fatigans biting out of doors near midnight than elsewhere.

Precipitin tests made by the Lister Institute, Elstree, England, had shown that man and birds were the commonest sources of blood for *C. p. fatigans*. Therefore, during a survey of the microfilaria index in blood-fed mosquiros, we determined at the same time whether mammalian or avian blood was present in their stomachs by observing the morphology of the erythrocytes. Preliminary experiments with *Aedes aegypti* in the laboratory had shown that mammalian and avian blood or a mixture of the two could be readily identified for up to about 26 hours after the feed (Fig. 1 and 2).

The survey, which involved the examination of 1558 blood-fed mosquitos, showed that, of those caught indoors, 93.3% had fed on mammalian and 6.7% on avian blood or on avian and mammalian blood, whereas for mosquitos caught out of doors the figures were 89.5% and 10.5%, respectively. The differences are not significant at the 95% probability level.

The only mammal, other than man, on which *C.p. fatigans* is known to feed in Rangoon is the dog. However, precipitin tests had shown that the proportion feeding on the dog does not exceed about 10%, so it is reasonable to conclude that about 80% of *C.p. fatigans* in Rangoon, whether caught indoors or out of doors, feed habitually on man.

Further evidence in favour of the contention that *C.p. fatigans* feeds as much on man out of doors as indoors is provided by data on the microfilaria rate in the two sections of the mosquito population. Examination of 1987 inside-resting and 664 outside-resting mosquitos indicated microfilaria rates of 4.7% and 3.3%, respectively. These two rates are not significantly different at the 95% probability level.

Confirmatory evidence was obtained from a calculation made by Dr S. Hayashi, FRU Epidemiologist. From the infective rate in the mosquito and hence the probability that a mosquito will become infective after one blood-meal (p), the microfilaria rate among the inhabitants (m) and the susceptibility of *C.p. fatigans* to infection (s), the probability that a mosquito will select man as a source of blood (r) is given by

$$r = \frac{p}{ms}.$$

By substituting figures already determined by us, we arrive at:

$$r = 0.035/(0.049 \times 0.83) = 0.861.$$

In other words, between 80% and 90% of *C.p. fatigans* must feed on man in Rangoon if the other requirements of the formula are to be satisfied.

Examples of the Use of Simple Age-grading in the Assessment of *Culex pipiens fatigans* Populations *†

by BOTHA DE MEILLON [a] *and* Z. H. KHAN,[b] *WHO Filariasis Research Unit, Rangoon, Burma*

Detinova's method, which is based on the examination of the ovariolar tracheoles of the female, allows one to divide a female adult mosquito population into two groups—nulliparous and parous. In nulliparous adults, the ends of the fine tracheoles are closely wound up into bundles (Fig. 1), whereas in parous adults the fine tracheoles are extended (Fig. 2). The identification of these two categories is usually simple and after a little practice only a negligible percentage remains unclassified. Some mosquitos caught resting cannot be classified by this method because as soon as the oocytes develop much beyond stage II the tracheolar ends become obscured and the terminal knots or skeins tend to become unravelled, as in a parous adult. This complication is of negligible importance in catches made on bait.

* From the WHO Filariasis Research Unit, Rangoon, Burma. This investigation was supported in part by the United States Public Health Service Research Grant EF.00194.04 from the Division of Environmental Engineering and Food Protection to the World Health Organization.

† Since this note was prepared, work has been reported (Hayashi, S. (1965) *Jap. J. sanit. Zool.*, **16**, 250) which supports the conclusions drawn here.

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FIG. 1
APPEARANCE OF OVARY IN NULLIPAROUS *C. P. FATIGANS* ADULT

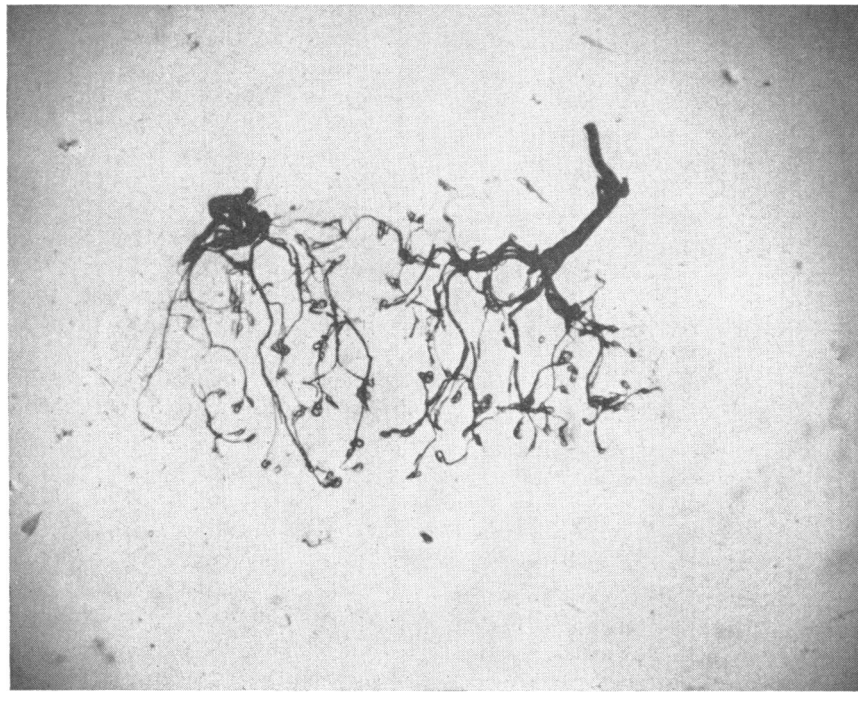
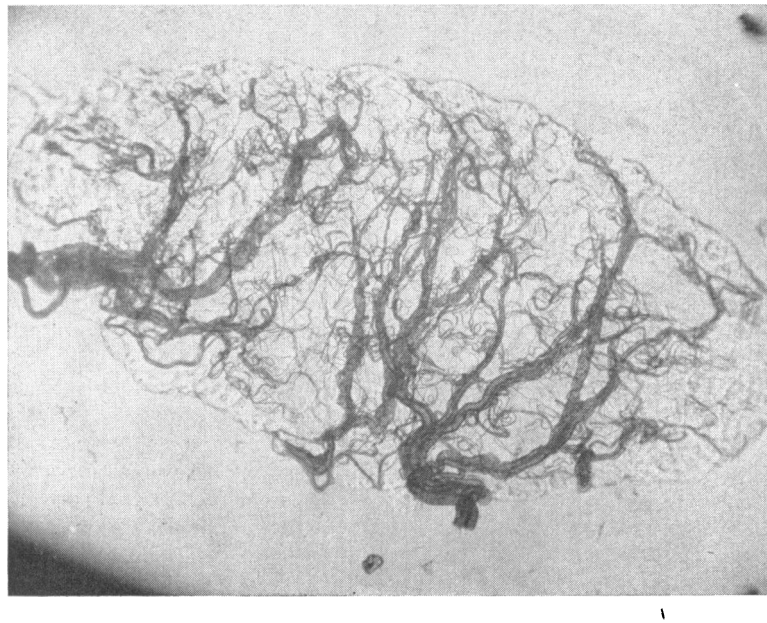


FIG. 2
APPEARANCE OF OVARY IN PAROUS *C. P. FATIGANS* ADULT



Before applying the technique in a routine manner we performed some laboratory experiments and found, as others have, that the method is simple and the results are reproducible.

Age-grading based on the counting of follicular remnants gives a more detailed analysis of the age structure of a population but is somewhat complicated and was not used in routine work. The technique calls for considerable skill and proved to be so time-consuming and the results so difficult to interpret that we could not adopt it in our programme.

The work described below shows that simple age-grading is a very sensitive index that readily gives indications of changes in the population structure. The amount of time and skilled labour that would have to be employed to demonstrate these changes by means of the more refined technique are not available in most field projects.

The methods employed in the Kemmendine Experimental Area for catching and handling, the times of catching and other details are described elsewhere in this issue.[c] Since catching stations were examined once every six weeks, Fig. 3A and 3B relate to the changes that took place between two consecutive catches and Fig. 4A and 4B to those between three consecutive catches.

The drainage systems

The Kemmendine Experimental Area,[d] which contains the ten catching stations in which mosquitos were collected for this study, may be divided into two main parts, the kutcha drain area and the pukka drain area.

The kutcha drain area. This contains catching stations 7, 8, 9 and 10 and is largely served by kutcha drains, which are simply channels dug in the earth. They vary in size, depth and length and in water-holding capacity. Towards the end of the dry season they tend to dry up, losing water by evaporation and seepage. They are mainly supplied by roadside standpipes, which serve as water-points for domestic use in the houses and also for laundering and bathing on the street. Some retain water throughout the year. Kutcha drains are easily blocked by rubbish thrown into them and by the spoil from rat holes dug in the banks. These drains are not easy to clean and to maintain. The uneveness of the bottom of these drains causes the water to collect in pockets that are not easily eliminated by simple cleaning.

The pukka drain area. The area occupied by catching stations 1, 2, 3, 4, 5 and 6 is mainly served by a system of well-constructed cement-lined drains called pukka drains. These, although also easily blocked by both man and rat, are rather easily cleaned and maintained. Once obstructions have been removed the water flows away easily because of the regular gradient and no pockets of water are left. Consequently these drains usually remain free of breeding *C.p. fatigans* for longer than the kutcha drains. They are supplied with water in the same way as the kutcha drains.

Effect of simple drain cleaning on parity and density

During the months of March to May, 1964, we recorded the cleaning of drains by local inhabitants and by the Corporation of Rangoon. Accurate recording is not easily achieved, but on the whole we consider our records to be reasonably accurate. Since the effects of drain cleaning are quite different in the kutcha and pukka drain areas, we shall deal with each separately.

The pukka drain area. Fig. 3A and 3B relate to catching stations 6 and 2, respectively, and show two types of results that follow larval control (by drain cleaning), but each indicates clearly the interdependence of density and parity.

In Fig. 3A density (number of adults caught per man-hour) dropped sharply after the cleaning of local drains in April and, since no newly hatched mosquitos were being added to the population, the percentage parous increased. Thus an increase in the percentage parous can be used as an index of recent successful disturbances of the breeding place.

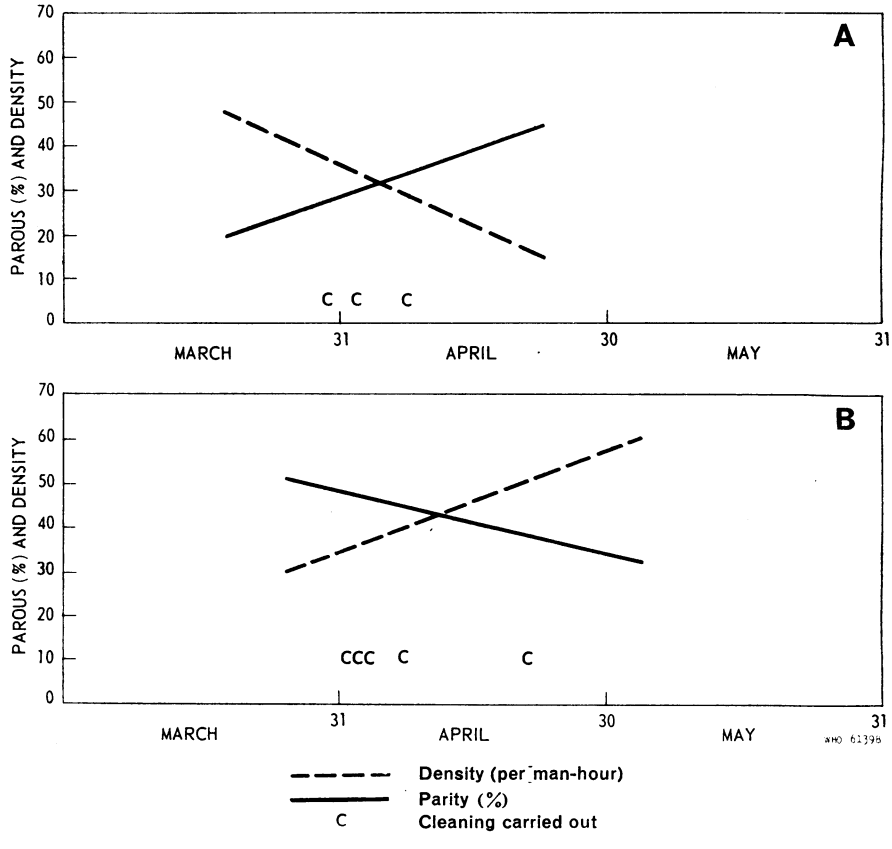
In contrast, Fig. 3B shows a sharp rise in density with a concomitant fall in the percentage parous. Here, obviously, there has been an influx of newly hatched mosquitos, which is reflected in the fall in the percentage parous. One interpretation is that cleaning was only partially successful and that new breeding places re-formed and contributed the young adults that resulted in a depression of the percentage parous. Another cause could be the sudden influx of newly hatched adults from elsewhere; however, we have no evidence that *C. p. fatigans* indulges in these mass flights in built-up areas.

Thus a fall in percentage parous can be used as an index of the re-commencement of breeding after control or possibly (although this is unlikely) to

[c] See the paper on page 91 of this issue.

[d] For a map of the area, see the paper on page 67 of this issue.

FIG. 3
PARITY AND DENSITY OF *C. P. FATIGANS* CAUGHT AT (A) STATION 6 AND (B) STATION 2 IN PUKKA DRAIN AREA



indicate the influx of a mass of newly hatched adults.

The rest of the catching stations in the pukka drain area gave similar results: stations 5, 4 and 3 resembled station 6 (Fig. 3A) and station 1 resembled station 2 (Fig. 3B).

The kutcha drain area. The kutcha drain area in which station 8 is situated was, to the best of our knowledge, never cleaned. The picture presented by this state of affairs is shown in Fig. 4A and it is clear that density and parity change only gradually, undisturbed by any interference with breeding places. Fig. 4A, then, might well represent the normal state of a stable population where natural losses and gains counterbalance one another in the absence of control measures or severe environmental influences.

Fig. 4B shows the results obtained where an attempt at cleaning has been made in the kutcha drain area (station 7): a small rise in percentage parous is accompanied by a small decrease in density. This is in line with our findings in the pukka drain area, but the effect is not so marked because, as we have already noted, drain cleaning in the kutcha area is difficult and largely ineffective.

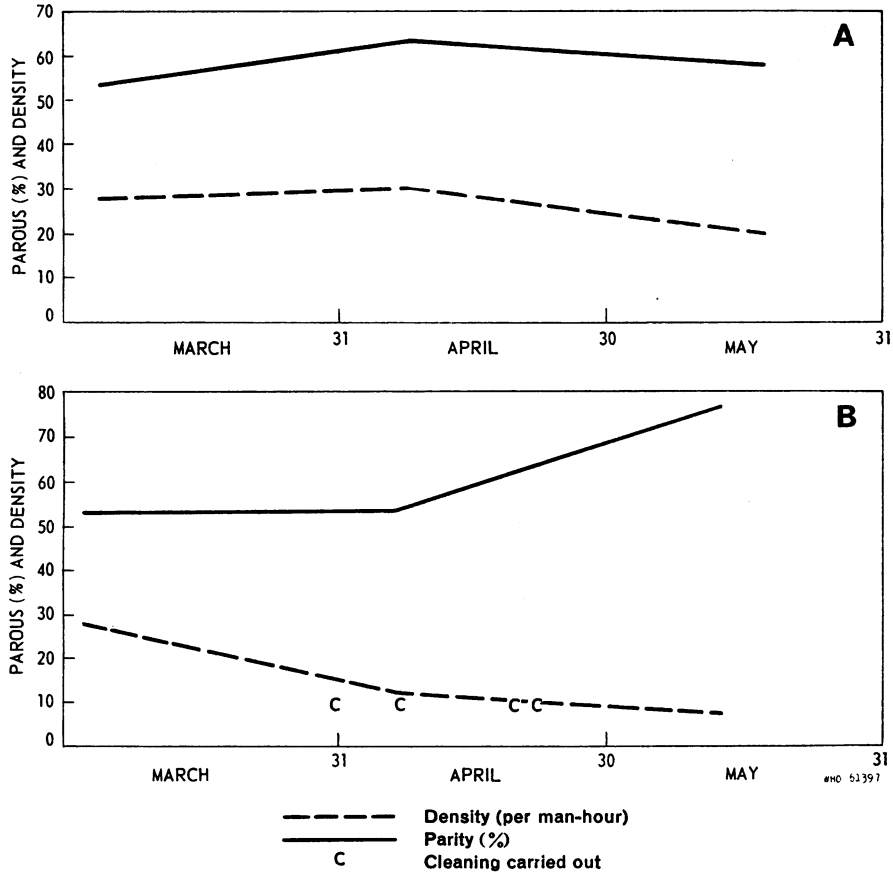
Naturally, the above conclusions, being based on field observation, will have to be put to rigorous experimental test before they can be accepted completely.

Use of parity in the analysis of sudden and untoward changes in field populations

Although the event now reported does not relate to *C. p. fatigans* but to *Aedes vexans*, it shows that

FIG. 4

PARITY AND DENSITY OF *C. P. FATIGANS* CAUGHT AT (A) STATION 8 AND (B) STATION 7 IN KUTCHA DRAIN AREA



parity can be used equally well to detect sudden invasions of recently hatched mosquitos of any species. This could be of practical importance, especially when a new prolific and unsuspected breeding source comes into operation.

On 3 June 1963 we obtained the following results in relation to *A. vexans* caught on human bait at one of the catching stations: early (18.30-20.00 hours), density (per boy-hour) 201, percentage parous, 1%; late (22.00-24.00 hours), density 23, percentage parous, 4%. Only negligible numbers of *A. vexans* were found in all catches made before and after this invasion. The low percentage parous shows that the adults were newly emerged and so all about the same age.

The sudden appearance of such a large number of adults in this condition must mean that they all emerged at about the same time and suggests the existence of a prolific breeding place. We knew that no breeding of *A. vexans* takes place in or near Kemmendine and strongly suspect that these mosquitos came from open country some miles away. The gradual build-up of a population of mosquitos, which might go undetected for some time, would definitely not show such a low percentage parous, which has been observed on other occasions when sudden invasion occurred.

A further example is provided by the analysis of the female *C. p. fatigans* mosquitos caught at stations 2 and 3 during routine catches in one week in May 1964, as shown in the table.

We do not know the precise cause of the dramatic differences, although, no doubt, they originate in some disturbance of the breeding places. Had

PAROUS AND INFECTED MOSQUITOS AMONG FEMALE *C. P. FATIGANS* CAUGHT IN TWO STATIONS IN ONE WEEK IN MAY 1964

Catching station	No. of mosquitos caught	Percentage parous	Infected	
			No.	%
2	800	24	16	2.0
3	249	94	14	6.6

examination for the percentage parous not been done one might have come to interesting but incomplete conclusions. It is clear that the large number of adults caught in station 2 are mostly recently hatched ones, whereas the situation at station 3 is just the opposite. As noted above, such figures provide information on the state of larval breeding and hence on the effect of control measures.

Of more than passing interest is the fact that, although the percentage infected (based on the number dissected) at station 3 is more than three times that at station 2, the actual number of mosquitos infected is practically the same. This aspect of infection and risk of infection is discussed more fully elsewhere in this issue.[c]

Conclusions

Simple age-grading, which gives the proportion parous, is a useful index for indicating changes in the population structure of *C. p. fatigans*. Since *C. p. fatigans* populations tend to manifest localized changes in breeding conditions, changes resulting from anti-larval measures, for example, are readily detected. In addition, sudden and unexpected changes in mosquito density can be explained by simple age-grading.

It seems that *C. p. fatigans* populations are localized but not static. They show continual short-term fluctuations, as regards both density and parity. These localized fluctuations are rapidly levelled out and in the course of a year give a misleading impression of unchanging stability.[c, e]

[e] See the paper on page 75 of this issue.

The Biting Cycle of *Culex pipiens fatigans* on Man in Rangoon, Burma, and the Microfilarial Periodicity *

by BOTHA DE MEILLON[a] *and* ANTHONY SEBASTIAN,[b] *WHO Filariasis Research Unit, Rangoon, Burma*

Some time ago four all-night catches of adult *C. p. fatigans* mosquitos coming to bite were subjected to dissection for parity and infection. As far as parity is concerned, no statistically significant trend could be demonstrated and it appears that the age structure of the biting population remains very much the same throughout the night. Infection followed the curve for density, as is to be expected.[c] Infection and parity will not be discussed further in this note. Density itself was shown to build up gradually to a maximum at about midnight. Although individual catches showed some variation, on the whole the above pattern was maintained.

In order to obtain further information, 14 indoor and 14 outdoor bait catches were made simultaneously. The results are reported here, together with the earlier data.

Materials and methods

According to our usual practice, two catchers were on duty for two to three hours at a time and were then replaced by the next team. This routine continued through the night. The team doing the first shift sometimes did the last one next morning; all other teams did only one spell of catching. For the indoor and outdoor catching we used one catcher in each situation and made use as a catching site of the quiet and undisturbed compound of the Bishop

* From the WHO Filariasis Research Unit, Rangoon, Burma. This investigation was supported in part by the United States Public Health Service Research Grant EF 00194.05 from the Division of Environmental Engineering and Food Protection to the World Health Organization.

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[b] Entomologist; seconded from the Directorate of Health, Burma.

[c] See the paper on page 91 of this issue.