to the observation of Gillies & Wilkes (1974) he found no evidence that host-seeking mosquitoes fly downwind, in fact *Culex nigripalpus* exhibited strong upwind flight. It appeared that hosts, or carbon dioxide, stimulated host-seeking at a distance of 15 m. Another approach is to place two identical traps at varying distances from each other, and then determine at what distance the size of the catch per trap is no longer decreased by the other, this would then be equal to twice the trap's range of attraction. Alternatively the numbers caught in traps set at increasing distances from a concentrated source of adults (e.g. isolated breeding sites) can be recorded. However, the decrease in numbers caught in the traps must be distinguished from the natural decline in numbers dispersing associated with increasing distance. A description and detailed account of how these latter two approaches were used to measure the distance of attraction of *Glossina pallidipes* is given by Dransfield (1984).

Tethered animals

Collection of tsetse flies from a tethered host, usually an ox, is a common sampling method for *Glossina* (see Glasgow & Phelps in Mulligan (1970) for references), but catching mosquitoes from bait animals not enclosed in any kind of trap has not been so widely employed. When, however, the method is used a common procedure is for one or more collectors to visit the tethered animal at intervals to collect with aspirators or test tubes mosquitoes that have settled on it and may in fact be feeding. In addition, mosquitoes that have not settled on the bait but are hovering around are sometimes caught in small hand-nets. An objection to direct catches from animals is that mosquitoes that may be attracted to the collectors while they are catching mosquitoes from the bait are likely to get included within the catch. Even if such mosquitoes are not immediately caught, having been attracted to the vicinity of the bait they may eventually settle on it, and be caught during subsequent collections, although in fact they have not been stimulated and attracted to the area by the tethered bait. The use of repellents may usefully reduce the numbers of mosquitoes attracted to the collectors, but at the same time may deter them from being attracted to the bait animal. Mosquitoes will normally be collected more quickly from bait animals during the day than during the night, consequently the likelihood of collecting those attracted to the collectors will be less.

Direct bait catches from tethered docile water buffalo or oxen have been used to sample mosquitoes in Taiwan (Hu & Grayston, 1962). Collections for a 3-hr period starting at sunset provided useful information on the relative abundance and seasonal prevalence of *Culex fuscocephala*, *Culex tritaeniorhynchus*, *Culex vishnui*, *Anopheles sinensis* and *Anopheles tessellatus*. In addition to these mosquitoes, 16 other species were caught. In Pakistan Aslam *et al.* (1977) and Reisen & Aslamkhan (1978) caught *Culex tritaeniorhynchus* and several other mosquito species for 15-min periods each hour of the night as they arrived to feed on buffaloes or cattle tethered to feed troughs. In later collections mosquitoes were caught from a tethered buffalo with the aid of torches and mouth aspirators, for a 30-min period starting 20 min after sunset (Reisen & Milby, 1986). Catches of *Culex tritaeniorhynchus* from cows were made from both inside and outside cow sheds in Korea (Ree *et al.*, 1969). In Malaysia, Wharton (1951*b,c*) collected mosquitoes attracted to tethered cattle, while in Trinidad Senior White (1952) made observations on *Anopheles aquasalis* feeding on a tethered ox, goat and a horse. In Jamaica Muirhead-Thomson & Mercier (1952) made routine collections of *Anopheles albimanus* from a tethered donkey, while in the USA Jones *et al.* (1977) collected mosquitoes from tethered horses and donkeys. In South Africa several mosquito species were successfully caught from tethered oxen (De Meillon *et al.*, 1957). In England in a study to identify the potential mosquito vectors of myxomatosis, mosquitoes were collected at 10-min intervals from a rabbit tied by a 1-m lead to a tree (Service, 1971*c*). In Australia Myers (1956) also collected mosquitoes attracted to rabbits, but immobilised them by pinioning them firmly to a board. Mosquitoes attracted to the rabbit were caught by carefully lowering a cone-shaped trap over the bait at intervals. This technique is somewhat similar to a drop-net catch. In Canada

Hudson (1983) used aspirators and torches to collect mosquitoes biting unrestrained calves, but when mosquito densities were high the cattle became restless and sometimes stampeded, and so they had to be tethered for mosquito collections.

In California Barnard & Mulla (1977) studied the diel feeding patterns, from 1400 or 1600 to 0745 or 0700 hr, of *Culiseta inornata* on a calf tethered to a stake in an open area and supplied with food and water. Throughout the night the bait was approached at 15-min intervals and the numbers of mosquitoes feeding counted; a procedure taking just 60–90 s. A 25-W lamp provided illumination. During daylight hours records of feeding were made hourly.

In Mali and Burkina Faso Touré & Coluzzi (1986) encircled cattle corrals with a fence of white mosquito netting. This was done by pushing vertically into the ground at 3-m intervals a series of 1.8-m tubular metal posts and attaching 1-m sections of metal tubing at an angle of 120° onto the tops of these posts. Mosquito netting was then hung from a series of rings placed at 30-cm intervals on both the vertical and slanting metal tubes. This netting barrier was erected about 1 m from the edge of a fence enclosing the cattle. Mosquitoes flew over this 2.3–2.5 m high barrier, and also through a 10–20 cm gap formed between the ground and the bottom edge of the netting, to feed on the encircled cattle. Large numbers of blood-fed females of the *Anopheles gambiae* complex, and other mosquitoes, were collected resting on the inside surfaces of the upright and inwardly leaning netting barrier.

In El Salvador periodic collections of *Anopheles albimanus* comprising mostly blood-fed individuals, were made from vegetation and from the beams and poles of corrals, having a roof but no walls, in which cattle were tethered. Regular 2-hr collections of mosquitoes attracted to these animals provided information both on the seasonal incidence of *Anopheles albimanus* and the variations in densities in different localities (Rachou *et al.*, 1965).

Animals that are too unmanageable to be tethered, such as most monkeys, can be anaesthetised with nembutal or some other suitable veterinary anaesthetic and placed or strapped on a board. In the Ivory Coast Cordellier *et al.* (1983) placed an anaesthetised monkey in a widely spaced mesh cage and had a person collect, with an aspirator, mosquitoes landing on it. It would also be possible to shoot dart-delivered anaesthetics into wild animals that are not easily approached and caught, thus enabling collections to be made from them while they are immobilised. Crans & Rockel (1968) caught mosquitoes attracted to turtles by tying nylon cord to a ring passed through a hole drilled in the edge of the carapace.

ANIMAL-BAITED BED-NETS

In addition to using bed-nets to trap anthropophagic mosquitoes they have been widely used to collect mosquitoes attracted to a variety of animals. Among their advantages over more elaborate traps are their cheapness, availability, simplicity, and ease of transportation to collecting sites. There are, however, a number of limitations (pp. 415–17).

The simplest arrangement consists of positioning an ordinary single or double-sized bed-net over a host and raising it a few cm from the ground to allow entry of host-seeking mosquitoes, but rather more complicated nets have sometimes been made. A few of these, such as the Wright & DeFoliart and Shannon nets, are described in this section.

In Nigeria Bown & Bang (1980) suspended a mosquito net about 30 cm above the ground over cages baited with one goat, two chickens, two monkeys and three rats. From eighteen 24-hr collections only 70 females belonging to six *Aedes* species were recorded. In South Africa Jupp & McIntosh (1967) placed an animal bait (goat, sheep) in a 2-in wire mesh cage in the centre of a bed-net 7 ft long, 5.5 ft wide and 6 ft tall supported on poles and raised 8 in from the ground. A large zippered panel allowed a collector to enter. Ten mosquito species were caught, the commonest being *Culex pipiens* (53%), *Culex theileri* (28%) and *Culex univittatus* (12%), this being the same order of abundance as obtained with CO₂-baited lard-can traps.

In Okayama City, Japan, when Sasa & Sabin (1950) baited standard US army mosquito bed-nets with a man and a variety of animals the most common mosquito caught was *Culex tritaeniorhynchus* followed by *Culex pipiens form pallens*, *Anopheles hyrcanus* and *Anopheles sinensis*. Surprisingly the mean catch of mosquitoes in a bed-net baited with both a chicken and a rabbit (588) was consistently greater than the mean catch obtained when it was baited with only a chicken (84) or rabbit (239), and also greater than the combined mean catches (323). Sasa *et al.* (1950) considered bait-nets were the most convenient and accurate collecting method for studying host preferences and temporal fluctuations in population size of the local mosquitoes. By not enclosing the bait animals within an inner mosquito net they gave the mosquitoes the opportunity of feeding on the bait, and used the rates of engorgement by different species on various baits to provide additional information on host acceptability.

In studies on vectors of simian malaria in Malaysia, mosquitoes were caught in nets baited with monkeys (Wharton *et al.*, 1963, 1964). The technique involved enclosing monkeys (*Macaca irus*) in a small cage covered with chicken wire, which did not prevent the mosquitoes from feeding on them (Fig. 5.7a). Two monkeys were enclosed when a small bed-net measuring 4 × 3 × 3 ft, having a 14-in wide 3-ft high opening on either side, was used, but four monkeys were exposed under larger nets measuring 6 × 5 × 4 ft with two 22-in wide and 4-ft high openings. There were no flaps to the openings. The traps were positioned either at ground level or at various heights in trees, and mosquitoes collected from them every 1–2 hr throughout the night. The larger nets with four monkeys caught about twice as many mosquitoes as did the smaller nets, and 5–6 times as many adults of *Anopheles hackeri*, the principal vector of *Plasmodium knowlesi*. Trap location was very important. More adults of *Anopheles hackeri* were caught in traps placed in a mangrove swamp where wild monkeys slept, than in traps sited immediately above the vector's larval habitats (Wharton *et al.*, 1963).

In later trials as many as 45 culicine and 18 *Anopheles* species were caught in monkey-baited bed-nets (Wharton *et al.*, 1964). Also in Malaysia Reid (1961)

compared the mosquitoes attracted by two men, a calf and two goats enclosed in 10-ft long, 7-ft high and 7-ft wide nets which had a 3-ft wide entrance in each long side which could be closed by a flap. The nets were positioned under a shelter without sides, and mosquitoes collected hourly from 1900–2300 hr. Results were expressed in terms of attraction ratios, for example the ratio of the numbers of a particular species caught on man compared with the numbers caught on a calf or two goats. Reid (1961) considered that although experimental conditions (e.g. sampling error, size of baits, different types of trap) might change the values of the ratios, their order of magnitude for different species would nevertheless remain more or less the same, and could therefore be used to compare results from experiments made at different times, in different localities and even in different areas. For example, although the man:calf ratio calculated for *Culex quinquefasciatus* and *Mansonia uniformis* caught in bait nets was 1.7:1 and 1:2.8 but 4.0:1 and 1:36 in catches with window trap huts, the two species were ranked in the same order of attractiveness by both sampling methods.

In Sabah, Chiang *et al.* (1984b) placed a bed-net (300 × 240 × 210 cm) having a 90-cm wide gap in each of the longer sides over a calf. Mosquitoes were collected for 15-min periods every 2 hr from 1800–0600 hr, or for 15 min every hour from 1800–2100 hr. Seventeen species were caught, including large numbers of *Culex gelidus*, *Aedes vexans* and *Culex tritaeniorhynchus*. In Malaysia Zairi (1990) baited a mosquito net (2.4 × 2.4 × 1.6 m) having a 1.2-m wide door panel closed by zippers on one side. A tarpaulin was placed over the net to protect against rain (Fig. 5.10b). Hourly, three collectors entered the net for 15 min to remove anophelines such as *Anopheles peditaeniatus*, *Anopheles sinensis*, *Anopheles lesteri paraliae*, and *Anopheles subpictus*.

In India Reuben (1971) caught over 21 mosquito species attracted to a man, a buffalo and a bullock and confirmed Reid's observations on attraction ratios. *Culex quinquefasciatus* preferred man, *Culex bitaeniorhynchus* was about equally attracted to all three baits, while the other species preferred animal baits to man. It seems unlikely, however, that attraction ratios will be universally applicable. Also in India Kulkarni (1987) caught 3007 female mosquitoes comprising 19 species of anophelines in bed-nets raised 30 cm from the ground and enclosing a cow tied to a stake. Mosquitoes were removed hourly and the most numerous were *Anopheles karwari*, *Anopheles jamesii*, *Anopheles maculatus* and *Anopheles splendidus*.

In Argentina Mitchell *et al.* (1985) used large (360 × 360, 210 cm tall) bed-net-type traps made of nylon tulle and baited with horses in a study on western equine encephalomyelitis. The trap was raised by ropes 30–46 cm from the ground to allow hungry mosquitoes to enter, and was lowered when mosquitoes were being collected. The bait animal, which was usually exposed from dusk to 1 or 2 hr after sunrise, was led in through a 1-m wide door-flap made in the middle of one side of the net. From just five trap-nights in one locality and three in another, 2752 and 6929 mosquitoes belonging to at least 20 species were caught; *Culex* (*Culex*) predominated at the two sites (45.8 and 95.7%). The commonest species were the *Culex pipiens* complex, *Aedes albifasciatus*, *Psorophora ciliata* and *Psorophora pallescens*. The authors reported that the trap performed better

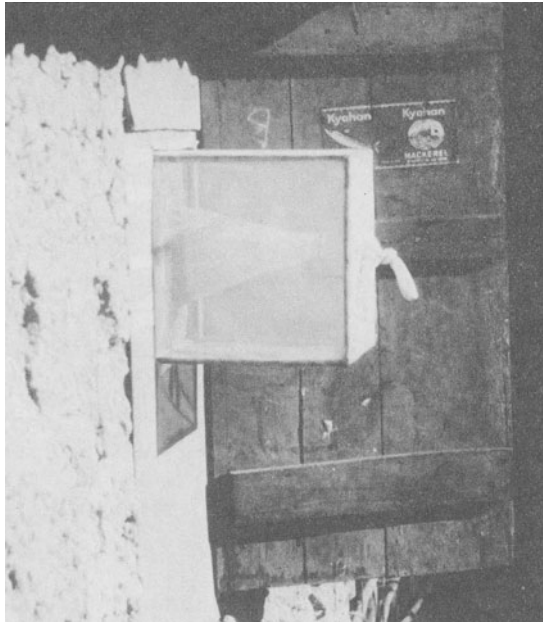


(a)



(b)

FIG. 5.10. (a) Typical night-time human bait catch outside a house (M. W. Service); (b) an animal-baited bed-net trap (photograph courtesy of Zairi bin Jaal).



(c)

FIG. 5.10—contd. (c) Muirhead-Thomson exit trap fitted to window opening of a house (M. W. Service).

than a portable, but still nevertheless cumbersome, Magoon trap, which had previously been used to catch potential virus vectors. In later studies Mitchell *et al.* (1987) collected 20 697 mosquitoes belonging to at least 28 species, but mostly *Culex* (*Culex*) unidentified species, over 3 years in these traps.

Wright & DeFoliart's bed-net

In Wisconsin, Wright & DeFoliart (1970) used a modified Gater-type bed-net (p. 378) made of saran cloth which is supported at each corner by wooden stakes and measures 6 ft in length, width and height. A heavy duty nylon zipper is sewn into one side and after the bait is introduced this is opened to about three-quarters of the distance to the top, and the flaps tied back to leave an opening about one-third of the total area of one side. Twenty species of bait animals were exposed in a variety of cages made of 1 or 2-in mesh and ranging in size from $5 \times 5 \times 16$ in to $11 \times 12 \times 20$ in; frogs, snakes and small rodents were enclosed in cylindrical cages 9 in in diameter and length made of $\frac{5}{16}$ -in hardware cloth. None of the cages prevented mosquitoes feeding on the bait animals. When deer were the bait, a $3 \times 2 \times 2$ -ft hutch was placed in a corner of a chicken wire pen enclosing the deer, and the bed-net suspended from the roof of the pen over the hutch. Bait animals were usually placed in the traps 2–3 hr before sunset and mosquitoes removed 2–3 hr after sunrise by a collector wearing protective clothing, including gloves and a helmet fitted with a bee net. In seven trials with un-baited traps 674 mosquitoes were collected, but only about 1.9% were engorged,

from which Wright & DeFoliart (1970) concluded that very few blood-fed mosquitoes entered the traps (but see pp. 415–17).

Shannon's bed-net

This bed-net was developed by Shannon (1939) in South America apparently independent of the more simple net of Gater (1935) and was usually baited with a donkey to catch day-time biting forest mosquitoes. It consisted of a large central compartment which in the original design was 130 cm wide, 200 cm high and 300 cm long and two identical smaller lateral compartments, 60 cm wide, 300 cm long but only 135 cm deep, thus leaving a 65 cm gap along the bottom (Fig. 5.11*a*). The tops and ends of all compartments were made of strong white muslin cloth, while the lateral panels of the central and two outer compartments were of mosquito netting. In addition, a small window of mosquito netting, 130 cm wide and 50 cm high, was inserted into one of the end panels about 25 cm from the top. A bait animal, usually confined to a pen or cage, is placed in the middle compartment, the lower edges of which are secured to the ground to prevent mosquitoes from entering it. These are collected from the two outer compartments.

Shannon-type nets made completely of cotton sheeting and with shorter side compartments are often used. Figures 5.12*a,b* show such a net in operation in Brazil, erected under a roof to protect against rain.

Shannon (1939) also described a one-compartment trap consisting of only the middle section of the typical Shannon net, with the roof made of muslin, but all four sides of mosquito netting. Mosquitoes are collected either resting on the outside of the trap or inside it when the lower edges are raised. This later modification gives a trap very similar to the bed-net of Gater (1935).

The Shannon-type of bed-net has not been so widely adopted as the simpler Gater's net, having mainly been used in South America (e.g. Forattini *et al.*, 1981). Trapido & Aitken (1953), however, introduced it to Sardinia in a mainly unsuccessful attempt to catch *Anopheles* with an unbaited net containing a light. In Malaysia these nets were not so effective as Gater-type nets baited with monkeys (Wharton *et al.*, 1963). Recently in South Africa Sharp *et al.* (1988) used Shannon (1939) nets baited with a man, goat or bovid, and at about 2100, 2400 and 0300 hr aspirated mosquitoes from the inside walls of the net. Only 133 mosquitoes, however, were caught from three night's collection.

Mpofu & Masendu trap

In Zimbabwe Mpofu & Masendu (1986) developed an ox-baited trap that is really a modified bed-net. It consists of a framework of light metal tubing to form a tent-like structure (Fig. 5.11*c*) that is covered with either mosquito netting or a khaki lightweight canvas. The latter is preferable in rainy weather as it helps protect the bait animal and mosquito catch. For extra stability guy ropes can be fitted to the four corners of the trap. One of the smaller ends has a vertical slit in the netting, or canvas, to allow the bait and collectors to enter. A gap (measurements not given, but probably about 25 cm) is left between the bottom of the net or canvas and the ground for entry of host-seeking mosquitoes. Over

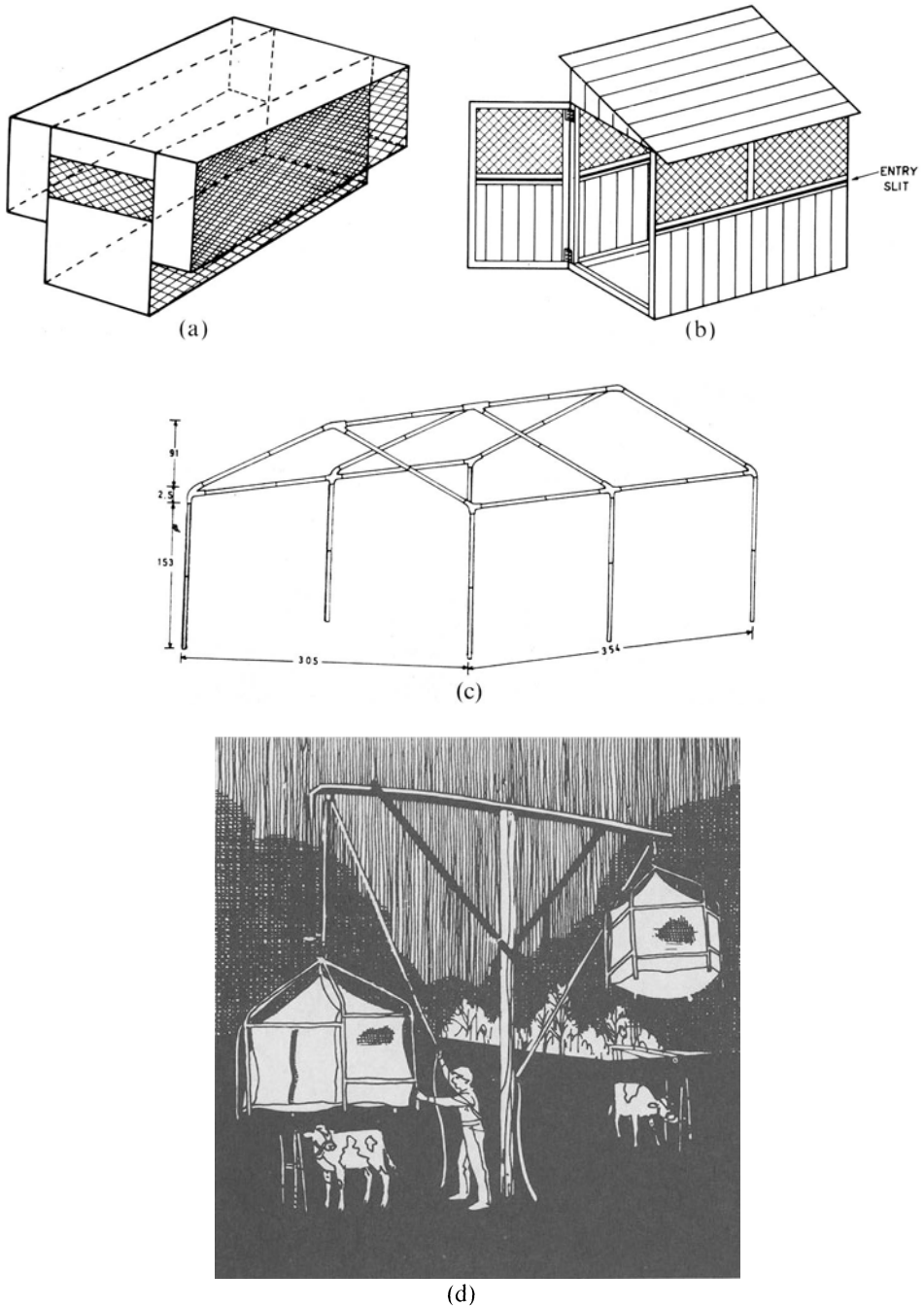
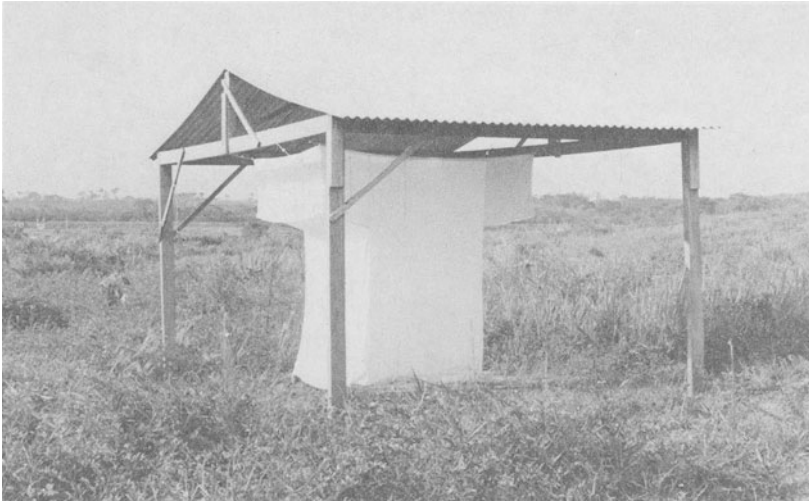
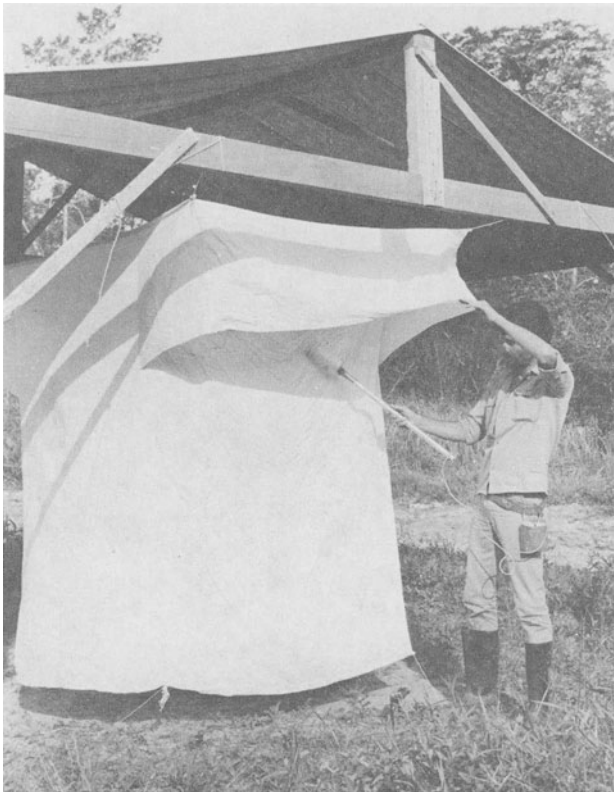


FIG. 5.11. (a) Shannon-type bed-net with central compartment for bait (Shannon, 1939); (b) Magoon-type bait stable trap (courtesy of World Health Organization); (c) tubular framework of animal-baited trap, measurements in cm (Mpofu & Masendu, 1986); (d) drop-net trap (Schmidtman et al., 1980).



(a)



(b)

FIG. 5.12. (a) Shannon trap protected against rain; (b) collecting mosquitoes from Shannon trap with battery-powered aspirator (photographs courtesy of O. P. Forattini).

a 3-month period in an area with apparently low mosquito densities 147 *Anopheles gambiae* complex were trapped from one such trap.

Limitations of bed-nets for human and animal baits

When Gater-type nets were used in Malaysia the mosquitoes caught in the outer nets remained active because they were continuously being stimulated by a bait upon which they could not feed, and this caused a number to escape through the two openings (Colless, 1959). To try to reduce this exodus, nets having only a single entrance were used, and the bait was not enclosed within an inner protective net. When a man was employed as the bait he collected throughout the night mosquitoes from the inside walls of the net on which they rested before attempting to bite. Catches of mosquitoes from human baited nets from which mosquitoes were continuously collected were compared with those from unbaited nets from which they were collected for about 20 min every hour. In one series of trials the mosquitoes which entered the net were given the opportunity to feed on the sleeping occupant. Surprisingly about 39–49% of the total catch of mosquitoes were collected from unbaited nets and in addition to unfed females, males, gravid and a few blood-fed individuals were caught. Because unfed females are more active than gravid individuals a number probably escaped from the unbaited net between the hourly catching periods. Hamon (1964) and Hodgkin (1956) have also reported the entrance of a few blood-fed individuals in bait nets. Wharton (1953) used the precipitin test to check whether engorged mosquitoes caught in his nets and trap huts had entered as recently blood-fed individuals or had fed on the bait.

Working in Malaysia Moorhouse & Wharton (1965) considered that the Gater-type net acted in part as an outdoor resting place for mosquitoes, especially for host seeking ones. For example, instead of mosquitoes resting on nearby vegetation prior to landing on the bait and feeding they rested in or on the nets. They also found that a number of mosquitoes escaped from their nets. They concluded that bed-nets did not give an accurate reflection of biting rates. When collections were made hourly from the nets the ratio of the numbers of mosquitoes caught in the nets to those caught in direct bait catches was 9:1 for *Anopheles letifer*, 1.8:1 for *Anopheles donaldi*, 2.3:1 for *Anopheles campestris* and 6:1 for *Anopheles maculatus*. Thus there was a bigger catch in the nets for all these species than found in direct bait catches on man.

When a Shannon net was baited with monkeys and baboons in Uganda, Haddow (1945a) found that although large numbers of *Coquillettidia fuscopennata* entered the nets, most escaped, especially around sunrise. Moreover, more than twice as many mosquitoes were caught biting a boy outside a net as were observed to enter it when it was baited with a boy, and about three times as many were caught as were retrieved from the nets in the morning. Larger catches have generally been obtained in Africa when the bait has not been enclosed within an inner net, and when the bottom of the net has been raised a few inches from the ground to provide an entrance for mosquitoes.

In Pakistan Akiyama (1973) found that in five trap-nights human-baited bed-nets raised a few inches from the ground caught 1570 female and 139 male

Anopheles, including 22 female and 15 male *Anopheles culicifacies*. On the same nights, however, no *Anopheles culicifacies* and only 16 female *Anopheles hyrcanus* group and 10 female *Anopheles pulcherrimus* were caught in direct bait catches. Half-gravid, gravid and blood-fed females were retrieved from the baited nets, but the engorged individuals contained bovid blood. Unbaited nets caught 172 mosquitoes. Clearly entry into the nets was either mainly accidental or in response to shelter, and not due to host attraction. The presence of large numbers of mosquitoes in unbaited nets is probably often due to them acting as efficient Malaise traps and consequently in these situations the catch comprises adults that have flown in indiscriminantly as well as those attracted by the bait. But when unbaited control nets catch only few mosquitoes, then almost all those collected from bait-nets can be considered to have been attracted by the bait. Nevertheless, this still does not necessarily imply that the different mosquito species are attracted in the same numbers or proportions as they are to the same animals outside nets. Colless (1959) found that when mosquitoes could feed on the bait there was often a disparity between the proportions of the different species caught in bed-nets and the percentage that fed on the bait. For example, large numbers of *Culex tritaeniorhynchus* were caught in nets baited with man but few took a blood-meal, whereas only a few *Culex quinquefasciatus* were caught but a high percentage were blood-fed.

In comparative trials in Burkina Faso more of most *Anopheles* species, particularly *Anopheles broheiri* and *Anopheles flavicosta*, were caught on human bait outside a net than from a man-baited net. With culicines about the same number of *Aedes* were attracted to man in and outside a net, but for other genera more mosquitoes were caught in bed-nets than in direct bait catches (Hamon, 1964). Fewer adults of most species were retrieved when collections were made every 2 hr instead of hourly. Another difference was that small variations were shown between the biting cycles of mosquitoes in direct bait catches and those caught in nets. Unbaited nets caught about 5.3 mosquitoes per night compared with 41.8 when they contained a man or large mammal. Hamon (1964) also reported that in Mauritania most of the mosquitoes that entered bait-nets in the early part of the night left before sunrise. In Nigeria more adults of *Anopheles gambiae* and *Anopheles nili* were caught in direct bait catches on man than in bed-net collections irrespective of whether the catches were performed in village huts or outside in the compounds (Service, 1963). There was no significant difference between the numbers of *Anopheles funestus* caught by the two methods. In a series of outdoor bed-net collections species other than the above three *Anopheles* formed 1.4% of the total catch, whereas in direct bait catches 'other species' formed as much as 49.1% of the catch. Furthermore, *Anopheles brohieri*, which was not collected from bed-nets, formed 13.8% of the mosquitoes in direct catches. Clearly different species may react differently to a bait enclosed within a bed-net, but in Morocco except for *Aedes* mosquitoes Bailly-Choumara (1973) collected the same species from man-baited nets as in direct bait catches, but in nearly all instances the numbers caught in nets were fewer. One of the first indications of the deterrent effect traps may have had on mosquitoes biting normally acceptable hosts was the observation of Hadow *et al.* (1948) in East Africa that although

Aedes africanus attacked monkeys avidly in the open forest they did not readily enter cages to bite monkeys. As a consequence sentinel monkeys had to be tethered to wooden posts fixed to tree platforms.

It is obvious that bed-nets may give heavily biased samples of the different species attracted to the bait, and that they usually attract and retain fewer individuals than can be caught in direct bait catches. But despite these limitations they can in many situations provide a cheap and easy method of catching mosquitoes attracted to man and other animals.

Drop-net cages

Drop-net cages which have been used for the capture of *Culicoides* attracted to animal baits and which could be employed in mosquito studies have been described by Schmidtman *et al.* (1980), Zimmerman & Turner (1983) and Hayes *et al.* (1984). Basically a cage of plastic screening fits over a framework of PVC or aluminium tubing to form a box-like trap (2–3 m diameter, 2–2.5 m high) which is suspended from a wooden crossbeam 5 m from the ground over an enclosed bait animal (Fig. 5.11*d*). A pulley system and winch attached to the upright poles supporting the crossbeam is used to lower and raise the trap. Hayes *et al.* (1984) used 15-min exposure periods before dropping the cage over the bait. Collectors crawled into the traps to remove the catch.

STABLE TRAPS

Stable traps were introduced in the study of mosquito biology later than bed-nets, and have mainly been used in the Americas. They were originally used to catch mosquitoes attracted to equines and bovids, but have since been baited with man, a wide variety of mammals, birds and even cold-blooded vertebrates. They are heavier and more permanent structures than bait-nets but lightweight models can be made so enabling them to be readily dismantled and easily transported. As with bait-nets and most types of animal-baited traps certain mosquito species may be reluctant to enter them. A check on the efficiency of a stable trap in catching mosquitoes that feed on a certain bait can be made by comparing the mosquitoes caught biting the bait outside and inside the trap. The efficiency and usefulness of stable traps may vary in different localities and for different mosquito species. Although de Zulueta (1950) found that with donkey-baited traps the smell from a previous night's bait did not attract any mosquitoes, this is a difficulty that may be encountered with stable traps, especially those with wooden floors. They tend to become contaminated with animal excreta and urine, so that the 'smell' of one animal persists after it has been removed and another introduced. Scherer *et al.* (1959) found that large numbers of mosquitoes were still caught in stable traps the day after a pig was removed; the numbers decreased on the following 2 days. The likelihood of mosquitoes being attracted to a lingering smell of a bait animal can be reduced by covering the floor with disposable plastic sheeting, or confining the bait to a cage that is afterwards removed. Alternatively the trap can be scrubbed out with clean water

and left open for a few days before a different animal is introduced, or a if a trap is used without a floor it can be moved to a new site after the conclusion of trials with one type of bait animal.

Stable traps have usually been used at ground level, but in Japan Flemings (1959) attached ropes to the top of small stable traps (36 × 36 × 24 in) baited with birds and pulled them up to various heights. Adults of *Culex tritaeniorhynchus*, *Culex quinquefasciatus* and *Culex bitaeniorhynchus* were caught in traps at ground level and all four heights, but *Armigeres subalbatus* was only caught in traps on the ground (Flemings, 1959). Scherer *et al.* (1959) also used small stable-type traps baited with birds and suspended at various heights in a study of Japanese encephalitis.

There are two basic types of stable traps, the Magoon and Bates (Egyptian) traps, but various workers have modified them to suit local conditions.

Magoon trap

Stable traps are usually credited with first being used in 1923 in Haiti by Payne, but earlier than this in Florida Metz (1920) caught *Anopheles crucians* and *Anopheles quadrimaculatus* in small wooden shelters baited with pigs and having a longitudinal slit entrance on two sides tapering from an 8-in to a 1-in opening. Stable traps were introduced into Puerto Rico in 1926 where they were modified by Earle (Earle & Howard, 1936), after which their popularity spread to other West Indian Islands, Colombia and Panama, and they became known as Caribbean traps. Only after Magoon had visited Puerto Rico was a detailed description of a modified and more portable trap published (Magoon, 1935). The trap in its original or modified form has now been used in many countries. Its size, construction and the materials from which it is made can be adapted to suit local conditions and requirements.

A useful trap is one with a base measuring about 3½ × 6½ ft, with one of the two longer upright sides about 7 ft high joined by a sloping and waterproof roof to the opposite vertical side which is about 5½ ft high (Fig. 5.11*b*). When animals such as donkeys and calves are used there is no need for a floor, but unless animals such as pigs and rodents are enclosed within a cage, a floor may be necessary to prevent them from burrowing and escaping. A door is fitted at one end of the trap, and another may be fitted at the opposite end so that larger animals can be removed without turning them round. Apart from the roof as much as possible of the trap should be made of plastic or wire mesh mosquito gauze to allow bait odours to escape, but the lower half usually has to be made of stout plywood or sheet metal on a wooden frame, to prevent the animals from kicking it to pieces. Mosquitoes enter the trap through a horizontal entrance slit (Fig. 5.13*c*) placed about halfway up the trap. It may extend completely round the trap, or be confined to the two longer sides. The construction of this entrance slit is the most critical part in building the trap. It is usually made from 4-in wooden planks placed to form a V-shaped trough with a 6–8 in wide opening to the outside converging to leave a ¾–1-in slit-like opening in the trap. The walls of the trap above the entrance baffle are made of mosquito gauze and those below of wood.

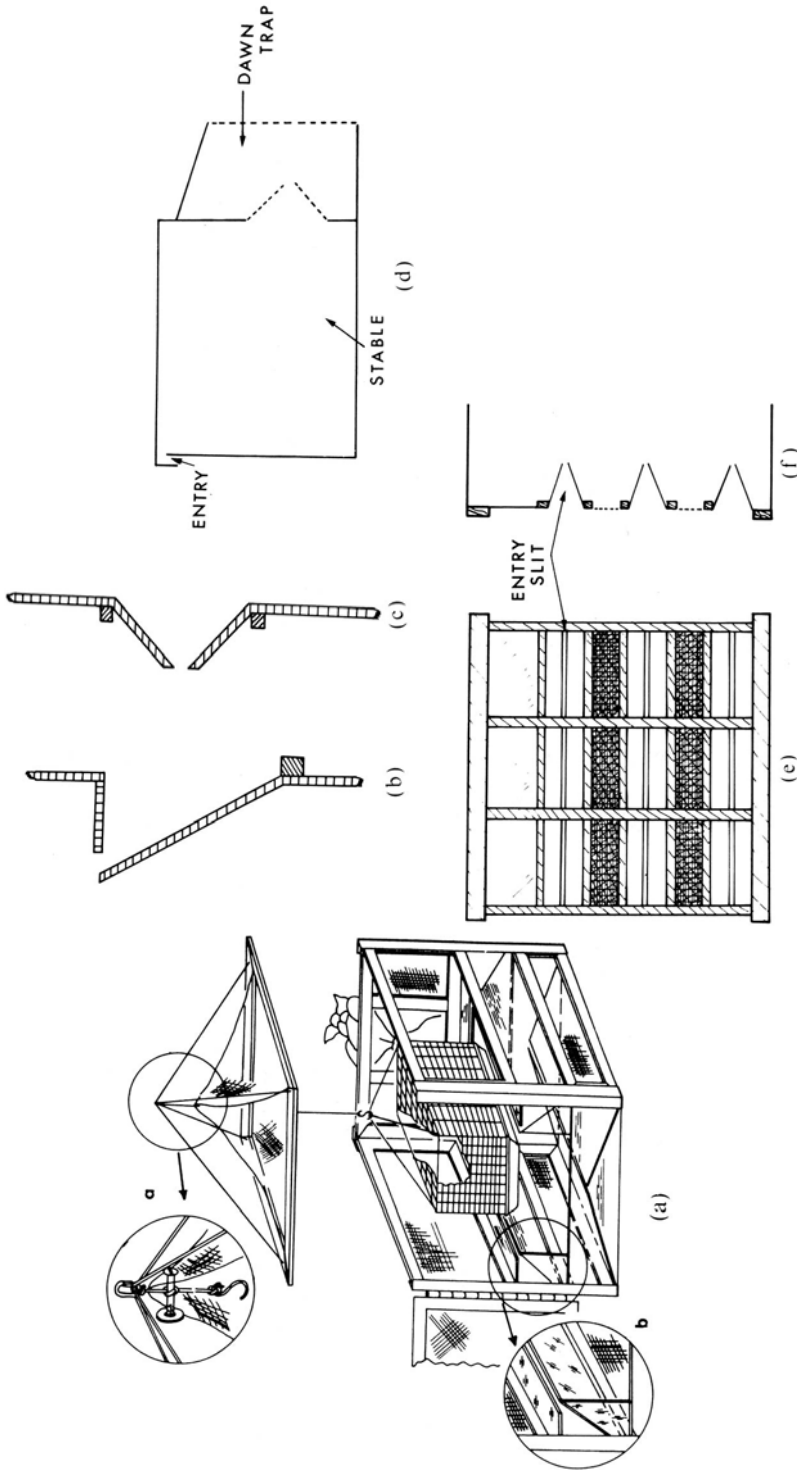


FIG. 5.13. (a) Miniature Magoon trap for rabbits with door open, and roof raised to give better view of the inside of the trap, a — details of roof and system for suspension of rabbit cage, b — details of entry slits (Garcia et al., 1988); (b) Bates (Egyptian)-type entry baffle for stable traps; (c) Magoon-type (i.e. horizontal) baffle for stable traps; (d) Shannon stable trap (dawn trap) (after Earle, 1949); (e)–(f) stable trap with entrance at three levels (after Roberts, 1965).

It is often convenient to make the trap demountable with the various sections fixed into position with bolts and wing nuts, enabling it to be readily transported to different sites (Fletcher *et al.*, 1988). Strips of plastic foam stuck along the edges of the different sections eliminate gaps through which the mosquitoes might escape. Wood chemically treated with insecticides or fumigant preservatives should be avoided as this may deter mosquitoes from entering the trap, or kill those that have already entered. A clear non-toxic varnish or paint can be used to protect the outside of the trap. Various predators such as lizards and spiders may enter the trap and will destroy the catch unless removed. Both Bates (1944b) and Bradley *et al.* (1949) recommended that permanent trap sites should have a concrete base surrounded by a water trough to prevent ants entering the trap and destroying the catch. The normal routine is to expose a bait animal in the trap for 12 or 24 hr, possibly with food or water, after which a collector enters the trap and carefully removes all mosquitoes resting on the walls and roof.

In Texas because the numbers of mosquitoes biting cattle were so great Kuntz *et al.* (1982) modified the Magoon trap to prevent trapped mosquitoes feeding on the bait animals. This was done by fixing a wooden collection box ($91 \times 182 \times 31$ cm) covered with plastic mesh screening over the lower part of each side of the mosquito-proofed stanchion housing a host animal (horse, calf, pig, dog, sheep). Two horizontal louvre openings ending in a 2.5-cm wide slit were positioned at heights of 7.3 and 48 cm on each box. Louvres were fitted with door panels which were closed at the end of the trapping period. Mosquitoes were removed through sleeve-type armholes fitted in the top and two ends of each box. Two traps were operated simultaneously 15 m apart for 2-hr periods after sunset, one in a flooded plain site and the other at a rice-farm site. Five mosquito genera were caught, and the most common species was *Psorophora columbiae* which formed 67.0 and 85.6% of the total catches from these two locations. At the floodplain site the next most common mosquitoes were *Culex salinarius* (12.8%), *Anopheles quadrimaculatus* (5.9%), and *Aedes taeniorhynchus* (3.8%), while on the rice-farm the next most common mosquitoes were *Anopheles quadrimaculatus* (6.4%) and *Anopheles crucians* (4.5%). In addition to the animals listed above, the stable traps were sometimes baited with 3 litres/min of carbon dioxide, or 4 CDC light-traps. Horse- and calf-baited traps attracted most mosquitoes, and except for CO₂ traps at the farm site, carbon dioxide and light-traps were not very attractive. No mosquitoes were caught in unbaited traps.

In Tennessee Hribar & Gerhardt (1986) compared the mosquitoes attracted to dogs in a modified Magoon trap and a Shemanchuk (1978) trap (see pp. 430–1). The Magoon trap measured 1.2×2.44 m and was 1.83 m high, with entry louvres on the two longer sides. Areas above the louvres were made of mesh screening while the part below the louvres was made of wood. The trap was mounted on a trailer to enable it to be easily transported to different areas. The Shemanchuk trap consisted of a 1.8×1.8 and 1.2 m wooden frame with a 1-m high sloping gable on top. The entire trap was covered with plastic mosquito screening. The four sides could be raised to allow hungry mosquitoes to enter. At about 1600 hr two dogs were placed in the Magoon trap and at about 2230 hr all trapped mosquitoes were removed, after which one of the dogs was tethered

under the Shemanchuk trap, while the other remained in the Magoon trap. The sides of the Shemanchuk trap were lifted for 10 min to allow mosquitoes to enter, then they were lowered and 5 min allowed for entrapped mosquitoes to feed on the host. The subsequent collection of mosquitoes took 5 min so the sides of the trap were repeatedly raised every 20 min from 1830–2230 hr.

Although the same five species were collected from both these traps there were some important differences. For example, *Aedes trivittatus* was collected about equally from both traps, but *Psorophora ferox* and *Aedes vexans* were more common in the Shemanchuk trap, while *Aedes triseriatus* and *Culex salinarius* were more commonly caught in the Magoon trap. The two dog-baited traps therefore ranked the species differently in abundance, and because of this the authors stressed the need for more than one trapping technique in epidemiological studies.

Ernst & Slocombe (1984) used a Magoon trap modified to take a dog (Ernst, 1982). The roof was made of plywood and most of the upper part of the sides consisted of mosquito screening. Entrance baffles were small V-shaped longitudinal slits placed directly beneath the screening on three sides of the trap. The dog was restrained in a wire cage. From 1980 to 1981 when the trap was operated on an unspecified number of occasions from evening to midday, 3310 mosquitoes belonging to 10 species were caught, including 1114 *Culex pipiens/restuans*, 739 *Aedes vexans*, 510 *Aedes trivittatus* and 569 *Coquillettidia perturbans*. In India Mahadev *et al.* (1978) using pig-baited Magoon traps caught a total of 33 mosquito species, but only 0.9 mosquitoes/trap-night. Their traps had two 0.75-cm horizontal entrance slits which unfortunately let some mosquitoes escape. They also used the portable bait trap of Rao (1957), which when containing a chicken caught a mean of 1.4 mosquitoes/trap-night.

In El Salvador Magoon traps were successfully employed to study the seasonal variations in the numbers of *Anopheles albimanus* and *Anopheles pseudopunctipennis* (Kumm & Zúniga, 1944), but Lofgren *et al.* (1974) were unsuccessful in their attempts to catch *Anopheles albimanus* in El Salvador in calf-baited traps. Lowe & Bailey (1981), however, developed a simple Magoon-type trap that proved very successful in catching this vector. Basically the trap consists of a wooden frame (1.2 × 2.4 m and 1.8 m high) with the bottom half on three sides covered with plywood and the upper halves covered with plastic mesh netting. Between the top and bottom sections there is a horizontal 15-cm wide slit-like entrance tapering to 2.5 cm, to allow entry of hungry mosquitoes. The roof is covered with plastic sheeting to protect against rain, and a door is sited at one end. A calf is tethered to a wooden support inside the trap. Two people could lift the trap into the back of a pick-up truck. The average catch of female *Anopheles albimanus* per night from four traps varied from 12–157, and was about 1.7 greater than the numbers collected per man-hour from stables. In Venezuela Gabaldon *et al.* (1940) found stable traps useful for sampling *Anopheles albimanus*, but not *Anopheles darlingi*. Sasse & Hackett (1950) used stable traps in Peru to study the host preference of *Anopheles pseudopunctipennis*.

Kay & Bulfin (1977) described a cheap and easily made sectional trap constructed mainly from angle-iron (Dexion) that can be readily transported and

fitted to cattle crushes in remote areas to convert them to small Magoon-type traps. The design prevents the entrapped mosquitoes from feeding on the cattle or other livestock bait.

In studying host preferences in Nigeria one Magoon trap was baited with a goat while another 45 ft away was baited alternatively with a sheep, a pig and two monkeys (*Erythrocebus patas patas*). About 33 mosquito species were caught, including 15 *Anopheles* species which formed about 85–93% of the catches of mosquitoes from the trap when it was baited with sheep, goat, and a pig. Only 16 mosquitoes were collected from the monkey-baited trap and 10 of these were *Culex quinquefasciatus* (Service, 1964).

In dengue studies in Malaysia Rudnick (1986) used relatively large Magoon traps which were baited with several (4–6) monkeys. Traps on the ground contained macaque monkeys (*Macaca* spp.) while those raised by winch and pulley to 75 ft in the tree canopy were baited with leaf-monkeys (*Presbytis* spp.). Numerous mosquito species (35) and genera (17) were collected in the high canopy traps, e.g. species of *Anopheles*, *Culex*, *Mansonia*, *Coquillettidia*, *Zeugomyia*, *Orthopodomyia*, *Armigeres*, *Heizmannia*, *Tripteroides*, *Aedes* and seven other genera. The most common mosquito was *Culex cinctellus*, but of the 3194 females caught in one locality only three had fed on the monkeys, whereas 16 of the 45 female *Aedes niveus* group were engorged. In some localities at least 53 species belonging to 22 genera were caught in *Macaca*-baited traps at ground level. In dengue studies in Malaysia Garcia *et al.* (1988) modified this small Magoon trap by constructing the roof, door, upper and lower sides separately from mitred aluminium screen moulding. The base of the trap measured 60 × 60 × 56 cm, while the panels were formed of plastic mesh screening held together by four vertical angle-iron corner supports. Four triangular pieces of mesh screening stitched together and fitted within an aluminium frame formed the roof (60 × 60 × 50-cm high). A hook and four lines supported the configuration of the roof (Fig. 5.13a). An animal bait cage was suspended in the centre of the trap by a nylon line from the roof hook, and 3 kg dry-ice placed in a 15 × 20 × 30-cm styrofoam box insulated with newspapers was placed on the roof and held in place by slipping the corners of the box under the four support lines. A 2-cm hole in the lower side of the box allowed carbon dioxide to flow down into the trap. Mosquitoes entered the trap through 1-cm wide baffles made of plexiglas and fitted to all sides of the trap, except on the end door. Another point of entry was the slit formed by two sloping (20°) panels of plexiglas that formed the bottom of the trap (Fig. 5.13a). Mosquitoes were removed with aspirators inserted through a cloth sleeve fitted to the rear end of the trap. In California these traps were baited with a rabbit and carbon dioxide and operated from 1400–1000 hr the following day, and mean catches of female *Aedes sierrensis* were 39.1, 79.9 and 355.4 depending on ecological location (Garcia *et al.*, 1989). The maximum overnight catch was 901 mosquitoes. Males hovered around the trap but relatively few were collected from inside it, the male:female ratio varied from 1:21 to 1:95. A Fay-Prince trap augmented with carbon dioxide caught similar numbers of female *Aedes sierrensis* as the rabbit-baited trap, but was more convenient to use because it was not encumbered with a live bait. More-

over, it was 15–20 times better at catching males. This is one of the few traps that employs both a bait animal and carbon dioxide. Landry & DeFoliart (1986), however, used mice and dry ice in a CDC-type trap but found that the addition of mice did not improve their catch of *Aedes triseriatus*.

Bates' (Egyptian) stable trap

Although Gabaldon *et al.* (1940) reported that in Venezuela less than 3% of the mosquitoes caught in Magoon traps escaped before they were collected shortly after sunrise, Bates (1944*b*) working in Egypt found that the trap was inefficient. A large proportion of mosquitoes caught escaped back through the entrance slits before collections were made in the early morning. He therefore made a new type of trap which he called the Egyptian trap, but which has subsequently also become known as Bates' stable trap. The principal difference between the Bates and Magoon traps is in the design of the entrance slit (Fig. 5.13*b*). This was modelled on the entrance baffle used by van Thiel *et al.* (1939) in their experimental work on host attractiveness, in which mosquitoes had to finally turn and fly upwards to gain access to their cages. A further difference is that unlike the original Magoon trap entrance slits were incorporated only in the two longer sides and not in the ends. Various sized traps were made, but finally standardised to measure 2 m long, 1 m wide and 1.75 m high; they were placed on a concrete base. A transparent roof was placed on the trap but this has not generally been used by later workers. The upper part of the entrance baffle consists of a 20-cm wide length of wood set at right angles to the side of the trap. The lower section of the baffle is 44 cm wide and is positioned 38 cm below and slopes upwards to leave a vertical 2-cm opening between the upper and lower lengths of wood.

In Egypt Bates (1944*b*) caught 4000–5000 mosquitoes/trap-night, including up to 1000 *Anopheles*. In comparative trials in Colombia Bates (1944*b*) clearly showed that many more mosquitoes were caught in Bates than Magoon traps, e.g. the catch of *Anopheles* (mostly *Anopheles rangeli*) was about 10 times greater. De Zulueta (1950) reported that very few mosquitoes escaped from these traps.

In Australia Kay *et al.* (1979*a*) baited Bates' traps with a man, a feral pig, a dog, two domestic fowls, a grey kangaroo and a calf. They used the Feeding Index of Kay *et al.* (1979*b*) to study host preferences (see pp. 467–8). At one site 44 626 mosquitoes belonging to at least 35 taxa were trapped from 360 collections, from another site 26 215 mosquitoes belonging to 15 taxa were caught in 90 collections. The most common species were *Culex annulirostris*, *Culex quinquefasciatus*, *Anopheles bancroftii*, *Aedes normanensis* and *Anopheles annulipes*. Although the traps were placed in very similar topographical situations there were considerable differences between catches in differently located traps. This paper provides a good interpretation of feeding preferences from baited traps and from the collection of blood-engorged mosquitoes.

Nelson *et al.* (1976) baited Bates' traps simultaneously with a jackrabbit and a chicken or pheasant, and after some 90–110 trap-nights more than 21 000 mosquitoes were collected, of which nearly 90% were *Culex tarsalis*.

In Canada Hudson (1983) caught 14 mosquito species from 12 nights operation of a Bates' trap, the most numerous being *Aedes vexans* (1325), *Aedes communis* group (358), *Culiseta inornata* (110), and *Culiseta alaskaensis* (94).

Roberts' stable trap

Roberts (1965) designed a modified Magoon-type trap which had better air circulation thus enabling bait odours to disseminate more efficiently from the trap. Another feature of the trap was that mosquitoes could enter it at several heights. The trap consists of a wooden framework, 7 ft long, 5 ft wide, 6 ft high, with a flat roof and a door at one end (Fig. 5.13*e,f*). The bait animal is confined in a stanchion to keep it in the centre and prevent it from damaging the trap. About the top 1 ft of all sides and door are covered with polythene sheeting, while the rest of the door and sections in between the baffles are covered with fine copper wire mesh screen. The Magoon-type horizontal baffles are built into the two long sides of the trap at ground level and at heights of 2 and 4½ ft. There are no baffle entrances in the end sections. In a comparison of the mosquitoes caught in light-traps and in steer-baited traps in Mississippi, 7381 mosquitoes belonging to 16 species were caught at light and 105 387 representing 23 species were caught in the stable traps. Only *Culex territans* and *Uranotaenia sapphirina* were caught at light and not in the stable traps.

In Wyoming Pennington & Lloyd (1975) baited Roberts' trap with a heifer, and collections were made at four irregular intervals throughout the diel. The numbers of mosquitoes in each collecting period were determined by either counting, or by weighing and estimating the numbers from the weight of a 100-mosquito subsample. From sixteen 24-hr collections 71 440 *Aedes melanimon* representing 44.34% of the total catch, 56 388 *Aedes dorsalis* (35.06%) and another six species were caught.

In the USA Jones & Lloyd (1985) found that about equal numbers of nine mosquito species were attracted to a 5–6-month-old ewe held in a Roberts' trap and to a CDC light-trap placed 300 m away and baited with 4.5 kg dry ice placed in a 1.37-kg coffee can with four 3-mm holes drilled in the bottom. This arrangement resulted in the dry ice releasing about the same amount of carbon dioxide as an adult bovine-sized animal (Morris & DeFoliart, 1969). Hayakawa *et al.* (1990) used a modified version of the Roberts' trap in which the entrance slits (10 cm) were much wider than the original 1.9-cm slits. Their cattle-baited trap caught more mosquitoes than did carbon dioxide-baited bed-nets.

Wright & DeFoliart's stable trap

In Wisconsin Wright & DeFoliart (1970) constructed two sizes of small Magoon-type traps which were baited with squirrels, rodents, reptiles and amphibia. The smaller size consisted of an 18 × 20 × 20-in wooden frame-work with the top and bottom made of ¾-in plywood, and all four sides, but excluding the door (11 × 11½ in), covered with 52-in mesh natural colour saran screening. A 4-in wide V-shaped plywood baffle leaving a ¾-in entrance slit was placed 4 in from the top of the trap and extended the entire length of all four sides. The larger

type of trap was similar in design except that it was 27 in long, and the door measured 12×13 in. In 17 checks with these small Magoon traps 190 mosquitoes were trapped but none was blood-fed.

Shannon's stable trap (= dawn trap)

Although sometimes referred to as a stable trap it is really a hybrid between a stable trap and an experimental hut containing a window-type exit trap. It consists of two parts, a wooden stable and a dawn trap (Fig. 5.13*d*). The stable has specially constructed lightproof overhanging eaves which leave a 6-in gap on three sides of the stable, through which mosquitoes can enter to feed on the bait animal. A close fitting door is provided to the stable which is dark inside, and may be painted black. The wooden dawn trap which measures about $3\frac{1}{2} \times 3\frac{1}{2}$ ft and 5 ft high is painted white inside except for the rear wall which is made of mosquito screening. It is fitted to the wall of the stable that faces the rising sun. Mosquitoes that have entered the stable at night to feed on the bait are attracted to the light coming from the dawn trap. They consequently fly through the 30-in wide opening of a screen mesh baffle fitted into the rear wall of the stable and pass through a $1\frac{1}{2}$ -in wide longitudinal slit into the dawn trap. The original description of the trap by Shannon (1943) is not very accessible, but a good account together with a diagram and photograph is presented by Earle (1949). These traps have mainly been used in Central and South America. Shannon (Earle, 1949) caught as many as 7145 *Anopheles aquasalis* in one night from a dawn trap, and in Trinidad Senior White (1952) collected mosquitoes in traps baited with man, ox, goats, horse and a pig.

Buescher *et al.* bird-baited trap

These traps were baited with various birds and used in Japan in a study of Japanese encephalitis (Buescher *et al.*, 1959). They are made of wood and resemble small Magoon stable traps, and measure 36×44 in and 40 in high, with a centrally pitched wooden roof. A 5-cm deep entrance extends the length of the two longer sides parallel to and 10 in from the base of the trap. The lower part of the entrance consists of an upwardly slanting strip of wood which leaves a 1-in vertical opening, as found in the Bates-type stable trap, about 18 in from the trap base. Because birds ate mosquitoes entering these traps a $\frac{1}{2}$ -in wire mesh frame is inserted inside the traps just below the slit-like entrance to confine the birds to the lower half of the trap. A small door in the upper part of the trap at each end allows aspirators to be inserted for the removal of the catch. A third small door is made near the base of the trap for introducing the bait animals and for facilitating feeding and cleaning out the trap.

Control traps without birds attracted no mosquitoes, but large numbers of *Culex tritaeniorhynchus* (4170) were caught from traps containing a Black-crowned Night Heron, a bird which also attracted large numbers of *Culex pipiens* (8712). However, the highest numbers of this mosquito were caught from chicken-baited traps (11679) (Scherer *et al.*, 1959). Both trap location and the microhabitat around the traps considerably affected the numbers of mosquitoes caught. Also, identical traps sometimes caught greatly different numbers of

mosquitoes, a difference that persisted even when both baits and trap positions were changed. This type of trap was also used in Mexico by Scherer *et al.* (1967).

Russell's calf-baited hut

In studying the flight range of *Anopheles culicifacies* in India Russell *et al.* (1944) built 80 calf-baited trap huts which they placed at various distances from the release point of marked mosquitoes. Each trap consisted of a small hut, 7 ft high, 6 ft long and 5 ft wide, built from a framework of *Casuarina* scantlings covered with palm matting. A thatched sloping roof reached to the ground on both long sides, and both ends were also covered with thatch. A doorway ($2\frac{1}{2} \times 1\frac{1}{2}$ ft) was left at one end for the entry of mosquitoes and a bamboo gate was fixed across this entrance to prevent jackals entering. Two strips of dark cloth were suspended from the roof to provide additional resting places for mosquitoes, which were collected by aspirators after a canvas curtain had been pulled across the doorway. Despite the success of the trap in catching 65 893 *Anopheles culicifacies* and 141 928 other species, including *Culex*, they have been little used as bait traps.

General considerations of stable traps

Bates (1944*b*) found that in 35 of 54 weekly catches the numbers caught on the first night were greater than those on the third consecutive night. This phenomenon had already been noted by Gabaldon *et al.* (1940) in Venezuela. When using Magoon traps they found that in any one location more *Anopheles* were caught on the first night than on either the second or third nights, but when the trap was moved only about 3 m a 'first night's catch' was obtained. The most likely explanation is that when a stable trap is first introduced into an area it catches, on the first night, both the local resting population that has built up over several days in addition to mosquitoes flying in from further afield. On subsequent nights the local population of hungry females having been depleted, only those flying into the area are caught. A change of trap location is likely to result in another high catch on the first night. In Colombia stable traps placed amongst grassy vegetation caught several times as many mosquitoes as traps no more than 10 m away, but sited in an area of cleared grass (de Zulueta, 1952). Presumably in the latter area there were fewer resting places for mosquitoes. Surprisingly, however, more *Anopheles darlingi* were caught in traps in the cleared area than in traps amongst grass. De Zulueta (1952) suggested that this was related to differences between the host seeking behaviour of *Anopheles darlingi* and the other species.

Although an average 1470 mosquitoes were caught per night in a donkey-baited trap in Colombia placed in savannah areas the species composition differed markedly from that obtained by drop-net collections (de Zulueta, 1952). For example, drop-net collections showed that *Anopheles* comprised 19% of the total mosquito population resting amongst the grass, whereas in stable traps *Anopheles* constituted as much as about 73% of the catch. Clearly culicines were inadequately sampled. Baiting the traps with a calf or fowls had no effect on

species composition. During four trials with unbaited traps, in which the door was sometimes left open, only 12 mosquitoes were caught, clearly demonstrating that mosquitoes were not just seeking shelter in the traps but were attracted to the bait animals. There was no evidence that the smell of a bait animal, such as a donkey, which might have lingered on from a previous night's trial attracted any mosquitoes into the trap; the bait had to be within the trap.

By regularly collecting mosquitoes from a donkey-baited trap de Zulueta (1950) was able to obtain the 24-hr biting cycles of several mosquito species.

Murphey *et al.* (1967) caught 6803 mosquitoes belonging to 14 genera in 2-ft cube Magoon traps baited with 19 different vertebrate hosts enclosed in wire mesh cages. It was considered that although the numbers caught might reflect the attractiveness of the different hosts, they were dependent on the size of the population and flight activities of the mosquitoes, whereas the percentage that actually fed on the bait was less dependent on these factors. Host preferences of different mosquito species were therefore assessed by using a host attractive index, obtained by multiplying the percentage that fed on the baits by the numbers caught in the traps and dividing by 100. An index of 8 or more was considered to reflect an attractive host, 3–7 a moderately attractive one and below this a poorly attractive host. This is a very simplified approach of comparing relative host attractiveness. It overlooks the fact that some species may find it difficult to enter the traps although they may be strongly attracted to the bait. Furthermore, the percentage feeding on a host confined within a trap may have little bearing on the proportion successfully obtaining a blood-meal from the same animal under natural conditions. In studying the host preferences of natural populations of mosquitoes Hess *et al.* (1968) used the forage ratio. This is obtained by dividing the percentage of blood-fed mosquitoes (caught from natural resting places) that have been shown by serological methods to have fed on a particular animal species or group of species by the percentage it comprises of the total available population of hosts in the area, i.e. availability of hosts is taken into consideration. A forage ratio of approximately 1 indicates neither preference nor avoidance of the indicated host, whereas ratios significantly greater than 1 indicate selective host feeding. The main difficulty of trying to apply this technique is that it is rarely possible to obtain reliable estimates of the relative proportions of the different hosts in an area. Furthermore, there may be seasonal changes in the proportions of available hosts. See also pp. 467–8.

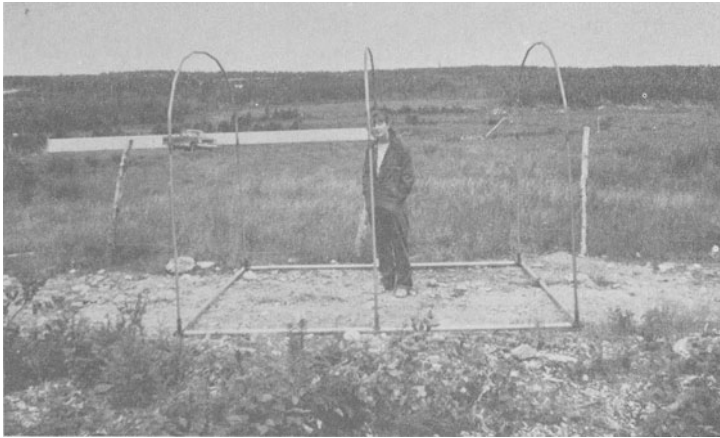
Stable traps usually contain mammals, but in the USA Blackmore & Dow (1958) used a small trap (4 × 4 × 4 ft) with Egyptian-type baffles baited with adult and nestling birds. A much higher proportion of *Culex tarsalis* caught in the traps had fed on nestling birds than on adults, but the difference varied according to the species of bird used. Mosquitoes probably found it easier to get blood-meals from nestling birds because they have fewer feathers and are more quiescent. As a result of these experiments Blackmore & Dow (1958) thought that birds nesting during the encephalitis season might be important reservoirs of infection. Flemings (1959) and Scherer *et al.* (1959) also used bird-baited traps in Japan in a study on Japanese encephalitis.

When different animals are placed close together hungry mosquitoes are given a choice of hosts and host preferences can be studied. If, however, they are widely separated there is no such choice, and a measure of only the relative attractiveness to mosquitoes is obtained. If the animals are placed too far apart, they may be in different ecological environments containing different mosquito populations. It has been shown that mosquitoes caught in human bait catches performed at different sites in a more or less homogenous habitat may differ (Service, 1971*b*). Under these conditions it may not be valid to make close comparisons between trap catches. In Japan small stable traps and other small traps baited with birds were used to collect *Culex tritaeniorhynchus* and *Culex pipiens* (Scherer *et al.*, 1959). Pronounced differences sometimes occurred between the numbers of mosquitoes caught in identical traps due to both trap location and microhabitat around the traps. In addition some traps consistently caught many more mosquitoes than others, even despite changing both baits and trap positions. Even more surprising was the fact that although similar numbers of *Culex tritaeniorhynchus* were caught in six bird-baited traps, two traps never caught mosquitoes infected with Japanese encephalitis virus (Scherer *et al.*, 1959). These same workers made some interesting observations on the attractiveness of traps without baits. They found that after a pig had been removed from a stable trap about the same numbers of *Culex tritaeniorhynchus* were caught on the following day. However, when a pig was removed for three consecutive days, the catch progressively decreased from over 2000 mosquitoes/night to less than 50. Similar but less well documented reductions occurred when man and birds were removed from stable traps for 3 days.

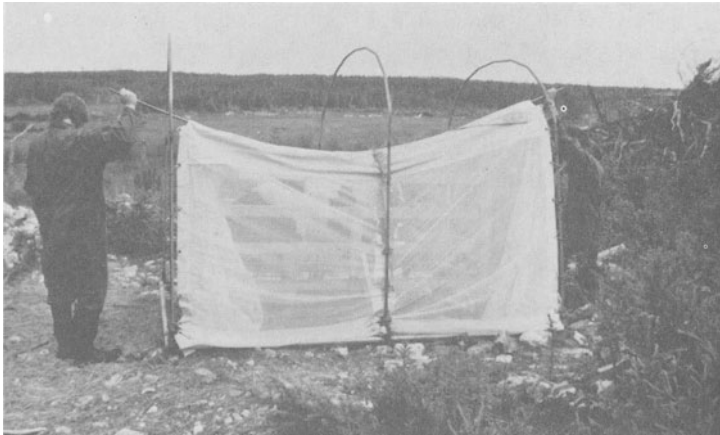
OTHER TRAPS FOR LARGE ANIMALS

McCreadie *et al.* trap

Because of the failure of mosquitoes to sometimes enter animal-baited traps employing baffles and cones, and because the suspension of drop-nets over a bait may deter some mosquitoes from approaching and biting Jones (1961) devised a tent-like net that was operated by springs and which flipped over an enclosed small animal, such as a sheep. For animals such as cattle McCreadie *et al.* (1984) developed a larger tent-like trap, in which the animal is placed in a wooden pen surrounded on the ground by a rectangular frame (2.4 × 3.0 m) of 4.3-cm diameter galvanised steel piping. Three hoops, each consisting of two straight and one curved section, of 2.1-cm diameter piping are fixed to this framework to form a 2.1-m high arc over the enclosed bait (Fig. 5.14*a*). A fine plastic netting tent is made to have a 30-cm canvas lower border and three 8-cm wide canvas strips sewn into the netting to lie directly underneath each metal hoop. Brass eyes and 5-cm diameter metal rings are sewn into these strips so that the net-tent is fixed to a series of rings which surround the three hoops arising from the basal metal frame (Fig. 5.14*c*). In operation the tent is collapsed on the ground along one side of the bait animal, then after a 10-min exposure period two collectors walk to the trap and rapidly pull the tent up and over the



(a)



(b)



(c)

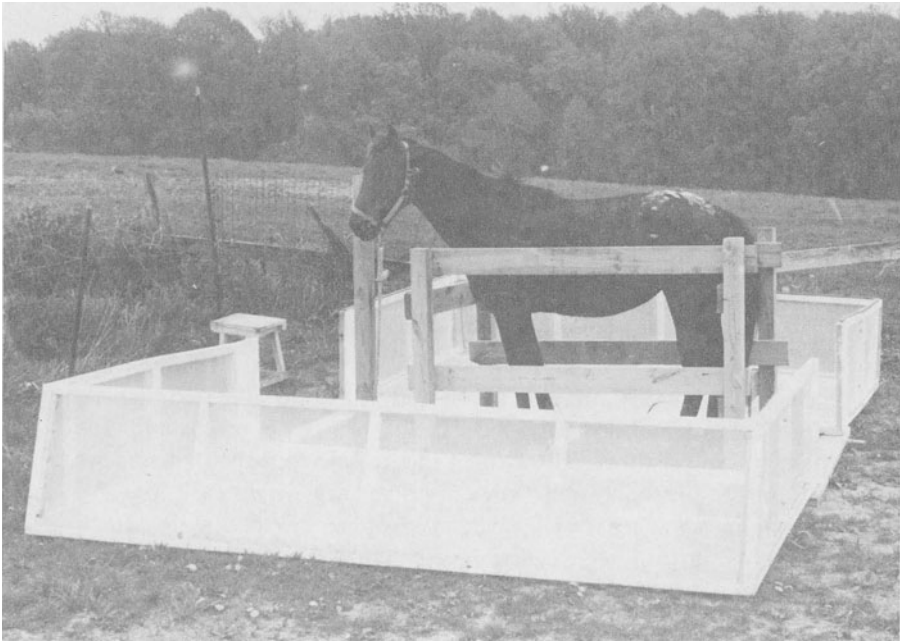
FIG. 5.14. *Bait-net of McCreadie et al. (1984) (photograph courtesy of J. W. McCreadie).*

three metal hoops (Fig. 5.14b) to enclose the animal and mosquitoes attacking it. This operation takes about 3 s and only a few mosquitoes hovering around the bait may escape. If required a further 10 min can be allotted for entrapped mosquitoes to feed on the host. A zipper sewn into one end of the tent allows a collector to enter and remove the catch with aspirators. McCreadie *et al.* (1984) used modified commercial Black and Decker Dustbuster hand vacuum cleaners as aspirators. In Canada when baited with a calf the trap caught 26 species of biting flies, including six mosquito species, the most common of which were *Culiseta impatiens*, *Aedes abserratus* and *Aedes punctor*. The authors suggested that smaller versions of the trap can be made for smaller animals, and that perhaps less conspicuous pens could be made so as to allow a more visible silhouette of the baits.

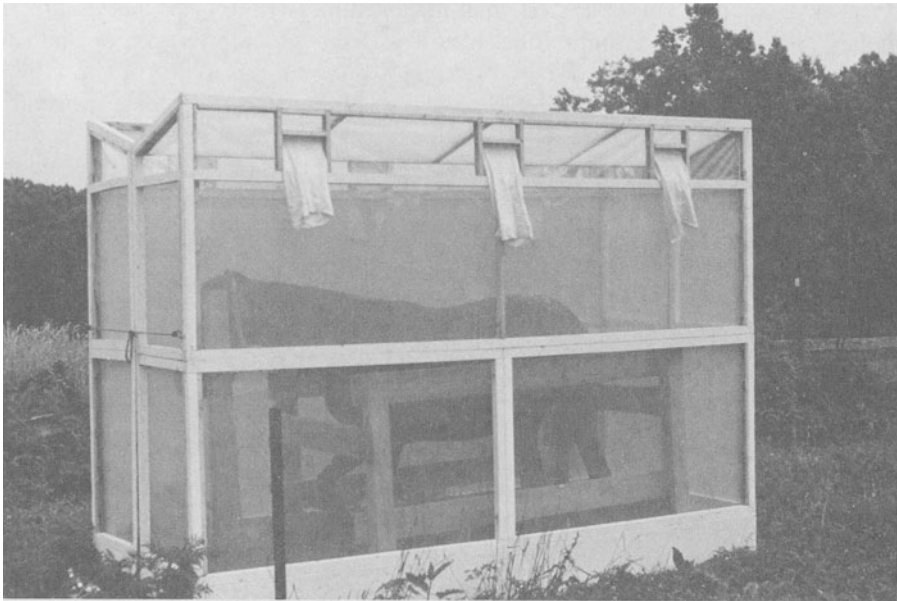
Later McCreadie *et al.* (1985) found that about double the numbers of simuliids (10747 vs 5720) were caught in their trap baited with a calf than in the carbon dioxide suction trap of Trueman & McIver (1981). Only a few mosquitoes were caught, the most common species being *Aedes abserratus*, *Culiseta impatiens* and *Aedes punctor*, of which the calf-baited trap trapped 363 whereas the carbon dioxide trap caught just 117 adults.

Because the collection of haematophagous insects from tethered baits is reported to be biased (Bennett, 1960; Zimmerman & Turner, 1983), Fletcher *et al.* (1988) preferred to collect biting insects from a horse-baited trap, which used the same principles as the one designed by McCreadie *et al.* (1984). Namely, the bait (horse) is restricted in a wooden stanchion surrounded by a wooden frame (4 × 10 ft and 7.5 ft high) (Fig. 5.15a). The upper and lower parts of the frame are covered with 40-mesh plastic screening, except that the upper 30 cm is covered with clear polythene sheeting and contains on each long side three collecting holes (6 × 8 in) with lengths of stockinette (Fig. 5.15b). The edges of the two long sides of the trap (left and right) are sealed with plastic foam weather stripping and when erected over the horse are held in position by elastic straps. Each side of the trap is hinged to a base frame of wooden boards (2 × 8 in and 6 ft long) thus allowing the two sides to be opened to expose the horse and when required raised and closed. The roof slopes down towards the middle to form a V-shape, this causes the entrapped mosquitoes to gather along the upper edges of the trap and facilitates their removal, when aspirators are inserted through the entrance ports. For ease of construction the trap is made in sections. The upper part is divided into two sections while the lower part comprises four 'L'-shaped sections. The horse is exposed to biting flies for about 15 min and is then enclosed within the trap, and after allowing 30 min for entrapped mosquitoes to feed they are collected. Although most biting flies (*Simulium*, tabanids, *Culicoides*) fly to the top of the trap and are readily collected, blood-engorged mosquitoes tend to rest on the sides so additional collection holes should be made along the middle of the trap and at the two ends.

Another somewhat similar trap is that of Shemanchuk (1978) designed specially for trapping *Simulium arcticum*, but which in Canada also caught *Aedes vexans*, *Aedes flavescens*, *Aedes fitchii*, *Aedes excrucians*, *Aedes punctor* and *Culiseta inornata*. The bait animal (100–450 kg steer or heifer) was closely confined in a metal stanchion bolted (Fig. 5.16a) to a plywood white-painted floor and surrounded



(a)



(b)

FIG. 5.15. Bait trap of Fletcher et al. (1988) (photograph courtesy of M. G. Fletcher).

by a wooden framework (170 × 245 cm and 180 cm tall). Nylon screening was permanently fixed to the roof but fixed to the sides with Velcro, so that it could be folded back onto the roof to fully expose the bait but quickly pulled down to enclose the animal and flies biting it (Fig. 5.16*b*). The distance between the stanchion and two longer sides of the wooden framework was 45 cm, while 52.5 cm separated the frame and stanchion at the two ends. After a suitable exposure period all sides were closed as quickly as possible, and after allowing 10 min for the flies to engorge on the bait, a person entered and collected the insects.

Portable traps (Mitchell *et al.*, Wilton *et al.*)

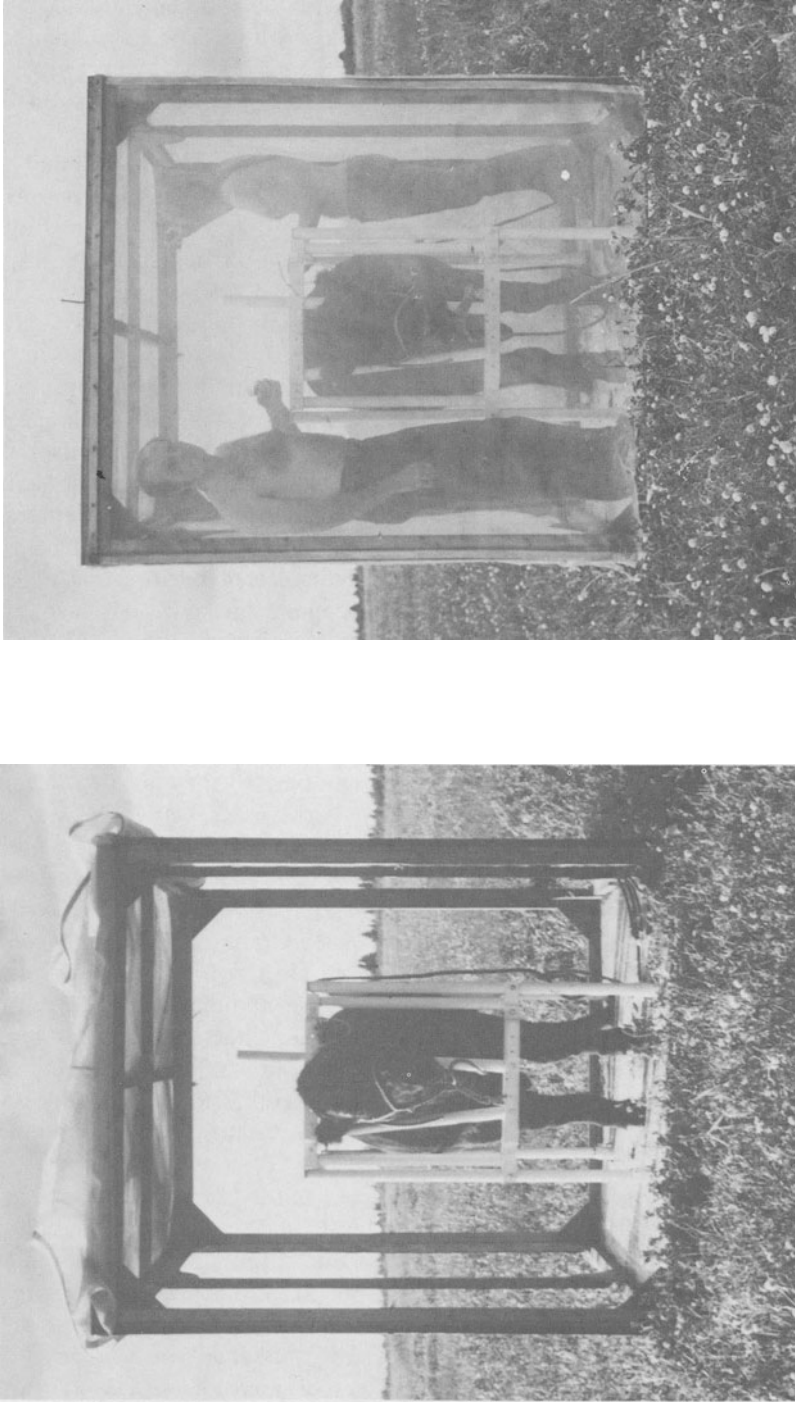
The Mitchell *et al.* (1985) trap is quite large (360 × 360 cm, 210 cm tall) and comprises stitched panels of nylon tulle suspended by ropes tied to the four corners and to trees or some other suitable supports. The bait animal is introduced through a 1-m wide door flap made in the middle of one side, and then secured to a stake. The ropes are pulled to raise the trap 30–46 cm from the ground. The following morning the trap is lowered to touch the ground and the bait removed, after which mosquitoes are collected. When used in Argentina 2752 and 6929 mosquitoes belonging to at least 18 species in six genera were caught after five and three trap-nights, respectively. *Culex* (*Culex*) species (45.8%) and *Aedes albifasciatus* (21.7%) predominated. The trap proved better than a Magoon-type trap.

In later trials with a horse as bait, Mitchell *et al.* (1987) caught over a year 2752 mosquitoes belonging to at least 23 species, but mostly unidentified *Culex* (*Culex*) species.

Wilton *et al.* (1985) considered that the usefulness of this portable trap of Mitchell *et al.* (1985) was limited because it was not self-supporting, but had to be suspended by ropes from nearby trees. To overcome this Wilton *et al.* (1985) modified two commercially available portable summer 'screen rooms' normally used to provide insect-proof living facilities out of doors. The smaller screen room measured 3.6 × 3.6 × 2.2 m and weighed about 14 kg, the larger room was 3.0 × 4.2 × 2.3 m and weighed some 15.8 kg. All four sides were made of mesh screen and were supported by tubular metal frames, the rooms had a nylon zipper entrance. To convert them to mosquito traps two 41-cm vertical slits were cut at the bottom of all four corners to enable the sides to be raised 20–30 cm above the ground and secured by spring clips for entry of mosquitoes. In Colorado when the larger trap was baited with a horse for six nights 2776 mosquitoes belonging to 11 species were caught, the most common of which were *Aedes vexans* (68.8%), *Culex tarsalis* (9.9%), *Aedes dorsalis* (9.5%) and *Aedes melanimon* (5.8%). The mean catch was 462.5 mosquitoes/trap-night compared to 367/trap-night with CDC light-traps supplemented with dry ice. A person exposed under the protection of a mosquito net in the smaller net on five nights from 1 hr before, to 1 hr after sunset caught 464 mosquitoes belonging to five species, again the most common was *Aedes vexans* (64.6%).

Dog-baited traps

In a study of the potential vectors of *Dirofilaria immitis* in dogs in Minnesota Bemrick & Sandholm (1966) constructed modified Magoon traps, in which the



(a) Steer-baited trap of Shemanchuk (1978) (photograph courtesy of J. A. Shemanchuk).
(b)

plywood sides (6×5 and 5×5 ft) were made in two sections to make the trap more portable. A V-shaped baffle with a $\frac{3}{4}$ -in slit opening extended completely round the trap at a height of 28 in from the ground. The large numbers of mosquitoes, especially *Coquillettidia perturbans* and *Aedes vexans*, which were caught in the traps were removed by the collector entering the trap through a door at one end.

A more portable dog-baited trap was made by Villavaso & Steelman (1970). This measured 36 in wide, 46 in long and 30 in high, but when dismantled and the sections stacked on top of each other was only $24 \times 30 \times 36$ in. Four dismantled traps could be transported in a $\frac{1}{2}$ -ton pick-up truck and each trap only took about 15 min to assemble. The basic design was that the dog was confined in a centrally placed compartment, about 38 in long and 30 in in width and height. Two removable mosquito collecting boxes (30×36 in and 8 in deep) formed the two long sides of the trap. The outside panel of each collecting box was made of 16-in mesh screen wire incorporating two $\frac{1}{4}$ - $\frac{1}{2}$ -in wide longitudinal entry baffles fitted at 10 and 20 in from the base of the trap. The inner wall of each box was covered with 16-mesh screening protected by a layer of $\frac{1}{2}$ -in wire mesh to prevent the dog tearing the screening. Mosquitoes which entered the two collecting boxes through the horizontal openings were prevented by the mesh screening from feeding on the dogs and were collected by inserting aspirators through 1-in square openings cut in the outside mesh screening. Villavaso & Steelman (1970) reported that in the USA 16 mosquito species were caught in dog-baited traps, and that as relatively large numbers of *Culex quinquefasciatus* (90% of the total catch) were obtained, the traps might prove useful for monitoring relative population densities of this mosquito in urban areas.

In Louisiana, Cupp & Stokes (1973) also caught 16 mosquito species in this type of dog-baited trap, the most common two species being *Culex salinarius* and *Culex quinquefasciatus*. Their procedure for removing mosquitoes from the collecting boxes was to place them in a plywood box and introduce car exhaust fumes for 5–6 min. About 90–95% of the mosquitoes which were knocked down and collected by aspirators recovered. Brock & Crans (1977) caught very few mosquitoes when using collapsible dog-baited traps of Villavaso & Steelman (1970) in New Jersey. By comparing the relative abundance of mosquitoes in light-traps with those of dog traps they concluded that the trap was biased in favour of *Culex* species, such as *Culex salinarius*, but was poor for *Aedes* such as *Aedes vexans* and *Aedes sollicitans*. They postulated that these *Aedes* species may find it more difficult than *Culex* species to enter the trap via their louvre-type openings.

In studying potential vectors of *Dirofilaria immitis* Lewandowski *et al.* (1980) constructed a special lightweight wooden dog-baited trap, having louvres of the Bates-type mounted on two opposite sides (Fig. 5.17a). Few details other than the figure were given. During two field seasons 2166 mosquitoes belonging to 14 species were caught; the most common of which were *Coquillettidia perturbans* (44%), *Culex pipiens* (17%), *Aedes vexans* (12%) and *Anopheles walkeri* (11%). In other studies on *Dirofilaria immitis* Walters & Lavoipierre (1982) built dog kennels in California measuring 90×120 cm and 120 high to the apex of the

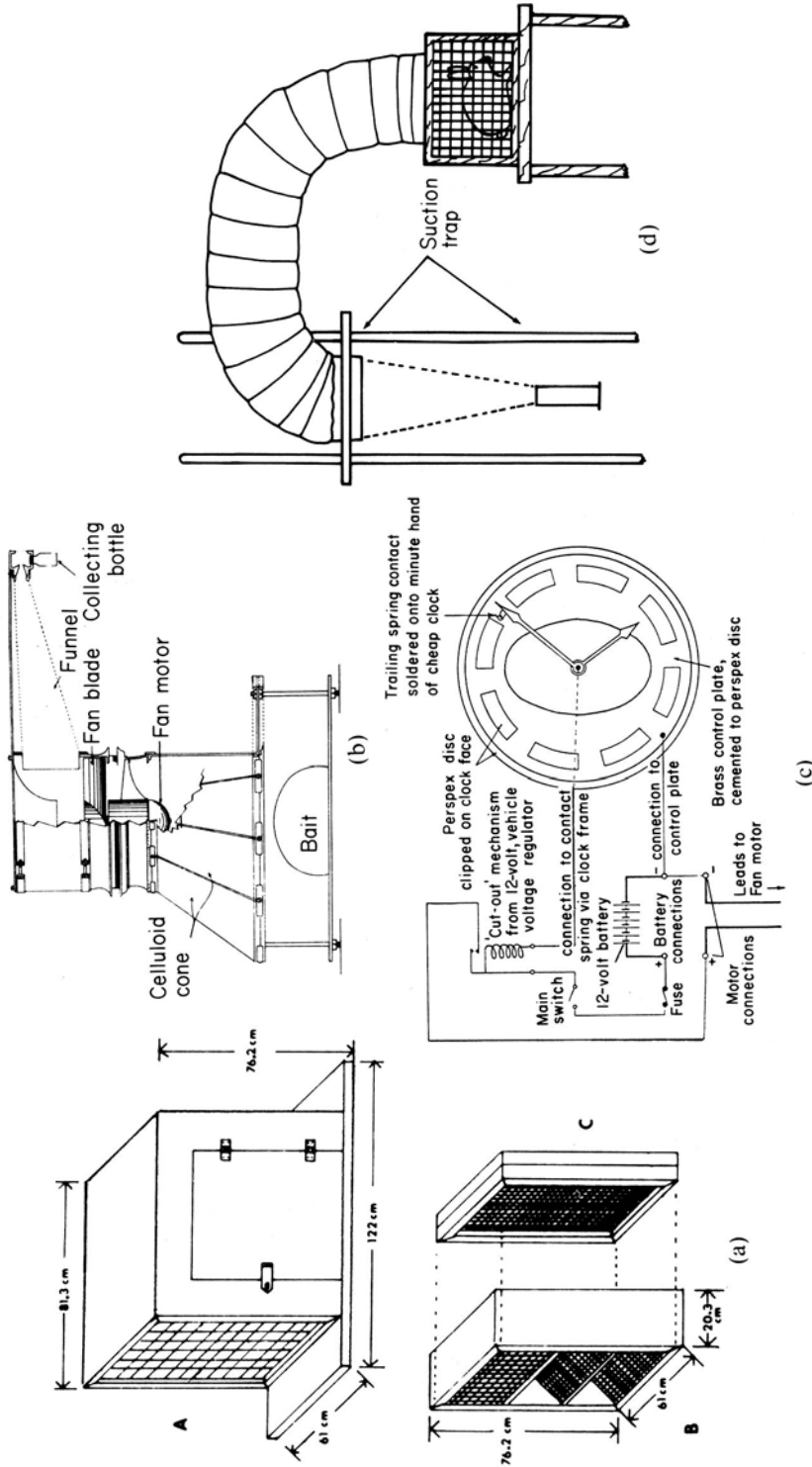


FIG. 5.17. (a) Dog-baited trap, A — dog compartment, B — mosquito collection chambers which are mounted on either side of dog compartment, C — removable screen frame which is removed to collect mosquitoes (Lewandowski et al., 1980); (b) small mammal trap (after Lumsden, 1957b); (c) electrical system and control plate used with Lumsden-type trap by Minter (after Minter, 1961); (d) suction trap used as a baited trap (after Service, 1977c).

sloping roof. There were two Bates-type baffles on three sides at heights of 45 and 72 cm. Only relatively few *Aedes vexans* and *Aedes sierrensis* entered the traps.

In Indiana Pinger (1985) used modified kennel traps of Klowden & Lea (1979) to study mosquitoes attracted to dogs. An inner restraining cage (69 cm long, 41 cm wide and 53 cm high) made from 2.54-mesh wire had a removable top which when in place was fixed by ties of insulated copper wire. The holding cage was placed inside an outer cage 91-cm long, 61-cm wide and 61-cm high having a plywood floor and 0.14-cm mesh screen sides. Two sheets of 97–61-cm plexiglas taped together and supported on wooden stakes formed a sloping roof over the cage. The ends of the outer cage had sleeves made from plastic dustbin bags with their ends removed, for replacement of the inner cage. Mosquitoes entered either through a 2-cm eave gap between the plexiglas roof and outer cage or through a 2-cm slit on each side of the outer cage positioned 24.5-cm from the ground. The dog was exposed in a trap from 1800–2100 hr. Over 2 years 14 species of mosquitoes belonging to five genera were caught, the most common were *Aedes trivittatus* (66%), *Culex pipiens/restuans* (5%), *Culex erraticus* (72%) and *Aedes vexans* (26%); the numbers in parentheses are the percentages engorging on the dog bait. Results confirmed other studies that *Aedes vexans* is not very attracted to dogs, whereas dogs are a good source of blood-meals for *Aedes trivittatus*. Pinger (1985) also noticed that mosquitoes often hovered around the dog-baited kennel traps as if experiencing difficulties in entering them through the horizontal 2-cm wide slits; other times adults were seen exiting the traps.

SMALL ANIMAL-BAITED TRAPS

There are an ever increasing number of traps designed to catch mosquitoes attracted to small mammals and birds, and more rarely to amphibia and reptiles. I have had to be selective in describing such traps. In fact I have omitted accounts of the traps of Sommerman & Simmet, Laarman and Minar, which were described in the first edition of this book (Service, 1976), because they have rarely been used and do not appear to offer any great potential.

Baited suction traps

Lumsden trap

When mosquitoes attracted to tethered baits are collected there is always the possibility that some of them have been attracted not to the bait but to the collectors. To obviate this the bait can be left unattended and the mosquitoes caught in a trap, but it is well known that there may be differences between the readiness of different mosquito species to enter traps and frequently traps into which mosquitoes have little difficulty in entering are those from which a high proportion escape. To overcome these difficulties Lumsden (1957*b*, 1958*b*) developed a trap which allowed mosquitoes as much access as possible to the bait but prevented their escape.

The bait animal is enclosed in a wire-mesh cage placed at the centre of a 70-cm diameter platform made of hardboard (Fig 5.17*b*). Some 15 cm directly above the enclosed bait is a 30-cm tall cone made from truncated sections of transparent cellulose acetate sheet separated by about 1-cm wide gaps. The upper end of the cone is attached to a 12-in diameter 'Vent-Axia' fan. This is connected to a 20-cm high metal cylinder which has a 45-cm long fine wire mesh funnel projecting laterally which terminates in a small collecting bottle containing the killing agent (potassium cyanide or pyrethrum). The trap is open around the platform containing the bait up to a height of 15 cm. The fan draws air upwards through the 15-cm opening and also through the 1-cm gaps between the cellulose sheets comprising the cone. The fan works for 2.5 min followed by an off period of 5 min; there are thus eight cycles per hour which are repeated throughout the catching period. This allows mosquitoes to settle undisturbed on, or near, the bait during the 5-min off-period, after which they are sucked up by the fan and blown into the killing bottle. Originally the fan operated through a 'Sunvic' hot-wire vacuum switch wired to the minute hand of an alarm clock which trails a hair-spring over a surface of aluminium foil (Fig. 5.17*c*). Cut sections in the foil corresponded to the 2.5-min on-periods (Lumsden, 1958*b*). Automatic switches with variable on- and off-periods, which can either be bought or made, can be used in place of this arrangement.

In Uganda when a 'Vent-Axia' fan that sampled 1755 m³ air/hr was used in a trap baited with a rabbit a maximum single night catch of 1061 mosquitoes was recorded by Lumsden (1957*b*). In five night catches a baited trap caught a mean of 489.8 mosquitoes (*Coquillettidia fuscopennata* comprised 57.6% of the catch), compared with 60.2 in an unbaited trap. When the trap was used at ground level and at a height of 3 m both the species composition and their relative frequencies differed. Lumsden (1958*b*) stated that most of the catch consisted of unfed mosquitoes, but gives no details on this aspect of the results.

Corbet & Ssenkubuge (1962) placed seven Lumsden traps, which were continuously operated for 24-hr periods and baited with monkeys, birds and rodents, at various heights on a steel tower in Zika forest, Uganda. The collecting bottle, which contained filter paper impregnated with trichloroethylene as a killing agent, was changed at hourly intervals so that diel periodicities could be studied. To try to eliminate any lingering smell of a previous bait the trap's hardboard base was painted with aluminium paint and the bait cage placed on a sheet of glass. Both base plate and glass sheet were wiped down before and after each trial. It was necessary to periodically check that the small entry hole connecting the wire mesh funnel to the collecting bottle was free of leaves and litter. It was considered that the relatively large number of mosquitoes that were caught in unbaited traps was due, in spite of the precautions taken, to attractive odours remaining from when the trap was baited. According to Corbet & Ssenkubuge (1962) this assumption is supported by the paucity of males and non-haematophagous insects in the traps, but catches of males in non-attractant suction traps are normally much smaller than females (Chapter 4). It was thought that some insects, especially larger ones might be able to escape being pulled into the trap by the fan. Over 32 species belonging to eight genera were caught

in the traps, most mosquitoes (1734) being attracted to birds, the fewest (146) being caught in lizard-baited traps.

The efficiency of the trap was tested by Haddow *et al.* (1962). They found that a number of mosquitoes that alighted on the bait escaped during the first 2–3 s when the fan came into operation and before it had gathered full speed. Some of these would then return to the bait when the fan switched off. With a continuously running fan more mosquitoes were caught. There were also marked differences between the percentages of *Coquillettidia aurites* caught each hour (1700–2100 hr) in traps and at human bait. During the hour after sunset for example 56% of the 182 females at bait were caught whereas in traps only 23% of the total of 214 females were caught. It was concluded that although the Lumsden trap could give interesting information on the mosquitoes in an area, it was inadequate for studying biting cycles.

Although the trap has the advantage that the bait is more exposed than in most traps and consequently more accessible to mosquitoes, it has not been widely used, probably due to its much greater cost compared with that for most alternative traps for small animals. It also requires electricity, but it could be operated from small portable petrol or diesel generators, or as shown by Minter (1961) from 12-V car batteries. However, in Florida Edman & Haeger (1977) used the Lumsden trap baited with a rabbit and chicken to determine the diurnal activity pattern of *Wyeomyia mitchellii*.

Modified Lumsden trap (Minter)

Minter (1961) modified the Lumsden trap to make it more robust and portable, independent of main's electricity and adaptable for baits varying in size from baboons to mice and lizards. When food and water is provided these animals can be left in the trap for long periods. The original paper should be consulted for detailed step by step construction, but the principal modifications are as follows. The bait is placed on a metal base plate and the perspex cone positioned above can be raised or lowered to accommodate animals of different sizes. To prevent bait animals, especially monkeys, becoming entangled with the fan a ½-in wire mesh screen is placed at the top of the perspex cone. A 12-in diameter 'Vent-Axia' fan blade is adapted to fit on the spindle of a 24-V d.c. motor that operates from a 12-V, 72 Amphr, heavy-duty vehicle battery. The motor takes approximately 3 A, and the fan speed is about 2000 rev./min. With this arrangement the motor is underrun, and when the trap operates for eight cycles of 7.5 min every hour (2.5 min on and 5 min off) the battery needs recharging every 72 hr. In practice Minter recharged them every 48 hr, either from a small 80-W petrol-operated recharging plant, or by using them in vehicles. Minter was interested in collecting small insects such as Psychodidae and Ceratopogonidae, but if mosquitoes are to be satisfactorily caught then a greater suction by the fan is probably needed. This can be achieved by connecting a 6- or 12-V battery in series with the standard 12-V one, or by substituting a 12-V motor.

The trap can be comfortably carried by three men and rapidly assembled. In addition to exposing various animals on the base plate of the trap Minter (1961) sometimes placed the hood directly on top of cages holding sentinel monkeys.

When these and other large mammals are used as bait they are usually anaesthetised.

These traps did not prove very successful when they were baited with two monkeys and hauled 20 ft up in trees in Malaysia (Wharton *et al.*, 1963); bed-nets caught considerably more mosquitoes.

De Kruijf (1970) used the Lumsden trap in Surinam but only after further modifications were made to it. Laarman (de Kruijf, 1970) found that a considerable number of mosquitoes were not blown into the collecting cage but remained within the cylinder housing the fan and in the laterally projecting funnel leading to the collecting bottle or cage. To overcome this a much wider diameter metal funnel was used, which was closed by a plastic cover weighted by a copper bar. When the fan operated this cover was blown up to allow mosquitoes to be discharged into the collecting cage, but when the fan was inoperative it returned to a vertical position thus preventing mosquitoes escaping. Other modifications were that a ten- instead of a five-bladed fan was used, but the fan speed was reduced to 1200 rev./min as this was sufficient to suck in mosquitoes, but reduced damage to them. According to Laarman these modifications caught about 70% of the mosquitoes attracted to exposed baits (de Kruijf, 1970). Three-hour operation with rats as bait animals produced an average of 67 *Culex portesi*, compared with 50 caught in a No. 10 Trinidad trap over a 24-hr exposure period.

For the battery-operated version of the Lumsden trap and other battery-operated traps, the very cheap and simple electronic timers described by Kimsey & Brittnacher (1985) and incorporating integrated circuits can be used to provide intermittent suction. The on- and off-periods are variable from microseconds to hours, power consumption is low (3.5 mA at 6 V) and the timer operates over a voltage range of 4.5–17 V d.c.

Service trap

This trap has only been used in a study of the role of mosquitoes in the transmission of myxomatosis in Britain (Service, 1971c). A commercially available 23-cm diameter 'Johnson-Taylor' suction trap which segregates the catch into time intervals was adapted to catch mosquitoes attracted to a rabbit. A full description of the suction trap is given in Chapter 4. In preliminary trials a rabbit was placed in a wire mesh bait cage directly above the fan inlet, but the trap became inoperative due to excreta falling into it. To overcome this the rabbit was confined to a 38 × 48 × 50 cm cage placed about 35 cm above the ground near the suction trap having all four sides and top covered with 1-in mesh wire netting. The end of a 1.75-m length of 25-cm diameter flexible tubing which was attached to the top of the fan rested on top of the cage (Fig. 5.17d). The fan operated through an automatic timing device on a repetitive cycle of 3 min on and 7 min off. Mosquitoes were sucked up the flexible tubing down through the fan into the collecting magazine, killed by pyrethrum, and the catch segregated into 50-min intervals. When the fan was operated on eight nights without any bait in the cage only two unfed *Aedes cantans* were caught. When a rabbit was exposed in the cage for 20 nights 383 *Aedes cantans*, 88 *Aedes geniculatus*, 1 *Aedes rusticus*, 77 *Anopheles plumbeus*, 1 *Anopheles claviger*, 5 *Culiseta*

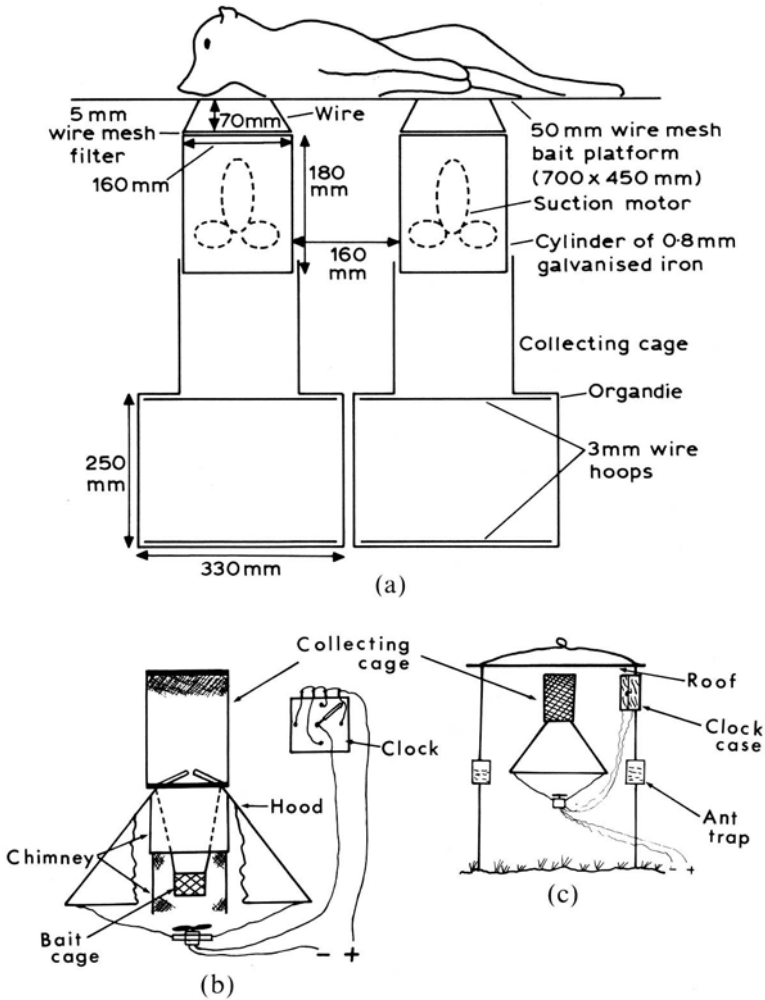


FIG. 5.18. (a) Suction bait trap of Jupp (1978); (b) and (c) blower trap (after de Freitas et al., 1966).

annulata and 18 *Coquillettidia richiardii* were caught. The dropping discs showed that most (64–77%) of the three more common mosquitoes were attracted to the rabbit between 1740–2010 hr, only 10% of the catch was collected after 2100 hr. Although the trap was successful it is unlikely to be widely used because of its cost, although a much cheaper version could be made by omitting the segregating mechanism and delivering the insects into a killing bottle.

Nasci & Edman trap

Nasci & Edman (1981b) converted a New Jersey light-trap into a baited suction trap by raising the rain shield to a height of 38 cm, and inserting a concave aluminium collar extending 10 cm outwards from the air intake to maximise

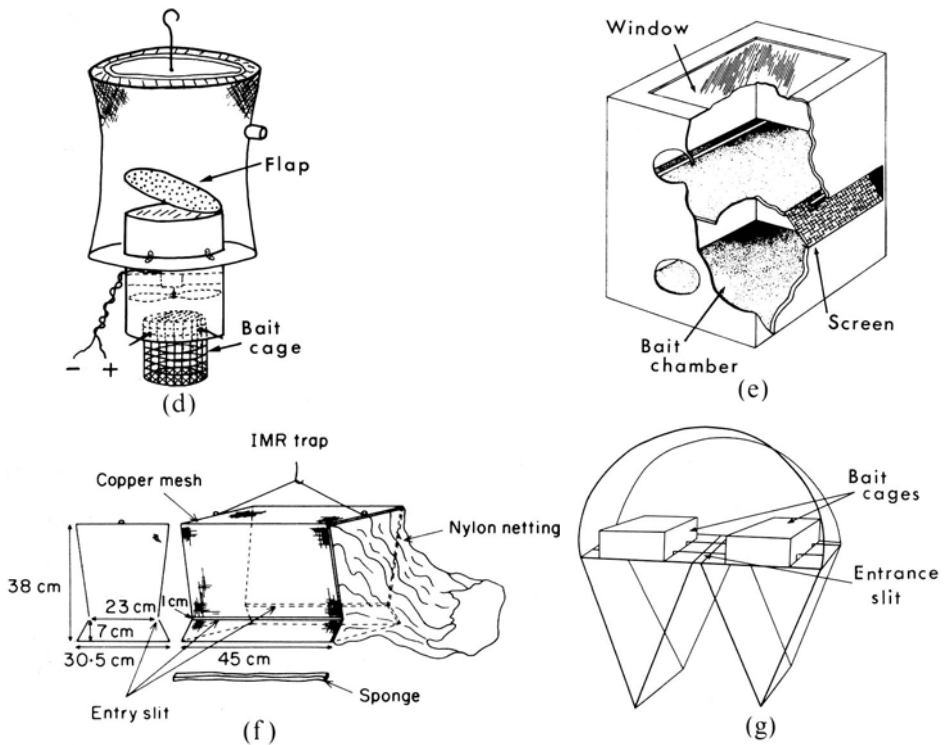


FIG. 5.18—contd. (d) Baited suction trap (after Davies, 1973); (e) bait trap of Hibler & Olsen (1965); (f) IMR bait trap (Chiang et al., 1986); (g) Trinidad No. 10 trap (Service, 1969b).

suction. After removing the bulb a pigeon was placed in a 23-cm tall, 18-cm diameter cylindrical mesh bait cage positioned over the motor supports. Double screening of the bait cage prevented mosquitoes biting the bait. A timer switched the suction trap on for 1 min and blew the catch into a cyanide jar, this was followed by a 4-min off-period. Power came from a 12-V battery attached to a d.c.-a.c. converter. A total of 133 *Culiseta melanura* were caught from six trap-nights.

Means trap

In the USA Means (1968) used a 6-V car battery to operate, for 1 min out of every 15 min, a fan to suck down into a Kilner (Mason) jar filled with alcohol mosquitoes attracted to baits (birds, small mammals, reptiles, amphibia) enclosed in a hard cloth or chicken wire tube placed above a fan. From 48 trials 2614 mosquitoes belonging to 10 species were collected.

Jupp trap

Working in South Africa Jupp (1978) reported that some mosquitoes, especially the *Aedes furcifer/taylori* group, were reluctant to enter large lard-can-type traps

baited with a monkey. He therefore tied an anaesthetised (intramuscular phencyclidine hydrochloride) baboon or vervet monkey to a 5-cm mesh wire bait platform, which was either supported on poles 1-m from the ground or raised by rope to a height of 10-m. Two rubber-bladed suction fans mounted on two 8-W 'autofan' motors housed in two galvanised cylinders painted black were suspended by wires beneath the platforms, so that their openings were 7 cm below the platform (Fig. 5.18a). Power was provided by a 12-V car battery. A 5-mm mesh screen on top of each cylinder excluded larger unwanted insects. Mosquitoes were sucked down into two organdy netting cages. Apparently good numbers of males as well as females of the *Aedes furcifer/taylori* group were caught.

Jupp & McIntosh (1990) used these traps and a later modification in which two unanaesthetised monkeys were placed in a wire mesh cage ($60 \times 45 \times 30$ cm) positioned over the two suction fans. Traps were suspended 10 m above the ground and operated for 2 hr after sunset. From a total of 240.5 trap-hours 9174 mosquitoes belonging to at least six species were caught. The most common being *Aedes furcifer* males (4825) representing a mean of 20.1/trap-hour, and *Aedes furcifer/cordellieri* females (3995) resulting in a mean of 16.6/trap-hour. In contrast a mean of 40.2 *Aedes furcifer/cordellieri* females were caught per man-hour in human bait catches. Clearly the monkey-baited trap was the better for collecting *Aedes furcifer/cordellieri*. Two hundred and seventy-one *Culex poicilipes* also entered the trap.

Blower trap of de Freitas et al.

The collection in the morning of mosquitoes from under the hood of a sentinel trap is not very satisfactory as mosquitoes that are attracted to the bait but do not remain under the trap until the morning are missed. To get more representative collections a trap was devised in which mosquitoes attracted to sentinel mice were automatically trapped at intervals (de Freitas *et al.*, 1966).

Mice are exposed in a $10 \times 10 \times 12$ -cm cage of wire mesh suspended from an aluminium hood, 62 cm square and 32 cm high (Fig. 5.18b,c). A 26-cm tall, 21-cm square, chimney having the lower half made of nylon netting and the upper half of aluminium is placed underneath the hood and surrounds the bait cage. A 19-cm fan blade is fixed to the spindle of a 6-V d.c. motor enclosed in a plastic bag and placed under the centre of the bait cage. It operates from a 6-V car or gel cell battery. The updraft of air produced opens a pair of 11.5-cm square plastic foam trap doors fitted to the top of the aluminium hood, and which fall shut when the fan is not operating. Originally the minute hand of a clock, which was housed in a small box underneath an aluminium roof, was wired to the positive lead from the car battery to the fan motor, so that electrical contact was made for a period of 90 s every 15 min, but the timer described by Kimsey & Brittnacher (1985) could be used (p. 439). During the short exposure period mosquitoes attracted to the sentinel mice are blown up through the open doors into a 23-cm square, 30-cm high, plastic mesh collecting cage placed on top of the hood. The complete trap is suspended by wires under a large flat protective aluminium roof, which if attached to pulleys allows the traps to be used at various

heights. The trap can also be mounted on metal rods. Wires or rods supporting the trap need to be greased to keep out ants. The collecting technique differs from that employed in the Lumsden (1958*b*) trap in that mosquitoes are blown and not sucked into the trap.

In Brazil these traps have proved exceptionally useful in collecting large numbers of mosquitoes. For example, de Freitas *et al.* (1966) caught 41 825 mosquitoes from a blower trap containing sentinel mice (mother and young) operated for 84 daily periods. The average catch was 498 mosquitoes per night. The identification of representative samples showed that about 96% of the catch comprised *Culex* species, of which about two-thirds were of the subgenus *Melanoconion*. The numbers caught were only slightly less when the fan functioned every 30 instead of 15, min, but the numbers of blood-fed mosquitoes increased from about 7 to 20%.

With the conventional type of hooded sentinel bait cage, without a fan, a number of mosquitoes undoubtedly return to the neighbouring forest after feeding on the mice. De Freitas *et al.* (1966) pointed out that if sentinel mice become infected and developed viraemia they serve as artificial reservoirs and mosquitoes feeding on them will become potential vectors. The blower trap reduces the likelihood of any such transmission occurring in the forest. In studies in Bush Bush forest in Trinidad this risk was reduced by never exposing sentinel mice for more than 14 hr in No. 10 Trinidad traps, thus preventing them becoming viraemic (Aitken *et al.*, 1968*a*).

This trap with little or no modification could be baited with a variety of other small animals or dry ice, and if a bigger fan was fitted and the trap modified it could be used to collect mosquitoes attracted to larger animals such as rabbits or even monkeys. Such a trap would probably be simpler to construct than that of Lumsden (1958*b*).

Baited suction trap (Davies)

One of the important features of this trap is that mosquitoes do not have to pass through any baffles or restrictive entrances to get at the bait since it is relatively exposed. The trap is made from readily available materials, and being cheap it should not be so liable to theft as are more sophisticated traps, although the motor cycle battery needed to operate it will be attractive. The trap was briefly referred to by Davies (1971), but a complete description together with diagrams did not follow until some 2 years later (Davies, 1973).

The trap consists of four basic components, a cylindrical net collecting cage, a small metal tubular fan housing, a wire mesh bait cage and a time switch which can be made from a cheap clock (Fig. 5.18*d*). The collecting cage is made from nylon or terylene netting and is 20 in long and 12 in in diameter except that at both ends a curtain wire threaded through a ½-in hem reduces the diameter to about 10 in. A 12-in circular piece of ¼-in plywood with a wire loop handle is inserted in the top of the cage as a convenient lid. A short length of plastic tubing, such as a vial with the bottom removed, is cemented into the middle of the cage so that an aspirator can be inserted for removal of the catch. An 8-in long, 6-in diameter metal tube (e.g. a 5-lb dried milk tin with both ends removed) is

mounted in a 12-in disc of plywood placed in the bottom of the trap. A 6-V d.c. motor is held by aluminium brackets or straps midway inside the tube. Ferrous mountings should be avoided as they may interfere with the magnets of the motor and hence its operation. A 2- or 3-bladed 6-in diameter plastic propeller with a 4-in pitch, such as used in model aircraft, is fixed to the spindle of the motor. The top of the metal tube is closed by a circular flap of ¼-in polystyrene foam, cemented only at one point, so that when the motor operates, the updraft of air forces the flap open. It falls back into place when the motor stops. A bait cage about 3 in in diameter and length made from ½-in galvanised wire mesh is supported at the bottom of the fan housing by two long meat skewers running through it at right angles.

Wiring the motor to a 6-V motor cycle or gel cell battery through a clock allows the fan to automatically operate for 30–45 s every 7–10 min. This is achieved by first removing the case, face and hands from a cheap clock and dismantling the alarm if one is fitted, and also the gears operating the hands. One of the gear trains between the main spring and escarpment will make one revolution about every 7.5 min, and this has a small stainless steel or copper pin soldered to its shaft or one of its spokes. A length of fine copper wire is inserted through the clock mechanism so that it makes contact with this revolving pin once every revolution; the rest of the wire must be insulated from the clock body. The duration of contact between the pin and copper wire governs the duration of the on period of the fan, and can be altered by adjusting both its angle and tension. Trial and error will give the desired contact time. Because the motor is likely to consume 1–2A, arcing between the contacts will occur if the switch is placed directly in series with the battery and motor. To overcome this a 50–100-Ω small switch relay is wired into the circuit. The clock together with the switch relay should be mounted in a small waterproof box fixed either to the lid of the net cage or to the side of the battery, which itself should be protected against rain by being placed in a plastic bag. Alternatively a more simple and cheap electronic timer could be used (Kimsey & Brittnacher, 1985). A metal or plastic roof can be placed over the complete trap.

Tikasingh & Davies (1972) compared the efficiency of this trap, the Trinidad No. 10 trap (Worth & Jonkers, 1962), the CDC light-trap and the No. 17 trap (Davies, 1971) in the rain forests of Trinidad. The baited suction trap caught more than twice as many mosquitoes (*Culex* species, mainly *Culex portesi*) as the No. 17 trap and about four and eight times as many as the CDC and No. 10 traps. Although the suction trap caught more mosquitoes, because of its comparative bulkiness, and the fact that under field conditions it sometimes broke down, the No. 17 trap with no working parts was considered the best practical trap.

Davies (1973) pointed out that trap dimensions are not critical, in fact bigger traps can be made for baiting with larger animals and in fact he used a large version in studying the attraction of *Culex portesi* and *Culex taeniopus* to various hosts in Trinidad (Davies, 1978). For this the bait cage (15.2 cm diameter, 8.9 cm high) was made of wire mesh having 1.3-cm squares. Half of this cylindrical bait cage protruded below the overhead fan housing into which mosquitoes were sucked up by a 22.9-cm diameter propeller powered by a 6-V d.c. motor. A

time switch activated these motors for approximately 45 s every 7.5 min. Power to each trap was standardised by connecting them in parallel to a single 12-V battery by 15.24 m of identical cable; the resistance of the cable reduced the 12-V to 6-V at the outlet. Six traps were suspended 90 cm from the ground 13.5 m apart along the circumference of a 27-m diameter circle. Bait cages were boiled in 10% bleach solution after use to eliminate any residual odours.

In all trials one trap was baited with two white mice to serve as a standard, and an adjustment factor, based on the ratio between the mean catch on the two mice and 50 mosquitoes/night, was calculated for each experiment to compensate for seasonal variations in mosquito population size. The mean catch, as well as its standard error, was then multiplied by this correction factor. Neither *Culex portesi* nor *Culex taeniopus* were attracted to crabs, toads or lizards exposed in the traps, but all rodents, bats and birds exposed attracted varying numbers of mosquitoes. The best bait for *Culex taeniopus* was the opossum (*Didelphis marsupialis*), while *Culex portesi* was attracted to a wide range of rodents and marsupials. High attraction to a bait did not necessarily result in high feeding success, the most extreme case was the high degree of attraction of *Culex portesi* to the grass mouse (*Akodon urichii*), yet only 6.08% engorged on the mouse.

Hibler & Olsen trap

To attract haematophagous insects in considerable numbers to small baits Hibler & Olsen (1965) developed a trap in which a number of small bait animals were confined in a small space to produce a concentration of host odour and heat. Basically the trap consists of a lower bait chamber about 2.5 ft long, 2 ft wide and 1.5 ft high made of plywood or some other suitable material. The upper 6 in of the two longer sides are made of fine nylon netting that slopes inwards leading to a ½-in slit in the floor on either side of the upper compartment (Fig. 5.18e). The upper compartment is of similar dimensions to the lower, but has a clear plastic insertion in the roof. A number of bait animals are inserted through a small doorway into the lower compartment, and mosquitoes attracted to the trap settle on the sides and eventually crawl or fly up through the two slits into the top compartment. Being attracted to the light most rest on the plastic part of the roof from where they are removed by inserting an aspirator through a cut-out section in the top compartment. If mosquitoes need to be given the chance to engorge on the host, a single bait animal can be placed in the top section, in addition to those housed in the bottom compartment. When this type of trap was baited with an American Black-billed Magpie in Colorado and hoisted about 15–20 ft above ground level the mean catch of mosquitoes during May–September, varied from 2–32/trap-night.

IMR bait trap

Basically this trap (IMR = Institute for Medical Research, Kuala Lumpur) consists of a rectangular cage 45 × 30 cm and 38 cm high having a sheet metal base. The sides are covered with copper mesh except that one of the smaller ends (30 × 38 cm) has a nylon netting sleeve attached for introducing the baits and removing the catch (Fig. 5.18f). The two longer sides are bent inwards at

7 cm from the metal base plate making an angle of 145°, and along the apices of these angles are horizontal 1-cm wide slits for mosquitoes to enter. When these traps were baited with either a chicken or pigeon near Kuala Lumpur, Malaysia a mean of 9.1 and 5.3 *Coquillettidia crassipes* were collected, respectively. There were no statistical differences between the numbers caught in these traps, in No. 10 Trinidad traps or in lard-can bait traps (Chiang *et al.*, 1986). A number of other species were collected including *Culex quinquefasciatus* and *Aedomyia catasticta*. Klinkaewnarong *et al.* (1985) trapped 172 mosquitoes during seven nights when one trap was baited with a chicken and another with a gerbil in their studies on vectors of *Cardiofilaria nilesi*.

No. 10 Trinidad trap

This has proved to be one of the most versatile traps for small animals and also for dry ice, although it has not been as widely used as merited. The frame of this double-baited trap is made from 1/8-in galvanised or stainless steel wire. The various sections can be soldered or tied together, or with some ingenuity the entire framework of the trap can be made by bending a single length of wire into the appropriate shape (Worth & Jonkers, 1962). Each end section of the trap is formed by bending the wire to form a 'W' and then the outer limbs of the 'W' are connected overhead by two semicircular pieces of wire (Fig. 5.18g). The two end sections are linked by two 16-in horizontal lengths of wire running from the outer top ends of the 'W'-shaped wire frame, and by two similar strips separated by a 1-in gap connecting the central apex of the two 'W'-shaped frames. This gap forms the entrance for the mosquitoes. The framework of the lower part of the trap is covered with rustless wire mesh while the upper part supported by the two semi-circles of wire is covered with white nylon mosquito netting which is sewn on to the framework except at the front end. A 'touch and close' fastener such as 'Velcro' is sewn on to the wire mesh at the front end of the trap and also along the bottom edge of the mosquito netting cover. Alternatively the top cover can be made as a bag that is dropped over the cage and held in position by a large strip of rubber such as cut from an old motor car inner tube. However the cover is fitted, it should not be made of cotton mosquito netting as this tends to become mouldy and fluffy, causing the holes to become occluded, and poor ventilation results in poor catches.

The trap is normally baited with small vertebrates contained in a small mesh cage supported on wire supports in each section of the trap, but the trap has also been successful when baited with dry ice contained in plastic bags or polystyrene boxes (Service, 1969b) (see Chapter 6). The trap is normally suspended from a tree and protected from rain by a horizontal sheet (18 × 24 in) of metal. Mosquitoes enter from below through the long vertical centrally placed slit. They are collected by inserting an aspirator underneath the bottom edge of the mosquito netting cage covering the trap. Worth & Jonkers (1962) found that over a test period of several days only about half a dozen mosquitoes entered a trap containing bait cages and food, but no animals. When the trap was completely covered with green plastic mesh no mosquitoes were caught even when baited with mice.

These traps have mainly been used in Trinidad where they have proved to be exceptionally useful, although to some extent they have been replaced with the simpler No. 17 Trinidad trap (below). In preliminary trials in Trinidad they caught up to 20 different species, the maximum catch being 1929 mosquitoes (Worth & Jonkers, 1962). Traps baited with mice caught more mosquitoes than Shannon-type traps baited with chickens (Aitken *et al.*, 1963). In later studies traps containing mice and White Leghorn chicks were used at both ground level and at 55 ft up in the tree canopy. At ground level chick-baited traps caught 21 species and mice-baited ones 25 species, but chick-baited traps caught more mosquitoes; 33 species were collected at human bait. Fewer mosquitoes were caught in traps in the canopy than at ground level (Aitken, 1967). The traps have proved very useful in virus isolation studies in Trinidadian rain forests where Aitken *et al.* (1968*b*) caught 25–50% of the mosquitoes used in virus isolation experiments in these traps. When baited with various rodents, lizards and chicks, 42 mosquito species, including those of *Limatus*, *Aedes*, *Mansonia*, *Psorophora* and *Wyeomyia* were caught, but *Culex* species, especially *Culex nigripalpus* formed the bulk of the catches (Aitken *et al.*, 1968*b*). In contrast very few mosquitoes were caught in traps baited with mice in England (Service, 1969*b*), although when about 0.5 lb of dry ice was placed in a polystyrene box in each section of the trap nine species, including *Anopheles*, were caught. The maximum overnight catch from a single trap was 105 females.

In Kenya Chandler *et al.* (1976*a,b*) baited Trinidad traps with six white mice, and from 34 trap-nights caught 787 mosquitoes belonging to 19 species, of which *Mansonia uniformis*, *Mansonia africana*, *Culex poicilipes*, *Culex univittatus* and *Culex antennatus* formed the bulk of the catch. From 56 trap-nights at another site 7696 mosquitoes were caught, with again the first three species comprising most (91%) of the catch. In other trials traps contained two young chickens in each V-shaped section and were suspended from trees in a heronry near a rice irrigation scheme at heights of 1.9, 7.4 and 11.7 m. From 54 nocturnal trap catches 364 mosquitoes belonging to 12 species were caught, of which the *Culex univittatus* group formed 77.7% of the total (Chandler *et al.*, 1976*a*).

Worth & Jonkers (1962) considered that only few of the mosquitoes caught escaped by 'blundering out', but Service (1969*b*) found that despite mosquitoes entering No. 10 traps much more readily than they did cylindrical traps with conical entrances a greater proportion escaped, although a few of these re-entered the trap. If mosquitoes are allowed to feed on the baits then they become less active and fewer escape, but it is not always desirable to let them engorge.

No. 17 Trinidad trap (Davies)

This trap was developed as a small, simple and cheap trap to collect mosquitoes attracted to small rodents (Davies, 1971). It consists of four distinct parts—lid, net cage, bait cage and spreader ring (Fig. 5.19*a*). The lid is a ¼-in thick 12-in diameter piece of plywood with a small central hole through which string or wire is fixed for suspending the trap. The upper surface can be painted, usually black, to protect the wood, while the lower surface is painted white. The net

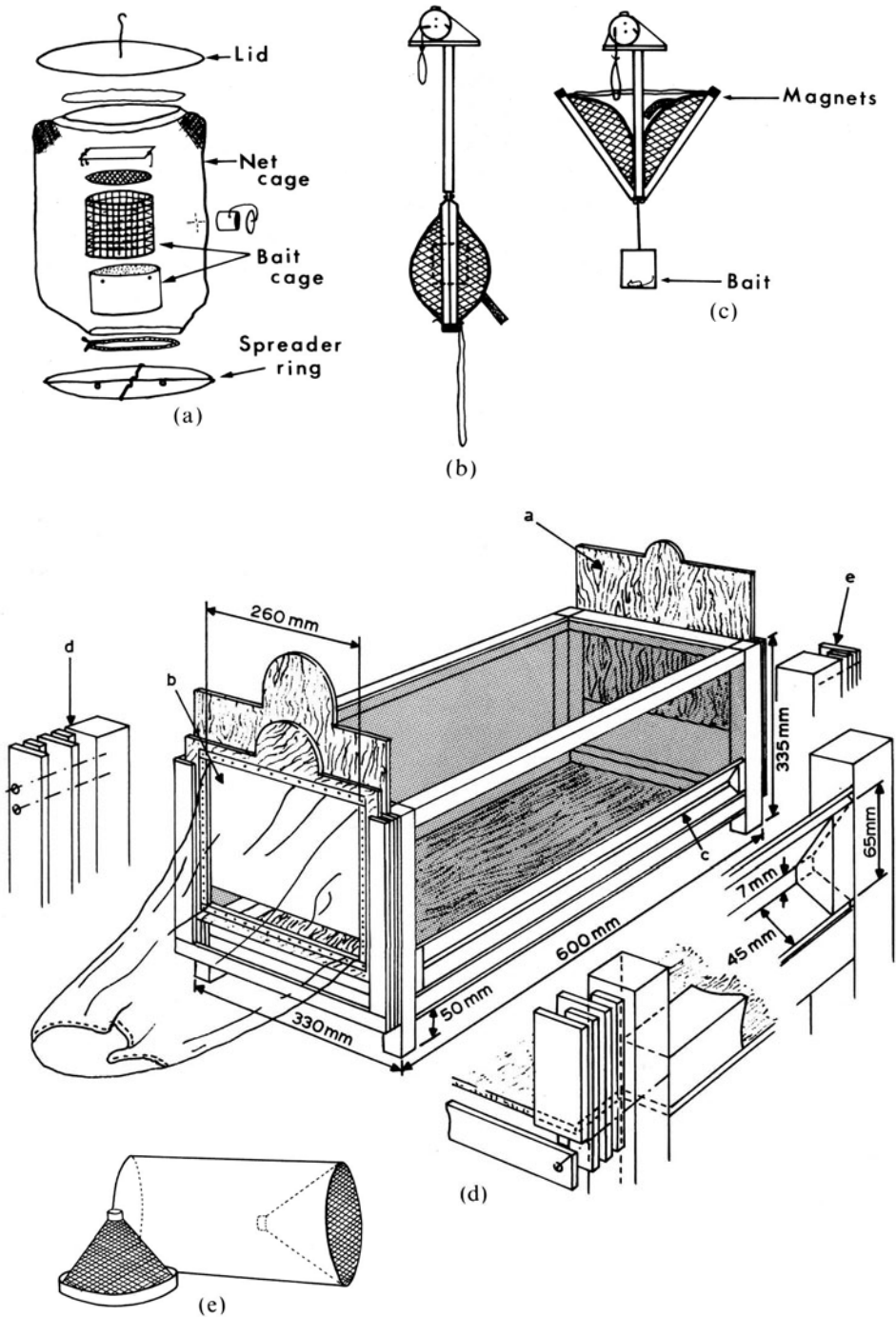


FIG. 5.19. (a) Trinidad No. 17 trap (after Davies, 1971); (b) and (c) flap trap closed and open (after Worth & Jonkers, 1962); (d) bait trap of Dégallier et al. (1983); (e) cylindrical lard-can-type trap (after Service, 1969b).

cage is made from a piece of 38×18 in terylene netting with a $\frac{1}{2}$ -in hem along both longer edges, by sewing the two shorter sides together to form a tube. A 28-in length of flexible curtain wire is threaded through the top hem and a 9-in length of $\frac{1}{4}$ -in wide elastic through the bottom hem. A short section of a 1-in diameter plastic tube having a removable top or cork is cemented into a slit made in the side of the bag. This provides a simple but efficient opening for inserting an aspirator. Alternatively a slit-like opening furnished with a touch and close fastening such as 'Velcro' could be used. The bait cage is made from a 1 US quart motor oil tin cut in half and with both ends removed to give a 4-in diameter, $2\frac{3}{4}$ -in long cylinder. Half-inch galvanised wire mesh screening rolled round to form a 4-in diameter, 13-in long cylinder is slipped down over the bait tin for about $\frac{1}{2}$ in and held in position by adhesive tape, small bolts or self-tapping screws. A disc of $\frac{1}{4}$ -in mesh screen is wired inside the wire cylinder about $1\frac{1}{2}$ in above the top of the tin to form a floor for the bait cage, and a piece of plywood or galvanised metal is hooked on top of the wire cylinder to form a roof. A piece of 16-gauge galvanised wire is placed underneath the middle of the tin that forms the bottom of the bait cage, and is bent to form two U-shaped slots to accommodate the rim of the tin. Two such pieces of wire are then placed at right angles to each other across the spreader ring, this being a 12-in diameter circle of stiff wire, which supports the cage within the trap. Mice, or other small baits, are confined within the 2-in high space between the roof and the wire mesh floor of the bait cage.

Davies (1971) found that there was a greatly reduced catch if the trap bag was made of nylon mosquito netting with round holes. The most efficient traps had a bag of terylene netting with square holes and 22 meshes to the inch. It was also important that the floor of the wire mesh cylinder was made of finer mesh than its sides. If two or more adult mice are used the bait chamber should be divided to prevent fighting. One man can carry 10 net bags and trap lids in one box and 10 bait cages packed in another box to the field.

Davies (1971) reported that in Trinidadian forests a trap baited with two adult mice catches up to 200–300 mosquitoes per night belonging to about 30 species; this represents about half the catch obtained by a No. 10 trap of Worth & Jonkers (1962) containing four mice. The portability, simplicity and cheapness of the No. 17 trap together with its efficiency have been responsible for its popularity in Trinidad and the almost complete phasing out of the double baited No. 10 trap. In comparative trials of four different traps the No. 17 trap has been identified as the best (Tikasingh & Davies, 1972; see pp. 444, 447). This trap was regularly employed in the extensive trapping programmes undertaken at Belém, Brazil, and has also been used for short periods in British Honduras, but since then has rarely been used.

Degallier trap

This consists of a rectangular wooden box (6 cm long, 33 cm wide and 33.5 cm tall) with the top and two long sides covered with metal mesh screening (Fig. 5.19*d*). The wooden bottom is covered with a removable sheet of metal to facilitate removing excess food and faeces from the cage. The two ends have removable

sliding wooden panels, and a sleeve of mosquito netting is attached to one end. A horizontal baffle entrance with a 7-mm slit is fixed on the two long sides (Fig. 5.19d). In trials in French Guyana the trap was baited with a variety of rodents and a small species of monkey (Degallier *et al.*, 1983). Twenty-nine mosquito species belonging to 12 genera were trapped, the most common species were *Culex portesi* and *Culex spissipes*. The only *Aedes* trapped was *Aedes serratus*, while the only *Anopheles* was *Anopheles nimbus*, although in human bait catches five species of each genus were collected.

Flap trap

This trap was described by Worth & Jonkers (1962) in the same paper as the No. 10 Trinidad trap.

A cheap clock having an alarm winding key that turns when the alarm rings is placed on a small wooden platform nailed to the top of a 20-in tall wooden pole (Fig. 15.19b). A small animal mesh cage (e.g. a semi-cylindrical test tube basket) is suspended by a wire some 5 in below the pole (Fig. 5.19c). Two similar flap-like structures are made by screwing two semi-circular plywood sides on to a heavy 16 × 20-in wooden frame and covering the outer surface with fine mesh wire or plastic screening. All wooden parts are varnished to prevent warping. One end of each flap is either hinged or fixed with open screw eyes to the bottom of the upright wooden pole. To set the trap both flaps are lifted and held in position by a long narrow loop of wire, which is firmly fixed to one flap but only lightly attached by a bent nail to the other. A smaller loop of wire is tied to the alarm key and passes right round the horizontal wire loop holding the flaps in the open position. Now, when at any pre-set time the alarm rings the key turns and pulls its loop upwards. This disengages the horizontal loop from the bent nail on one of the flaps, whereupon both flaps rapidly fall and enclose the bait cage. Worth & Jonkers (1962) attached strong magnets to the edges of the frames of the flaps to provide extra momentum and to hold them together when they had fallen. Mosquitoes are removed by inserting an aspirator through a small netting sleeve attached to one or both flaps. The trap is protected by a galvanised cover or hood.

Because it is known that some mosquito species are 'trap shy' Worth & Jonkers (1962) thought that more representative samples of mosquitoes attracted to bait animals were likely to be caught in these traps, but it is unlikely that the trap is free from sampling bias. For example, some species may enter the bait cage more readily than others, and these will more likely be caught by the falling flaps than mosquitoes which hover around the outside of the cage. Species may also show differences between their ability to escape capture by the descending flaps. The trap has rarely been used and there is little information on its efficiency in the field, but in Trinidad six traps baited with mice were used to obtain diel activity rhythms. The first trap was in operation at 0600 hr and the flaps automatically closed at 0800 hr, and then at 2-hr intervals the flaps on the other five traps descended. At 1800 hr, when the last trap had closed, mosquitoes were removed from all the traps which were then re-set to get an activity cycle during the night (Aitken *et al.*, 1968a). Despite their careful operation, continuous

human bait catches proved to be more useful because catches were segregated into hourly, not two hourly, periods.

Lard-can traps

Birds or small mammals (Dow *et al.*, 1957; Downing & Crans, 1977; Lounibos & Escher, 1985; Lounibos & Linley, 1987) instead of dry ice can be placed in the metal cylindrical traps of Bellamy & Reeves (1952). In trials in England a rabbit or young chicken not enclosed in any restraining cage was placed directly into a metal cylinder, 35 cm long and 25 cm in diameter, with inverted wire mesh funnels at both ends (Fig. 5.19*e*). With rabbits nine mosquito species were caught, the most common being *Coquillettidia richiardii* but relatively large catches of *Aedes detritus* were also obtained. At night relatively large numbers of unfed females of both *Culiseta morsitans* and *Culex pipiens* were trapped, species known to be almost entirely ornithophilic in the area, but few (0.6–2.3%) fed on the rabbits (Service, 1969*b*). In contrast when the traps were baited with pullets very few mosquitoes entered them, and moreover none engorged on the birds. The reasons why mosquitoes which normally feed on birds entered a rabbit-baited trap are not understood, but the results emphasise the caution needed in interpreting trap catches in terms of natural host preferences.

A better technique of exposing bait animals in cylindrical traps is to restrict them to a small section of the trap. For example, bait animals can be placed in a small screen cage soldered to the floor of a trap and provided with a small door underneath to allow their easy insertion and removal (Dow & Morris, 1972). Ehrenberg (1966) introduced a pigeon through a hinged flap door in the side of the cylinder, and the two mesh cone entrances at the ends opened into a screened cage, thus preventing biting on the host. Downing & Crans (1977) found these traps very useful in catching *Culex* mosquitoes in New Jersey, recording a mean catch of 55.9 per night. In addition to *Culex pipiens*, which formed 73.0% of the *Culex* caught, small numbers of *Culex salinarius* and *Culex restuans* were obtained, as well as a very few *Aedes*, *Culiseta* and *Coquillettidia*. Slaff & Crans (1981) using a pigeon-baited Ehrenberg trap found that most host-seeking *Culex salinarius* were trapped during the first 2 hr after sunset. Mitchell & Millian (1981) made lard-can traps in three sections. The two longer end sections had conical entrance funnels which were separated by a shorter removable circular bait cage of equal diameter having both ends made of mesh and held in position by 'snap-on' clips. A curved hinged flap in the side of the circular cage allowed the bait (a chicken) to be introduced and removed.

Nayar *et al.* (1980) also modified lard-can traps. They cut a 15 × 25-cm hole in the side and screened it from the interior by a fine mesh cage (16 × 26 cm and 12 cm deep). The bait animals, 1–3-week-old chicks, were confined to a wire cage attached to the lid, which was fitted within the mesh cage, and then the lid was inserted into the can and fastened. Thus, it was possible to introduce and remove the bait without opening the trap. The outside of the trap was sprayed with matt black paint. A 6-cm cube (approx. 500 g) of dry ice was placed in a 12 × 12-cm styrofoam box hung outside the baited trap. When these traps were suspended 1–2 m above the ground many hundreds of *Culex nigripalpus* were caught.

Edman *et al.* (1985) believed that because 6.9 and 9.6 times as many *Culex nigripalpus* were caught in lard-can-type traps containing an unscreened chicken as in one in which it was protected, provided evidence for the existence of an 'invitation pheromone' (Ahmadi & McClelland, 1985; Alekseev *et al.*, 1977; see p. 353).

In California Dow *et al.* (1957) confined birds and other bait animals to cages placed in a fine mesh screen recess inserted in the top of a cylindrical trap. The outside opening of the recess was closed with a hinged cover. In later experiments mosquitoes were given the opportunity of feeding on the birds, which were placed in a $\frac{1}{2} \times 1$ -in wire mesh cage introduced into the trap through a hinged trap door provided with a 3-ft cloth sleeve. To minimise the effect of trap position, four identical cylindrical traps were suspended at right angles to each other about 5 ft from the ground on 3-ft booms, which slowly rotated horizontally. The testing procedure was set out as a Latin Square. Basically each individual bait was exposed in a different trap each successive night, until each bait had been placed in all the traps. The numbers caught in the different traps were transformed to $\log(x + 1)$ for an analysis of variance. The percentages which had engorged on the baits were subjected to an inverse sine transformation (Bartlett, 1947). It was found that the numbers of *Culex tarsalis* attracted to different birds were directly correlated with the size of the birds and not species, whereas engorgement rates were independent of attraction rates and size of the birds, but were related to the species and also to different birds of the same species. A density-dependent phenomenon was observed, namely an increase in catch size of *Culex tarsalis* resulted in a decrease in the proportion feeding.

In studying the host preferences of mosquitoes in Massachusetts Hayes (1961) baited cylindrical 120-lb capacity lard-tins with 25 different vertebrate species, including birds, bats, rabbits, squirrels, snakes, turtles, frogs, toads and salamanders. Small animals were placed in a small hammock, $10\frac{1}{2}$ in wide, $6\frac{1}{2}$ in deep, made of nylon mesh (28 strings/in) and edged with a 2-in muslin collar with four button holes. The hammock was inserted through a 6-in diameter hole cut from the top of the cylinder and held in position by spreading the collar over the outside of the cylinder and covering it with a $1\frac{1}{2}$ -in wide metal gasket bolted to the cylinder. A metal cover was bolted over the pocket on the outside of the trap to prevent mosquitoes reaching the bait without entering the trap. Except for the turtles, which were placed unrestrained within the hammock, baits were immobilised. Birds with their feet bound together were placed in the toe of a nylon stocking and then placed on their backs in the hammocks; amphibians were also held in a stocking. A wad of cotton wool on the floor of the trap underneath the hammock absorbed urine which was generally produced by the animals. Moderately sized mammals and snakes were restrained in galvanised $\frac{1}{2}$ -in wire mesh cages before being placed in the hammock, but animals such as rabbits, rats and squirrels which were too large for the hammock were placed in cages on the floor of the trap. Following an exposure period the metal cover over the hammock was removed and the baits taken out, after which mosquitoes in the trap were anaesthetised and collected. After use the entire trap, including the nylon hammock, was washed to remove bait odours.

Because Blackmore & Dow (1958) had found that if birds were relatively active in cylindrical traps they inhibited mosquitoes from getting a blood-meal, Reeves *et al.* (1961) restrained bait chickens in nylon stocking sleeves before placing them in a bait cage on the floor of the trap. When the chickens were not enclosed within a stocking restrainer an average of only 30.3–47.3% fed on them, whereas when a restrainer was used engorgement rates increased to over 90%.

In Texas, Easton *et al.* (1968) compared the mosquitoes collected in cylindrical traps, made from 110-lb capacity lard-cans, with those caught in Malaise traps of Townes (1962). Larger bait animals, such as Californian jackrabbits and Audubon's cottontail rabbits, were enclosed in 6-in diameter, 15-in long cylindrical wire restraining cages, while smaller animals were placed in a 15-in long rectangular box made of ¼-in wire mesh. Each trap had the screw top of a Mason (Kilner) jar soldered over a 2-in diameter hole cut from one side of the trap. A plastic cone was cemented inside the screw lid so that when the glass jar was screwed in it projected into it. The baited traps were placed amongst shade, which usually resulted in mosquitoes caught in the traps flying into the collecting jars. This procedure enabled the catch to be easily removed. Because artificial light failed to attract mosquitoes into the jar at night, catches were removed only during the day. With the exception of *Culex erraticus*, and possibly *Culex quinquefasciatus* and *Aedes trivittatus*, the Malaise traps were much more efficient in collecting mosquitoes and other haematophagous flies than the cylindrical traps. When the cylindrical traps were baited with jackrabbits all trapped mosquitoes fed on them, but none fed on smaller mammals enclosed in the ¼-in mesh cages, possibly because of the reduced aperture of these cages, or the greater movement afforded to the smaller animals.

To avoid bias resulting from trapping in different sites, the procedure that had been used by Dow *et al.* (1957) was largely followed, i.e. the traps were suspended from horizontal arms and slowly rotated. The test was also based on the experimental layout of a Latin Square. By exposing 25 vertebrates in 113 trials, 15 182 mosquitoes belonging to 13 species were caught. The catch consisted mainly of *Culiseta melanura*, *Culex salinarius*, *Culex pipiens* and *Aedes canadensis*, all of which were attracted to amphibians although only *Culex salinarius* and *Aedes canadensis* fed on them. *Culiseta melanura* and *Culex pipiens*, but not *Culex salinarius* and *Aedes canadensis*, were much more common in bird-baited traps than in those containing mammals.

When Brockway *et al.* (1962) used dry ice as bait they found that if four cylindrical traps were mounted at right angles to each other the trap facing downwind caught 61.3% of the total catch of mosquitoes. This was about four times as many as caught in the upwind trap. Further experiments confirmed that traps facing into the wind caught most mosquitoes (Bailey *et al.*, 1965). The most critical evaluation of wind as a factor in operating cylindrical bait traps was made in Florida by Dow & Morris (1972). Their traps which were baited with two Leghorn pullets, and divided into two equal parts by a vertical wire mesh partition, were suspended horizontally about 1 m from the ground. By an arrangement of pulleys, the traps could be orientated parallel to the wind, at right

angles to it, at a random fixed angle to it, or made to continuously rotate at a speed of 0.2 rev./min about a vertical axis. Results showed that revolving traps caught the most *Culex nigripalpus* and *Psorophora confinnis*, while those facing into the wind caught the fewest *Culex nigripalpus* and traps at right angles to it the least *Psorophora confinnis*. In discussing practical considerations Dow & Morris (1972) concluded that if large numbers of mosquitoes are required two ordinary traps set in any random direction should catch more mosquitoes than a trap maintained in any of the orientations they tested. However, if suitable power was available a revolving trap would probably catch most mosquitoes.

Edgar & Herndon (1957) constructed a large aluminium hexagonal trap with six separate compartments and entrances for baiting with animals, carbon dioxide, light or other attractants in the laboratory. The trap revolved around a central pole to overcome directional bias when the compartments contained different attractants. Although this trap has not apparently been used in the field it might be worth evaluating.

In South Africa lard-can traps baited with a rodent caught 232 *Culex rubinotus* during 17 trap-nights, compared to 17 and 14 when baited with pigeons and bats (Jupp *et al.*, 1976). Also in South Africa, in addition to using lard-can traps containing birds enclosed within a nylon mesh restrainer McIntosh *et al.* (1972) constructed larger cylindrical traps, 76 cm long and 43 cm in diameter, baited with monkeys restrained in wire cages (20 × 20 × 25 cm). Traps were exposed at both ground level and at a height of 12 m in the gallery forest. Twenty culicine species were caught in the monkey-baited traps, and 22 culicines and one anopheline in the fowl-baited traps; in human bait catches 25 culicine and seven anopheline species were collected. The traps were not very efficient in catching *Aedes* or *Anopheles* mosquitoes, and some species caught at human bait which failed to enter the traps readily fed on monkeys in the laboratory. Similar large cylinders (44-gal drums) baited with monkeys contained in expanded metal cages were hauled up 20 ft in Malaysia, but caught relatively few mosquitoes (Wharton *et al.*, 1963). In Zaire, Laarman (1959) caught 91 *Anopheles theileri* in large empty petrol drums baited with porcupines, but only six and one adult when they contained a monkey or rabbit.

In Florida lard-can-type traps were baited with chickens on eight nights near a drainage canal where the predominant species in emergence traps were *Mansonia dyari* (89.7%) and *Mansonia titillans* (6.2%). A total of 3993 female *Mansonia*, of which 78.3% were *Mansonia dyari* and 21.7% were *Mansonia titillans*, were caught in the bait traps (Lounibos & Escher, 1985). The differences in the proportion caught as emerging adults and as blood-seeking females may indicate that *Mansonia titillans* is more strongly attracted than *Mansonia dyari* to chickens, that the former species entered the traps more readily than *Mansonia dyari*, or that the bait traps were sampling over a larger area than the drainage canal.

Emord & Morris (1982) reported that they often observed female *Culiseta melanura* resting on the funnel entrances of lard-can traps, but not entering them, while Mitchell & Millian (1981) consistently reported the entry of blood-fed mosquitoes into their chicken-baited traps.

Emord & Morris trap

Animal-baited lard-can traps have not always attracted many mosquitoes (Emord & Morris, 1982; Main *et al.*, 1966; Stamm *et al.*, 1962), and because of this Emord & Morris (1982) designed an alternative small animal-baited trap, based on the CDC light-trap. They took a standard CDC trap and removed the light source and made the following modifications. A semi-cylindrical cage (6 × 4.5 × 4 in) made from 0.5-in mesh wire screening wrapped round a plywood D-shaped base was attached by a metal hook to a 8 × 0.25-in threaded metal rod fixed to one side of the CDC trap. The hinged top of the bait cage was also made from wire screening. A 1.5-in thick layer of household sponge was placed on the floor of the bait cage to protect the bait (sparrow) from the cold, and also to facilitate cleaning the cage. (Clearly other bait animals could be put in the cage). Traps operated from four 1.5-V torch batteries fixed to the CDC trap (Fig. 5.20a). The collecting container consisted of a plastic 1-quart frozen food storage container with a section from the bottom and three sections from the side removed and replaced by plastic mosquito screening. These screenings, and a cloth sleeve fixing the container to the trap, were embedded into the plastic with a soldering iron.

The trap was evaluated against a sparrow-baited lard-can trap and a CDC light-trap with 3–4 lb dry ice suspended nearby in a cloth bag. Significantly more *Culiseta melanura*, *Culiseta morsitans* and *Culex pipiens/restuans* group were collected in the bird-baited CDC trap, whereas more mammalian-feeding *Aedes vexans* and *Anopheles* spp. and *Coquillettidia perturbans* were caught in the CO₂-CDC trap; the lard-can trap performed the worst. Emord & Morris (1982) concluded that the greater exposure of bait in their trap over the lard-can trap, and the difficulty some species have in entering the latter, made their trap more effective in collecting several species.

Several others have found the Emord & Morris trap effective. For example, Howard *et al.* (1983) in studies on the vectors of eastern equine encephalomyelitis baited these traps with house sparrows, and placed them in 12 different types of habitats, at ground level, at heights of 5 and 10 m and in the tree canopy. From a total of 641 trap-nights spread over 32 nights, 15077 female mosquitoes belonging to at least 15 species were caught, the most common of which were *Culiseta melanura* (32.0%), *Coquillettidia perturbans* (18.9%), *Culex pipiens/restuans* group (12.8%), *Aedes canadensis* (11.1%) and *Culiseta morsitans* (10.8%).

In studying the feeding patterns of Swedish mosquitoes Jaenson (1985) and Jaenson & Niklasson (1986) used Emord & Morris (1982) traps placed 1 m above ground, and the net traps of Jupp & McIntosh (1967) which were made of white netting measuring 1.5 m³, and having the sides rolled up to provide a 20-cm opening all round. Both sets of traps were baited with a rabbit, guinea pig, hen or a dove, and in addition there were unbaited traps. The traps were operated for three 24-hr periods each week for 5 months. At least 17 species belonging to *Anopheles*, *Culex*, *Culiseta* and *Coquillettidia* were caught; the most common species were *Aedes communis*, *Aedes excrucians* s.l., *Aedes diantaeus*, *Aedes intrudens*, *Aedes cinereus*, *Aedes cantans* and *Culex pipiens*. The Emord & Morris traps proved to be more efficient than the net traps.

The percentage of engorged mosquitoes in the net traps (13.5%) was on average nine times greater than engorged females collected from the suction baited traps (1.5%). Surprisingly there was considerable movement of mosquitoes between traps. For example, of the 17 blood-meals identified serologically as

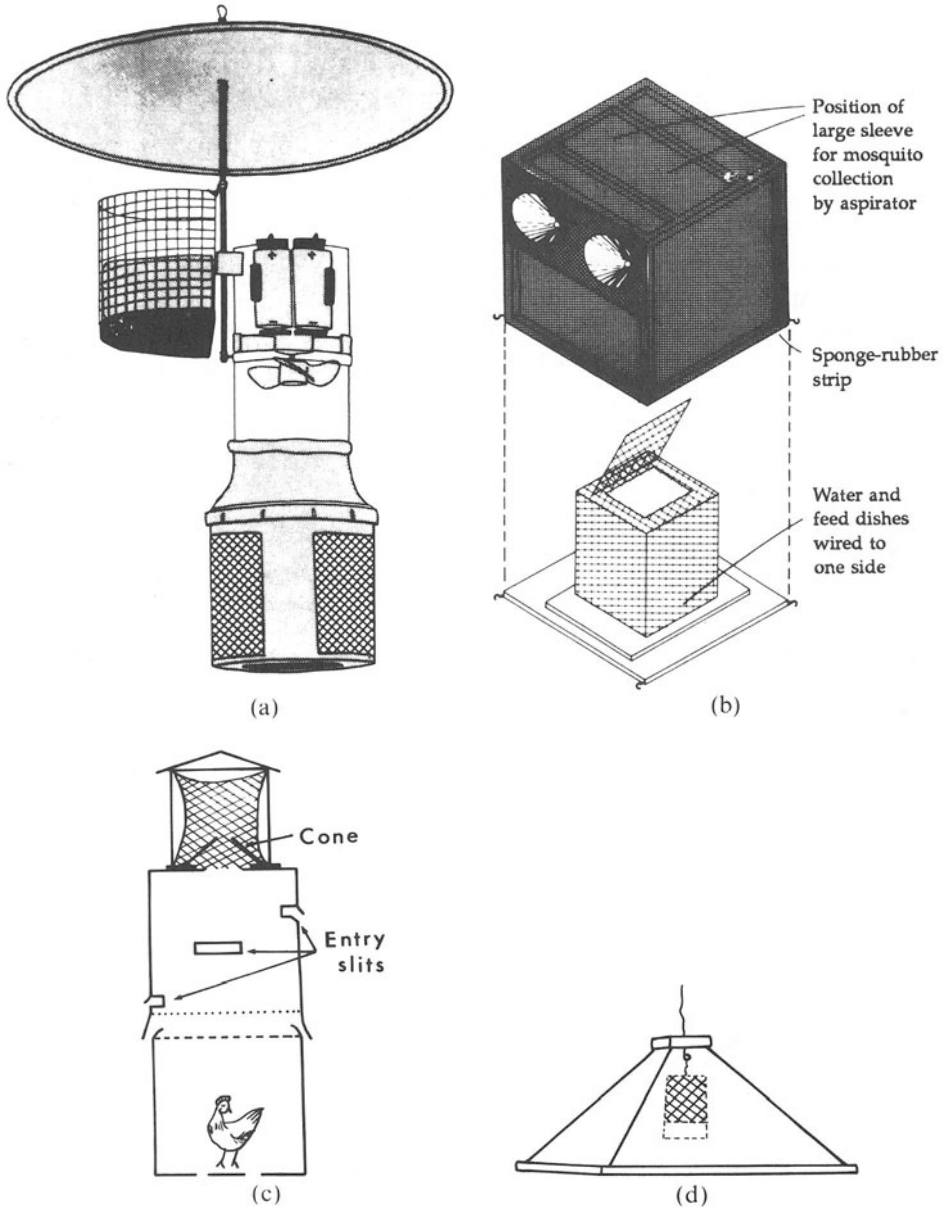


FIG. 5.20. (a) Host-baited CDC trap (Emord & Morris, 1982); (b) duck-baited trap (Meyer & Bennett, 1976); (c) bantam trap (Pillai & MacNamara, 1968); (d) sentinel trap with aluminium hood.

guinea pig blood, only five were collected from guinea pig traps, furthermore only 8% of blood-fed *Aedes* tested from the bird-baited net trap contained avian blood possibly indicating that birds are difficult to feed upon, and from 23 blood-fed *Aedes* mosquitoes collected from rabbit-baited Emord & Morris traps only five had fed on rabbits. Some *Aedes* caught in the traps had fed on cervids and/or cattle. Another problem in interpreting the results was that relatively large numbers of mosquitoes were caught in unbaited traps. For example, large numbers of male *Aedes diantaeus* and small numbers of male *Aedes communis* and *Aedes intrudens* were caught in the Emord & Morris trap; males were also collected in the baited net trap of Jupp & McIntosh but generally in smaller numbers.

The Emord & Morris traps caught considerable numbers of *Culex pipiens* and *Culiseta morsitans* confirming the view of Emord & Morris (1982) that these traps are efficient in catching these species. In contrast the net traps caught many fewer *Culex* and *Culiseta* mosquitoes.

Using rather similar traps, that is modified CDC light-traps normally baited with carbon dioxide (Pfundner, 1979), but baited with a white mouse, dry ice or with both attractants, Landry & DeFoliart (1986) working in Iowa found that location of the traps was very important in determining the numbers of *Aedes triseriatus* caught.

Howard *et al.* (1989) successfully used a chicken-baited Emord & Morris trap to catch *Culiseta melanura* and *Culiseta morsitans*.

Meyer & Bennett trap

This is a duck-baited trap, but can be adapted to hold other animals. The bait cage consists of a 30 × 30 and 35-cm high chicken wire cage nailed to a wooden board (35 × 45 cm), there is no wire bottom. A hole is cut from the top to allow the bait to be introduced, after which it is covered with a piece of chicken wire netting. Food and water dishes are wired to one of the sides of the cage. The mosquito collecting cage is a 60-cm cube wooden frame covered with fine plastic mosquito netting on five sides, the bottom being left open (Fig. 5.20*b*). Four polythene 25-cm diameter funnels with their stems cut off to leave about 5-cm diameter holes are glued to fit into four 25-cm holes cut from two panels (30 × 60 cm) of plywood fixed at the top of the cage and on two opposite sides. Finally, a mosquito netting sleeve is sewn into the top of the cage for removal, with an aspirator, of the mosquitoes.

In Canada during 15 weeks when traps were exposed from about 1800–0900 or 1100 hr on 37 trap-nights they caught 7235 mosquitoes belonging to at least 13 species. The mean catch per night was 188 mosquitoes (range 1–1445). The most common species were *Coquillettidia perturbans* (6333), *Anopheles walkeri* (330), *Culiseta morsitans* (257) and *Aedes cantator* (132). Species not found in larval surveys nor in human bait catches were also caught. About 30% of the mosquitoes had engorged on the duck, the only species not feeding on the bird was *Culex territans* (Meyer & Bennett, 1976).

In their studies in the USA on the vector of *Plasmodium elongatum* Beier & Trpis (1981) used a slightly enlarged version of this trap to accommodate a penguin. From 63 trap-nights in 1978 they caught 739 female mosquitoes, while

in 1979 they caught 455 mosquitoes from 69 trap-nights, all but two were *Culex pipiens/restuans*.

Silon trap

This is based on the ramp trap (Gillies, 1969). It consists of a 150 × 60-cm pyramidal part composed of white mosquito netting which narrows to a slit (30 × 4 cm) opening into a rectangular (30 × 60 cm) mosquito netting collecting chamber (Labuda & Nosek, 1978). In practice two such traps were fitted one above the other and were supported by two vertical iron rods, while two shorter rods held up the collecting chamber. Several such traps formed a circle around a bait animal (ram or ducks), and over five catch-days 3313 mosquitoes were caught, the prevalent species being *Aedes vexans*, *Aedes sticticus* and *Aedes cantans*. In later studies Labuda and Kožuch (1980) found that more *Aedes vexans* and *Aedes rossicus* were caught in bird-baited traps placed at 0–60 cm, than in those at 60–120 cm, but about equal numbers of *Aedes sticticus* were caught in traps at these two heights.

Bantam fowl trap (Pillai & MacNamara)

The need for a small, portable avian trap in which the catch of mosquitoes is kept separate from the bait animal led to the development in New Zealand of the bantam trap (Pillai & MacNamara, 1968). A large oil drum (e.g. about 12-gal capacity) is cut horizontally into two halves (Fig. 5.20c). The lower half forms the bait chamber and its top cut edge is bent inwards slightly so that the upper half rests neatly over it. Chicken wire mounted in a 4-mm thick wire frame is fixed 1 in below the cut upper edge of the drum by three equally spaced screws. A few ¼-in holes are drilled into the floor of the drum to serve as drainage holes and prevent fouling of the drum during use. A 6-in diameter hole is cut from the centre of the top of the upper half of the cut drum, and two 3/16-in bolts are welded by their heads, one either side of this hole some 11 in apart. Two ⅛-in thick brass plates, 2½ × 1 in, are drilled at one end and slipped over the two welded bolts. A wing-nut screws each plate down and holds in position a plexiglas cone mounted on a square plastic base plate. An 8-in cube mosquito netting collecting cage suspended over a wire framework is positioned over the cone. The diameter of the smaller aperture of the cone is not given, but appears to be about 2 in. Mosquitoes that pass through the cone into the collecting cage are retrieved by aspirators inserted through two 5-in long, 3-in diameter cloth sleeves. An ⅛-in thick 12 × 12-in sheet of plastic is clipped on the top of the wire cage to give some protection against rain.

Three 6-in long, 1-in wide entrance slits for mosquitoes are cut into the top drum, one about 2 in from the top, another on the opposite side about 2 in from the lower cut edge and the third about equidistant from these and midway up the drum. Fine wire mesh is soldered over the bottom of the top drum to prevent captured mosquitoes descending into the bait chamber. The bait is placed with food and water, and usually a small amount of straw and leaves, in the bait chamber. The upper chamber is placed on top and the collecting cage and plastic cone are held in position by the two brass plates and wing nuts. The upper and

lower halves of the drum are more firmly held together if a rope is passed through three metal rings fixed to the top of the upper half and to the bottom of the lower half of the drum. The trap, together with a bantam, weighs less than 15 kg, is less than 36 in high and can be raised from the ground by pulleys to sample mosquito populations at different heights.

During three year's trapping in New Zealand the trap caught large numbers of *Culiseta tonnoiri* and *Culex pervigilans*; in fact as many as 1326 *Culiseta tonnoiri* were collected in one night from a single trap. A nightly catch of 500–1000 mosquitoes per trap is not unusual during the peak mosquito season (Pillai & MacNamara, 1968). Although intended as a bantam-baited trap, a variety of other vertebrates could be exposed in the trap.

Sentinel animals

Smithburn *et al.* (1949) introduced the method of exposing immigrant animals, such as monkeys, into an area and regularly bleeding them to detect the presence of circulating arboviruses. Rudnick (1986), for example used sentinel monkeys in modified Magoon traps during dengue studies in Malaysia. This procedure is now widely adopted in arbovirus studies, and in addition to monkeys, various rodents, marsupials, deer, baby chicks and mice are used as sentinels (Andre *et al.*, 1985; Artsob *et al.*, 1983; Crans, 1986; LeDuc, 1978; Reisen *et al.*, 1990; Vigliano & Carlson, 1986), and in India Mani *et al.* (1991) used donkeys as sentinels for Japanese encephalitis and West Nile viruses.

Apart from bleeding the sentinels, the mosquitoes attracted to them are frequently caught and tested for arboviruses.

A common procedure involves placing sentinel mice, usually consisting of a mother and a number (six) of new-born infants, in a 10 × 10 × 12-cm wire test tube basket which has the top covered with wire mesh and the bottom and lower quarter of the sides with aluminium sheeting (Causey *et al.*, 1961). Wood shavings are provided for bedding, and dry pelleted food and a water bottle are added. The basket containing the mice is suspended by a wire hook underneath a 56-cm square aluminium hood which tapers to a 10-cm square top, and is 38 cm deep (Fig. 5.20*d*). To prevent ants entering the trap the wire that suspends the hood from a tree branch should be coated with grease, oil or a permanently sticky adhesive. Some of the mosquitoes attracted to the sentinel mice rest on the basket or underside of the aluminium roof and can be periodically removed with aspirators. Alternatively a single collection can be made in the mornings by carefully fitting a screen over the bottom edge of the hood, and removing the catch by inserting an aspirator through a cloth sleeve in the middle of the screen. Obviously only a fraction of the mosquitoes attracted during the exposure period are caught in the morning.

Sentinel chicken shed

Domestic chickens are susceptible to various viral encephalides, such as St. Louis encephalitis, western equine encephalomyelitis and eastern equine encephalomyelitis, and show good antibody responses to all three. They are suitable as sentinels because of their widespread distribution, and because haemagglutination-inhibition, fluorescent antibody complement fixation and ELISA techniques can be used for

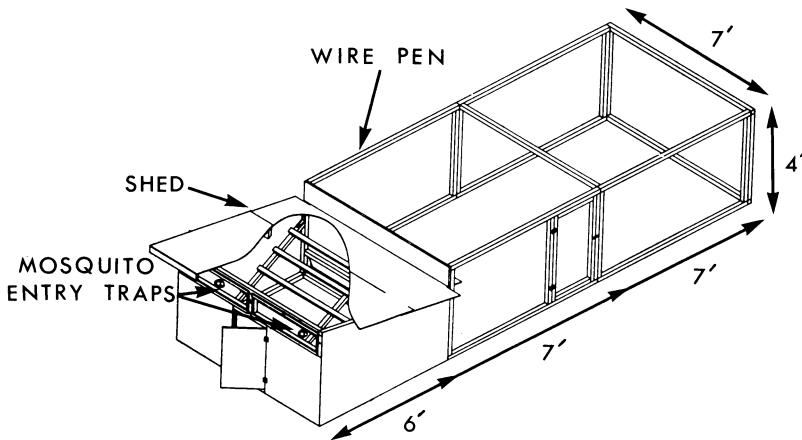


FIG. 5.21. Sentinel chicken shed and pen (Rainey *et al.*, 1962).

determining their antibodies (see Monath, 1988). Viral activity can be studied by making antibody surveys of farmyard chickens, but it is usually better if variables such as flock size and types of poultry shelters are eliminated. Furthermore, transmission indices may be required in areas where there are no farm flocks. For these reasons the sentinel chicken shed was developed in Colorado by Rainey *et al.* (1962). The complete trap consists of three basic parts, a shed, two removable mosquito traps and a chicken wire pen (Fig. 5.21). A 20-in wide, 33-in high doorway is made in front of the shed and a 10-in high door, that is normally raised to allow free entry of chickens into the shed from the pen, is hinged along its upper edge and extends across the entire back of the shed. A screened opening, 47-in wide and 24½-in high, is fixed above the hinged door to increase ventilation through the shed, which has been shown to increase the catch of *Culex tarsalis* fivefold. It also results in large catches of other *Culex* species, *Mansonia* and *Culiseta* mosquitoes, but there is no such marked increase in *Aedes* species.

Two removable mosquito entry traps are fitted to the top of the front wall of the shed and incorporate Bates'-type baffles (Fig. 5.13*b*). The pen, which is pushed up against the back of the shed, is 14 ft long, 7 ft wide and 4 ft high and is made from chicken wire fixed to a wooden framework. To prevent predators entering the pen the floor can also be covered with chicken wire. A 23 × 43-in doorway is placed in one side of the pen to provide access. If the shed and pen are made in prefabricated panels, two men can apparently assemble, or dismantle, a trap in about an hour (Rainey *et al.*, 1962).

Hayes *et al.* (1967) found these traps useful in studying the ecology of arboviruses in Texas, while Shemanchuk (1969) in Canada used similar traps, but modified to include an entry trap to catch mosquitoes before they could feed on the birds, and an exit trap to collect those leaving the trap. In Malaysia a sentinel chicken shed trap collected a mean of 12.02 *Culex vishnui* and smaller numbers of *Culex quinquefasciatus* (6.15) and other *Culex* species per day (Wallace *et al.*, 1977).

Anthrophagism and zoophagism

It is often wondered whether in a species having adults that feed on more than one kind of host, for example humans and cattle, there is any inherited trend involved in selecting the host, or whether the host fed upon is largely a random event, determined mainly by host numbers and availability. To try and determine this Rawlings & Curtis (1982) studied the feeding behaviour of *Anopheles culicifacies* species B in Sri Lanka by a series of bait catches conducted from 1830–2100 hr on a cow in a hut, and on five men sitting in an adjacent house. Mosquitoes caught biting the cow were marked with magenta fluorescent dusts while those caught in human bait catches were dusted yellow: all mosquitoes were released outside the huts at the end of the catch period. On six subsequent evenings mosquitoes caught on either baits were checked for markings, and unmarked mosquitoes marked with the appropriate colour, after which all were released. On the final seventh evening the mosquitoes were collected and killed. A total of 1150 and 188 mosquitoes were caught biting the cow and men, respectively. Recaptures of marked adults were small. Only two originally biting a cow were caught at human bait, and just four firstly caught on men were later caught biting the cow. It was tentatively concluded that there were no distinct anthrophagic and zoophagic populations of *Anopheles culicifacies* species B, at least in their area.

Similarly in Malaysia using the same methods Loong *et al.* (1990) found there were no separate populations of *Anopheles maculatus* feeding on cattle and people.

In Sabah Hii (1985) and Hii & Vun (1987) also used mark–recapture methods to study the feeding preferences and behaviour of *Anopheles balabacensis* and *Anopheles donaldi* on buffaloes and people. Mosquitoes feeding on four men were caught, dusted with blue powder and released, while those feeding on a buffalo some 33 m away were marked green and then released (Fig. 5.22). On subsequent nights when returning to feed mosquitoes were recaptured and treated as previously. In summary it was found that adults of both species tended to return to the same types of host, that is contrary to the findings in Sri Lanka and Malaysia there appeared to be two behaviourally distinct populations, one preferring bovids the other people. Similarly in Thailand Nutsathapana *et al.* (1986) found that there was a statistically significant tendency for adult *Anopheles minimus* to return to the hosts on which they were first caught, thus showing host-preference heterogeneity in the population.

IDENTIFICATION OF BLOOD-MEALS

Formerly mosquito blood-meals were identified mostly by the interfacial precipitin (ring) test, and it is sometimes still used (Anderson & Gallaway, 1988; Chandler *et al.*, 1975a; Irby & Apperson, 1988; Nasci & Edman, 1981a; Ritchie & Rowley, 1981; Snow & Boreham, 1978), but a variety of other techniques have been employed, including occasionally complement fixation, latex agglutination, and especially in China (Huang & Luo, 1986; Shihai & Jun, 1989; Wang, 1986) cellulose acetate or agar gel counter immunoelectrophoresis. Useful reviews of

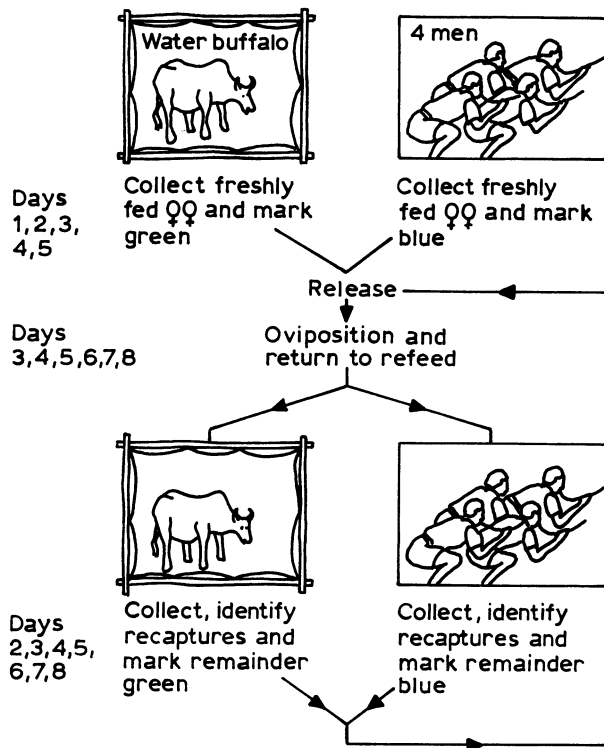


Fig. 5.22. Flow diagram for a mark-release-recapture experiment to determine host choice between man and buffalo (Hii & Vun, 1987).

available methods are given by Boreham (1975), Tempelis (1975), Washino & Tempelis (1983), and more recently by Pant *et al.* (1987) and WHO (1987).

Collins *et al.* (1986) described a modified gel diffusion method for identifying mosquito blood-meals in which 16 blood-meals on a microscope slide (7.6 cm) can be tested simultaneously against two hosts (e.g. cow and man), and using a 10-cm long slide up to 25 blood-meals can be tested. This method has been found useful in India for identifying blood-meals (Anon, 1988, 1989).

An unusual method of identifying the source of blood-meals is to mix the engorged mosquito stomach with an approximate volume of 0.1 saturated solution of ammonium oxalate and let the mixture dry on a microscope slide. The shape of the haemoglobin crystals which form are compared with crystal shapes from blood of various animals (Washino & Else, 1972). Although this is a simple and inexpensive technique it has been little used.

Increasing use is now being made of the enzyme-linked-immunosorbent assay (ELISA) techniques, either direct (Beier *et al.*, 1988) or indirect (Burkot *et al.*, 1981, 1988; Service *et al.*, 1986). Beier *et al.* (1988) developed a two-step method using antihuman peroxidase conjugate and antibovine phosphate conjugate that allowed a test on a single mosquito for two hosts using the same microtitre plate

well. Finally, a system was developed to allow a single mosquito to be tested by the ELISA method for the presence of malarial sporozoites and for identification of its blood-meal. Beier *et al.* (1988) argued that because host-specific antisera are not required the direct method is easier than indirect methods (e.g. Service *et al.*, 1986). However, I believe that the indirect method is much cleaner, and moreover it should not require any blocking agents such as milk powder, gelatin, BSA or casein.

Irby & Apperson (1989) studied the rate of blood digestion in *Aedes aegypti* by immunoblot analysis using polyclonal antisera to follow the degradation of various serum proteins. Their findings are pertinent to immunological methods used to identify blood-meals.

I consider one of the best techniques is the sandwich (indirect) ELISA (Service *et al.*, 1986) which can be used without a plate reader. Blood-engorged mosquitoes can be smeared on to filter paper and stored for months or even years over a desiccant (e.g. phosphorous pentoxide, silica gel) or kept in a refrigerator or deep-freeze. Alternatively blood-fed mosquitoes can be stored in gelatin capsules (Tempelis & Lofy, 1963), although this is not necessary in this instance because contrary to the findings of Eligh (1952) and Roy & Sharma (1987) no proteins appeared to be lost when blood-engorged mosquito abdomens were squashed on to filter paper (Service *et al.*, 1986). The sandwich technique is sufficiently sensitive to identify blood in half-gravid mosquitoes, and also in most three-quarter gravids. A dipstick method could have certain advantages (reduced costs, increased speed) over an ELISA method employing microtitre plates, especially if a single nitrocellulose filter paper strip (the stick) has sections coated with different host antibodies that can be dipped into an eluted blood smear to test against several hosts simultaneously. At the moment, however, although microdot and dipstick methods have been evaluated (Abdulaziz & Pal, 1989; Lombardi & Esposito, 1986; Roy & Sharma, 1987), a system as efficient and reliable as a good ELISA microtitre plate method has not been found. However, recently Hunter & Bayly (1991) working with simuliids described a very interesting modified ELISA test using a biotinylated second antibody and a streptavidin-biotinylated peroxidase complex. Using this approach sensitivity was considerably increased. The method deserves evaluation in situations where the blood contents in mosquitoes is small.

Heller & Adler (1980) used the pyridine haemochromogen test on the Turin Shroud to detect the presence of dried blood, and Boorman (1986) adapted this method to detect blood in Ceratopogonidae preserved in either alcohol or formalin. Basically insects, or just their guts, are ground up with a single drop of 99.5% hydrazine in the shallow wells of a porcelain plate, after about 5 min a single drop of formic acid is added. This results in a puff of white 'smoke'. When the reaction has subsided two more drops of acid are added and the plate examined under ultraviolet light. If blood is present there is a bright pinkish-red fluorescence, the intensity of which increases after 10–30 s irradiation. Harrington (1990) adapted this test for detecting the presence of blood in old museum Hemiptera.

Day *et al.* (1982) pointed out that although mosquitoes may feed differentially on nestling and adult birds (Blackmore & Dow, 1958), it is not known whether

mosquitoes penetrate burrows and nests and feed on suckling mammals. Specific steroid-binding proteins (SBP) such as alpha-fetoprotein (AFP) are found in the serum of newborn and juvenile mammals of many species and serve to bind maternal steroid hormones such as oestrogen. Laboratory experiments demonstrated that radiolabelled estradiol (a steroid) could be detected in mosquito blood-meals up to 18 hr post-feeding. Day *et al.* (1982) suggested that this method may be able to identify feeding on suckling mammals, but the limitations of the method are stressed.

Simple agglutination tests can be performed on blood-fed mosquitoes up to 20 hr after feeding to identify whether they had fed on humans having A, B or O blood-groups (Bryan & Smalley, 1978). Boreham *et al.* (1978) used gradient-gel electrophoresis to identify different haptoglobin types in studies on host preferences between humans sleeping in the same houses. Identification of actual hosts can be useful in behavioural studies (see pp. 391–3). Although feeds on individuals can be identified by these methods the number of genotypes for these loci are few, and this makes the method of limited practical use. However, Coulson *et al.* (1990) have recently demonstrated that it should be possible to use DNA fingerprinting on mosquito blood-meals up to 10 hr post-feeding to identify blood from individuals. The method has, however, not yet been tested in the field.

The sensitivity and reliability of many immunological techniques depend on the specificity of high titre antisera produced by inoculation with sera of potential hosts. Numerous regimes are favoured for producing sensitive and specific antisera. Gill (1984) reports on an immunisation schedule of injecting 1 ml Freund's complete adjuvant plus 1 ml 2% Tween 80 in saline into or near the axial or inguinal lymph nodes of rabbits, followed a week later by a similar injection but this time incorporating the antigen (serum), and then a final similar inoculation given a week later.

Rubidium chloride has been used as a marker in studies on several herbivorous insects (Fleischer *et al.*, 1986; Pearson *et al.*, 1989; Stimmann, 1974; van Steenwyck *et al.*, 1978, 1979) and caesium has also occasionally been used (Moss & van Steenwyck, 1982). Kimsey & Kimsey (1984) were the first to mark mosquitoes with rubidium to detect arthropod blood-meals. They injected mice, chickens and lizards intraperitoneally with rubidium chloride, and found that a dose of Rb^+ of 500 mg/kg had no adverse effects on the hosts, nor the mosquitoes feeding on them. Blood-engorged mosquitoes were prepared by an acid digestion method involving the wet ashing technique of Smith (1953), except that there was no need to add a vanadium catalyst, and also that if mosquito samples were left for 3 days they could be digested without heat. Rubidium was detected by flame spectrophotometry using an atomic emission mode at 779.6 nm. All *Culex tarsalis* fed on rubidium-marked quail remained detectable for up to 6–7 days after they had fed. If atomic absorption machines are available (e.g. in medical and research laboratories) then the method is relatively cheap, and analytical procedures are simple and safe. No special preservation methods are necessary to keep blood-fed mosquitoes prior to testing and their shelf-life is indefinite. The authors point out that the technique is not a replacement for serological detection of hosts, but can be used as an adjunct, and where for

some reason other tests are not practical. For example, Anderson *et al.* (1990) injected chickens with rubidium and caesium in order to study multiple feeding by natural populations of *Culiseta melanura*. These two metals are easily distinguished by their emission wavelengths, and can be detected in mosquitoes for up to 3 days, but for this the dosage of caesium has to be higher than that of rubidium. The authors stress the value of the method in quantifying multiple feeding on hosts that are serologically indistinguishable.

Boreham (1976) pointed out the possibilities of introducing pathogens with blood-meals from insects squashed and dried on filter paper. He proposed a variety of methods to deal with this, such as 1-hr immersion of the papers in diethyl ether, heat of 60°C for 1 hr, or exposure to UV light. However, these treatments may be ineffective against pathogens in whole insects sent for blood-meal identification. There would be no danger of infection with HIV if blood smears, or blood-engorged mosquitoes, have been dried for a day or more, because the virus is not viable in a dry state.

Interrupted feeding

Klowden & Lea (1978) cited several studies that indicated mosquitoes may take multiple blood-meals during a single gonotrophic cycle, while Magnarelli (1977) showed that 4.9% of mosquitoes caught at human bait in Connecticut already contained small amounts of blood. Cupp & Stokes (1976) found that 12.5% of blood-meals identified from *Culex salinarius* were from a mixture of hosts. In Colorado Mitchell & Millian (1981) found that 1.5% of *Culex tarsalis* caught in animal-baited lard-can traps already had some blood.

The proportion of blood-meals that are taken from more than one host depends on the probability of two or more hosts being selected by a hungry mosquito and the probability of the blood-meal being interrupted. Burkot *et al.* (1988) studied mixed feeding by species in the *Anopheles punctulatus* complex in Papua New Guinea. They elaborated on the model proposed by Boreham & Garrett-Jones (1973) for estimating the proportion of cryptic mixed blood-meals. The proportion of cryptic mixed blood-meals can be derived from the proportion of unmixed and patent mixed meals and the probability of feeding on these hosts (Boreham & Lenahan, 1976; Boreham *et al.*, 1978, 1979; Bryan & Smalley, 1978; Port *et al.*, 1980). It can also be measured more directly by ABO blood groups or by serum protein haptoglobins, because from this the probability of a meal on a host species being interrupted can be estimated (Boreham & Lenahan, 1976; Boreham *et al.*, 1978, 1979; Bryan & Smalley, 1978). For simplicity Burkot *et al.* (1988) considered just a two-host situation, a human and a non-human host. If it is assumed that blood-feeding is interrupted just once, then the proportion of blood-meals that are patent mixed will be

$$Q(1 - Q)(I_H + I_N) \quad (1)$$

in which Q = probability of humans being the host; I_H = probability of a human feed being interrupted; I_N = probability of a non-human feed being interrupted. The proportion of patent mixed feeds increases as the probability of interruption increases. However, the greatest proportion of patent mixed feeds will be when

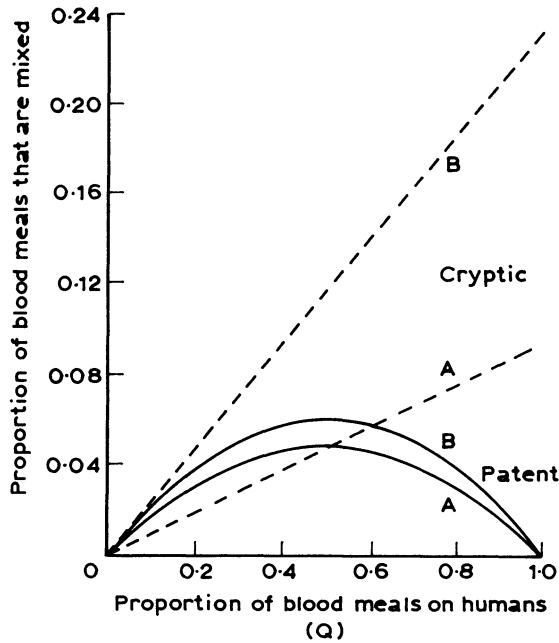


FIG. 5.23. Influence of interrupted feeding on the proportion of patent- and cryptic-mixed blood-meals. Solid lines show proportion of all blood-meals that are patent-mixed meals; dashed lines show the proportion of human blood-meals that are cryptic-mixed meals (Burkot et al., 1988).

there is an equal likelihood of the mosquito selecting a human or non-human host (i.e. when $Q = 0.5$) (Fig. 5.23).

The proportion of all blood-meals that are cryptic mixed on humans will be $Q^2 I_H$, and dividing by Q gives the proportion of human feeds that are cryptic mixed ($Q I_H$). That is the proportion of cryptic mixed feeds on humans increases linearly with Q for a given I_H (Fig. 5.23). Now, although the proportion of both cryptic and patent mixed meals increases as the probability of interruption increases, the overall proportion of meals taken on humans remains unchanged, although the proportion of mixed meals increases. What actually changes is the absolute number of blood-meals taken. Consequently if none of the feeds is interrupted then the HBI is a direct estimate of Q . If, however, interrupted feeds occur on both human and non-human hosts, then the HBI (as usually measured as the total numbers of meals positive for human blood) will overestimate Q . This estimate, however, can be corrected as follows:

$Q =$ proportion of feeds with only human blood $+ [I_H / (I_H + I_N)] \times$ total proportion of patent mixed human feeds.

So, if $I_H = I_N$ then Q is obtained by adding half of the proportion of patent mixed human feeds to the proportion of only human feeds.

Now, if the proportion of mixed feeds is measured, then the probability of a feed being interrupted can be estimated. But we must know either I_H or I_N to

estimate the other, or else assume that the two are equal (as Burkot *et al.*, 1988 did in their study) and so obtain a common estimate that is applicable to both. If we assume the latter; then from eqn (1) we have

$$I_H = I_N = \text{proportion of patent mixed blood-meals}/2Q(1 - Q).$$

FORAGE RATIOS AND FEEDING INDEX

In most studies on host preferences, as determined by identification of mosquito blood-meals, there is little or no information on the numbers of available hosts present in an area. To try to overcome this Hess *et al.* (1968) introduced the 'forage ratio' into mosquito studies. This is the percentage of engorged mosquitoes that have fed on a host of a particular species divided by the percentage this host species comprises of the total population of available host species in the area. Thus a ratio more than 1 should indicate preferential feeding on that host, whereas values less than 1 indicate avoidance of the host. A value of 1 or near 1, is said to represent more or less opportunistic feeding. This approach was used by Hayes *et al.* (1973) to investigate possible seasonal shifts in feeding patterns in Texas. In studying host preferences of *Psorophora columbiae* and *Anopheles crucians* Kuntz *et al.* (1982) carried out a census of the proportions of available hosts in a rice field area in Texas, and applied the forage ratio technique to results of precipitin tests performed on wild-caught adults. In Egypt Beier *et al.* (1987), Kenawy *et al.* (1990) and Zimmerman *et al.* (1988) used the forage ratio to study feeding preferences of various mosquitoes, including sometimes those caught as blood-feds in light-traps.

There are, however, problems with this approach. For instance the difficulty, or more usually near impossibility, of enumerating the numbers of possible hosts in an area, and the failure to take into consideration their ecology and availability to mosquitoes. Edman (1971) pointed out these as well as other deficiencies in trying to use the forage ratio. Attempting to overcome these difficulties Kay *et al.* (1979b) proposed the 'Feeding Index' (*FI*). This is defined as the proportion of feeds on one host with respect to another divided by the expected proportion of feeds on these two hosts based on factors affecting feeding. These factors include host abundance and size, their temporal and spatial concurrence with the mosquito species, and the mosquitoes' feeding success. Thus

$$FI = \frac{Ne/Ne^1}{Ef/Ef^1}$$

where Ne = numbers of feeds identified from host 1; Ne^1 = number of identified feeds from host 2; Ef = expected proportion of feeds on host 1; and Ef^1 = expected proportion of feeds on host 2. An index of 1 indicates equal feeding on both host species being compared, while smaller or larger values indicate a decrease or increase of feeds on host 1 compared to host 2.

As an example, Kay *et al.* (1979b) present data on *Culex annulirostris* feeding on dogs and fowl in an Australian village. The estimated dog population was 100 and the fowl population 80, thus the expected ratio of feeds based just on

their abundance would be 1:0.8, or 1.25. The actual analysis of blood-meals showed 35 dog feeds and 8 fowl feeds, that is observed feeding (Ne/Ne^1) is 35/8 = 4.38. Now, ignoring all other environmental factors the feeding index is calculated as 4.38/1.25 = 3.50, i.e. greater feeding on dogs than fowl. However, ecological and behavioural studies were able to measure the concurrence of these two hosts and *Culex annulirostris*. During the feeding times of the mosquito the proportions of dogs outdoors and indoors were 0.9 and 0.1, the proportions for fowls were 1.0 and 0; and finally for *Culex annulirostris* 0.92 of the population were feeding outdoors and 0.08 feeding indoors. Thus the concurrence for dogs to fowls is

$$\frac{(0.9 \times 0.92) + (0.1 \times 0.08)}{(1.0 \times 0.92)} = 0.91$$

showing that *Culex annulirostris* is more likely to encounter fowl than dogs. In animal-baited stable-trap experiments 96% of *Culex annulirostris* fed on dogs and 83% on fowl. So there is the following adjustment to be made to account for feeding success, 96/83 = 1.16 for dogs relative to fowl. Finally, the fact that a dog is about five times the weight of a fowl is taken into consideration. So, the expected comparative feeding rates for dogs with respect to fowl would be

$$1.25 \times 0.91 \times 1.16 \times 5.0 = 6.59$$

So the true feeding index (FI) = 4.38/6.59 = 0.66. A very different value from the crude feeding index of 3.5 obtained when environmental factors were ignored. Such calculations require much information on host availability, host size, and concurrence as well as the feeding success of mosquitoes on different hosts, and moreover the estimation of these parameters will likely be inaccurate. These difficulties were recognised by Kay *et al.* (1979b), who admitted that factors determining host selection were so complex that perhaps any such above analysis was of limited value. However, they believed that they had provided a framework on which a better understanding of mosquito host-feeding patterns might be built, and Kay *et al.* (1985) used the feeding index in later studies on *Culex annulirostris* and other species. Few other people, however, have tried to use this index although Burkot *et al.* (1988) used it to study feeding preferences of the *Anopheles punctulatus* complex in Papua New Guinea, and in India it was applied in studies on the feeding habits of *Culex tritaeniorhynchus*, *Culex pseudo-vishnui* and *Culex vishnui* (Anon., ? 1989).

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