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SAMPLING OF OUTDOOR RESTING POPULATIONS OF *ANOPHELES CULICIFACIES* AND *ANOPHELES FLUVIATILIS* IN GUJARAT STATE, INDIA¹

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INTRODUCTION. The resting habits of the malaria vectors are among the important indices in the assessment of the epidemiological capacity of the vector population. In another paper (Shalaby, in press), the outdoor resting anophelines collected from artificial pit shelters dug at four localities in Panchmahals district of Gujarat State in India, have been in-

vestigated. Twelve species of *Anopheles* were detected. They were: *Anopheles culicifacies* Giles, *A. tessellatus* Theob., *A. theobaldi* Giles, *A. fluviatilis* James, *A. barbirostris* v.d.w., *A. stephensi* Liston, *A. pallidus* Theob., *A. annularis* Wulp, *A. maculatus* Theob., *A. splendidus* Koidzumi, *A. jamesi* Theob. and *A. subpictus* Grassi. Of the 12 species, *A. culicifacies*, a major vector of malaria in Panchmahals district, ranked first in abundance and *A. fluviatilis*, an additional vector and the sole vector in some parts of Gujarat State,

¹ This paper is based on work carried out during the period that the author was a staff member of the World Health Organization.

ranked third. This paper presents investigation of the outdoor resting populations of these two species.

A. culicifacies is found in relatively large numbers indoors in Panchmahals district of Gujarat State. It was found tolerant to DDT in September 1959 (Rahman *et al.*, 1959 and Luen and Shalaby, 1962). In June 1960, the author confirmed the development of resistance to DDT in the mosquito population in the district. A progressive rise in the level of DDT resistance was shown (Shalaby, 1968). It should be noted that the transmission of malaria has been interrupted despite the development of DDT resistance in the vector. A decrease in the parasite rate in the human population has been indicated. The author undertook studies of some of the variables which may explain this unusual feature in the epidemiology of malaria in the district (Shalaby 1965a, b, 1966 and 1969a). On the other hand, *A. fluviatilis* has been found in a low indoor density in Panchmahals district. The susceptibility status of this species to DDT has not been investigated as the numbers of the mosquitoes were insufficient for any tests to be made.

This paper records results of comparative samples of the outdoor resting populations of *A. culicifacies* and *A. fluviatilis* taken in artificial pit shelters for a period of 10 months during 1961-1962. The purpose was to obtain information which might prove useful in the entomological assessment of the area.

METHODS. Eight artificial pit shelters were dug at three villages in Panchmahals district of Gujarat State namely, Motipura, Mandhra and Bhuval. Two pit shelters were dug at a regularly sprayed part of Motipura village and two in another part that was left unsprayed since June 1959 onwards. Four more pits were dug at Mandhra and Bhuval villages, two in each. These localities were under intensive residual DDT spraying for 12 years in the malaria eradication program of the district. The location and spray history of Panch-

mahals district were given elsewhere (Shalaby, 1966).

The sites for digging the pit shelters at the different localities were carefully selected. A shaded site under a tree or large bush was usually selected close to the village as a site for digging the pit. Muirhead-Thomson's (1958) technique of the pit shelters was followed. Accordingly, each pit shelter was rectangular in shape; 160 cm deep, 130 cm long and 120 cm wide. In each of the four vertical sides, about 50 cm from the bottom of the pit, a little cavity was dug about 30 cm deep. These four small cavities or niches would serve as attractive resting sites for the mosquitoes entering the pit shelters. The eight pit shelters were of the same shape and size. It was insured that the mouth of the pit shelters would be shaded from above either naturally by overhanging tree or bush or failing that, artificially by means of a framework covered with thatch awning and twigs as in the case of one of the sites at the unsprayed part of Motipura village.

The pit shelters were searched for the day-time outdoor resting anophelines once a week for a period of 10 months from October 1961 to September 1962. Collections from the pit shelters at Bhuval village started in November instead of October 1961. It was not possible to carry out the routine weekly collections from the various pit shelters at the various localities for a period of 2 months namely, July and August 1962. A total of 38 observations were conducted at the various sites during the entire period of study. Thirty-five observations were made at the sites in Bhuval village.

Searching the pit shelters for outdoor resting mosquitoes was normally done during mornings, 9-11 a.m. The mosquitoes were collected by means of sucking tubes and transferred to regular mosquito cages, 20 x 20 x 25 cm for transportation. At the field laboratory, the mosquitoes were anesthetized, identified and counted.

The *A. culicifacies* and *A. fluviatilis* captured were classified as to the sex. The sex ratios were estimated for both species at the various localities. Females of both species were further examined with respect to their stomach and gonotrophic conditions. Empty, gravid, fully fed and half-fed females were counted and their proportions estimated. These records were summed up on monthly basis at each of the four localities.

The climatological conditions had been taken into consideration. The monsoon rains fall from mid-June to mid-October. During this period the temperature averages 27°C and the relative humidity increases to 97 percent. In the winter months of December and January the temperature falls to about 18°C and the relative humidity averages about 53 percent. The temperature gradually rises in February and March until it reaches as high as 36°C in June, when the relative humidity is about 60 percent.

The inhabitants normally sleep outside their dwellings during the summer, from April to June. At other seasons, people and cattle occupy the same rooms at night.

RESULTS AND DISCUSSION. During the period of study, 3,377 adult *A. culicifacies* and 176 adult *A. fluviatilis* were collected from the eight artificial pit shelters at the sprayed and unsprayed parts of Motipura village and at Mandhra and Bhual villages. It is to be noted that the records obtained indicate that individuals of *A. culicifacies* have been captured during each time the artificial pit shelters were visited. The maximum captures, however, were during the period December to April. The density started to increase from November onwards, reaching the peak in December and January and declining gradually from April onwards when the temperature increases and the relative humidity decreases. Over 70 percent of the total outdoor catch had been taken during the period December to April. Shalaby (1966) in his study of the behavior of *A. culicifacies* in untreated and DDT-treated experimental huts at Motipura village, reported that the mosquito was found in

considerable density the year round, but December, January and February were the months of highest densities.

It seems that the fluctuations in the abundance of this species outdoors run in parallel lines with its density indoors. Similarly, it was noted that December, January and February were the months of relatively higher captures of *A. fluviatilis* from the artificial pit shelters at the various localities. More than 65 percent of the total outdoor catch had been taken during these 3 months. Evidently, this seasonal rise in the density of *A. fluviatilis* outdoors and of *A. culicifacies* indoors and outdoors occurs after the monsoon rains subside by mid-October. The numerous small pools and the rain water accumulations that are formed at the end of the monsoon season furnish suitable breeding places which yield a new supply of mosquitoes.

In general, *A. fluviatilis* is a rare species in Panchmahals district. Viswanathan and Rao (1943) reported that there are two geographic races of *A. fluviatilis* in India, one of which is outdoor resting, anthropophilic and low in density. Shalaby (1969b) reported an overall low density of this species in Panchmahals district. It was by the artificial pit shelter technique that it became possible to collect 176 specimens; a feature that could be attributed to an exophilic tendency in the mosquito. Capture from the pit shelters was the method of choice in collecting numbers of blood fed females of *A. fluviatilis* which were utilised to study the blood feeding pattern of this species by analysis of the blood meals by precipitin test (Shalaby, 1969b).

It should be noted that the majority of the captured *A. fluviatilis* were taken in the pit shelters at the unsprayed part of Motipura village which appeared to be more attractive to this species than the others, whereas the artificial pit shelters at the sprayed part of Motipura seemed to be more attractive to *A. culicifacies*. Of the 176 *A. fluviatilis* captured, 77 (over 43 percent) were taken in the two pit shelters at the unsprayed part of Motipura village.

Only 24 individuals were taken in the artificial pit shelters at the sprayed part of Motipura during the entire period of observations. *A. fluviatilis* numbering 35 and 40 had been taken in the pit shelters at Mandhra and Bhuval villages, respectively. On the other hand, of the 3,377 *A. culicifacies* captured, 1,062 specimens were obtained from the artificial pit shelters at the sprayed part of Motipura village and 771 were taken at the sprayed part. This could be attributed to the excito-repellency exhibited by *A. culicifacies* on contact with the DDT sprayed surfaces indoors as reported by Shalaby (1966), causing the exit of the irritated individuals in order to seek shelter out of doors. *A. culicifacies* numbering 849 and 695 were obtained from the pit shelters at Mandhra and Bhuval villages, respectively.

The sex ratios in the outdoor populations of both *A. culicifacies* and *A. fluviatilis* have varied considerably. The numbers of *A. culicifacies* males have generally slightly exceeded the females. At the sprayed part of Motipura and at Mandhra and Bhuval villages, the male proportions were slightly over 50 percent. The population taken in the pit shelters in the unsprayed part of Motipura gave more females than males. There, the ratios were 53.96 percent for the females and 46.04 percent for the males. In average, the sex ratios for the total outdoor collections of *A. culicifacies* were males at 53.06 percent and females at 46.94 percent. The situation was different in the case of *A. fluviatilis* where the female was the dominant sex. The proportions of the males were 19.67 percent and 22.08 percent among the outdoor population taken at the sprayed and unsprayed parts of Motipura, respectively; 31.43 percent in the population at Mandhra and 22.5 percent at Bhuval giving an average male proportion of 23.3 percent. At the same time, the proportions of the females were 68.57 percent at Mandhra, 77.5 percent at Bhuval and 83.33 percent and 77.92 percent at the sprayed and unsprayed parts of Motipura, respectively, giving an

average female proportion of 76.7 percent. Altogether, the male to the female ratio among the outdoor resting populations of *A. fluviatilis* at the various localities was little over 1:3.

The samples of the outdoor resting females of *A. culicifacies* and *A. fluviatilis* with respect to their stomach and gonotrophic conditions show that the empty unfed *A. culicifacies* form the bulk of the catch from the artificial pit shelters at the different localities. The proportions of the empty females were 70 percent, 62.02 percent, 53.54 percent and 30 percent at the sprayed and unsprayed parts of Motipura, Mandhra and Bhuval villages, respectively. In average, more than half (55.77 percent) of the 1585 females taken in the area were empty females. On the other hand, the proportions of the empty unfed *A. fluviatilis* females were 45 percent, 36.67 percent, 29.17 percent and 35.48 percent at the sprayed and unsprayed parts of Motipura, Mandhra and Bhuval villages, respectively; giving an average ratio of 36.30 percent. The gravid females were the least encountered from both the species. They were quite rare among the outdoor populations. In *A. culicifacies*, their proportions were 1.5 percent at the sprayed part of Motipura, 4.32 percent at the unsprayed part, 4.46 percent at Mandhra and 5.60 percent at Bhuval, with an average of 3.78 percent of the total outdoor collections.

Slightly higher but still low were the proportions of the gravid *A. fluviatilis* females. They were 5 percent at the sprayed part of Motipura, 11.66 percent at the unsprayed part, 20.83 percent at Mandhra and 6.45 percent at Bhuval, with an average of 11.11 percent of the total outdoor collections. The fully fed and half fed *A. culicifacies* females did not seem to occur in abundance. Their proportions were 13.07 percent at the sprayed part of Motipura, 14.42 percent at the unsprayed part, 23.10 percent at Mandhra and 31.77 percent at Bhuval, with an average of 19.62 percent for the fully fed females and 10.43 percent at the sprayed part of Motipura, 19.24 percent at the unsprayed

part, 18.90 percent at Mandhra and 32.63 percent at Bhuval, with an average of 20.83 percent for the half fed. The fully fed and half fed *A. fluviatilis* females were less abundant than the empty ones. Their proportions were 30 percent at the sprayed part of Motipura, 21.66 percent at the unsprayed part, 20.83 percent at Mandhra and 6.45 percent at Bhuval, with an average of 23.70 percent for the fully fed and 20 percent at the sprayed part of Motipura, 30.01 percent at the unsprayed part, 29.17 percent at Mandhra and 32.26 percent at Bhuval, with an average of 28.89 percent for the half fed.

The above clearly indicates that the artificial pit shelter technique proved to be of value in detecting and sampling the outdoor resting populations of *A. culicifacies* and *A. fluviatilis* in Panchmahals district. It was of particular value in the case of *A. fluviatilis*, since experience gained prior to this study had shown that the artificial pit shelters were most suitable for detecting outdoor resting *A. fluviatilis* at very low density. It should be emphasized that the artificial pit shelter collections should be supplemented with collections in natural outdoor resting places, to find out whether there is evidence that one type of shelter is more attractive to empty, blood-fed or gravid females than another. Muirhead-Thomson (1960) in his study of the use of pit shelters for sampling outdoor resting *A. pretoriensis* (Theob.) at Uzumba in Africa, showed that 88 percent of the total blood-fed and gravid females were taken from natural ravines and only 12 percent from artificial pit shelters. In spite of the fact that the findings obtained from the present investigation are of severely practical value, it is felt that further progress towards the interpretation of these findings might be made by studying the age composition of the captured mosquitoes, to find out the age of the mosquitoes the shelter sample represents. The determination of the age composition of the outdoor resting population of *A. culicifacies* would be of signifi-

cant value in this area insofar as it would help determine the validity of the disappearance of the old multiparous *A. culicifacies* females among the indoor population after resistance to DDT developed in the mosquito in June 1960 (Shalaby, 1965b).

SUMMARY. During a period of 10 months from October 1961 to September 1962 3,377 *A. culicifacies* and 176 *A. fluviatilis* were captured from 8 artificial pit shelters at 4 localities in Panchmahals district of Gujarat State in India.

The periods of highest outdoor captures were December–April for *A. culicifacies* and December–February for *A. fluviatilis*.

The average sex ratios were 53.06 percent for males and 46.94 percent for females in *A. culicifacies*, and 23.3 percent for males and 76.7 percent for females in *A. fluviatilis*.

Of 1,585 *A. culicifacies* females, 55.77 percent were empty, 3.78 percent gravid, 19.62 percent fully fed and 20.83 percent half fed. Of 135 *A. fluviatilis* females, 36.30 percent were empty, 11.11 percent gravid, 23.70 percent fully fed and 28.89 percent half fed.

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RELATIVE ABUNDANCE OF *CULEX PIPIENS* AND *CULEX RESTUANS* IN CATCH BASINS IN JEFFERSON COUNTY, KENTUCKY¹

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An epidemic of St. Louis encephalitis in Jefferson County, Kentucky, in 1956 prompted the establishment of a Mosquito Control Project as an arm of the Louisville—Jefferson County Department of Public Health. Details of the survey and control operations of this unit are reported by Covell (1968).

The survey of mosquito species in Jefferson County was begun in earnest in 1958 and continues today. Sampling for both larvae and adults is done with emphasis upon the breeding sites of *Culex pipiens*—the probable vector of SLE in the Louisville area.

Particularly important as potential breeding areas for *C. pipiens* are the storm sewer catch basins with which the streets in the city and suburbs are equipped. These basins have been constructed in Jefferson County over a period of many years, but they generally conform to the following description: The distance between the grating at street level to the bottom of

the chamber is about 6 feet; an inlet pipe empties into the basin 4 feet from the bottom; and on the opposite wall there is an outlet pipe that eventually empties into a main storm sewer (the outlet pipe is 3 feet from the floor of the chamber). The catch basins are thus designed so that the grit admitted through street inlets is collected at the bottom and not permitted to pass into the storm sewer system.

The data for this study were compiled from catch basin larval samples taken from about 30 selected basins scattered throughout the County from mid-May to mid-October during 1967, 1968, and 1969. A total of 1,408 larvae from 217 samples were determined and counted by Covell. Sanitarians who took the samples also measured water and air temperature at each locality, and larvicided these and all other catch basins at intervals each summer.

Only three species were identified from these samples: *Culex pipiens*, *Culex restuans*, and *Aedes vexans*. The first of these is well known as inhabiting catch basins (Carpenter and LaCasse, 1955; King *et al.*, 1960; Maddock *et al.*, 1963); *restuans* is perhaps less known as a catch basin breeder. *Aedes vexans* is extremely abundant in Jefferson County but is not nor-

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