A BIONOMIC STUDY OF ADULT *AEDES* (*NEOMELANICONION*) *CIRCUMLUTEOLUS* IN NORTHERN KWAZULU, SOUTH AFRICA

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ABSTRACT. Sixteen monthly visits were made to the Ndumu game reserve when resting *Aedes circumluteolus* mosquitoes were collected on the ground. The population density reached exceedingly high levels 7 or more days after the Usutu and/or Pongola rivers inundated their flood plains, and was related mainly to river flooding and far less to local rainfall. During dry months male *Ae. circumluteolus* disappeared while females persisted at very low levels. Blood-feeding and ovarian development occurred throughout the year and precipitin tests showed the preferred host was almost exclusively antelope (Bovidae), the dominant large mammal present. It was concluded that *Ae. circumluteolus* is a floodwater mosquito. Owing to the low density of females during dry months and low infection rates with arboviruses it is considered unlikely that *Ae. circumluteolus* would support viral transmission throughout the year. It is more likely that viruses survive in overwintering eggs and are transovarially transmitted.

INTRODUCTION

The Ndumu game reserve is regarded as situated in the tropical, eastern coastal plain of South Africa. This is based on the 18°C mean midwinter isotherm and the 500 mm isohyet (Poynton 1964). From April 1956 through December 1968 our Unit made a viral survey of the mosquito population. *Aedes (Neomelaniconion) circumluteolus* (Theobald) was shown to be the most prevalent mosquito and over the period yielded 75 isolates which included strains of 11 arboviruses (Worth et al. 1961, McIntosh et al. 1972a). *Aedes circumluteolus* was the most frequently infected species and laboratory tests had shown it capable of transmitting the flaviviruses Wesselsbron (Muspratt et al. 1957) and Spondweni, and the bunyaviruses Pongola and Bunyamwera among the viruses isolated (Arbovirus Unit, unpublished data). It was concluded at that time that *Ae. circumluteolus* was an important vector of arboviruses even if only in an incidental capacity (McIntosh et al. 1972a). Additionally, 2 strains of the phlebovirus Rift Valley fever virus had also been isolated from the species in another part of northern Kwazulu (Kokernot et al. 1957).

A study of the adult bionomics of *Ae. circumluteolus* was carried out at Ndumu from August 1967 through December 1968 to better understand its role as a vector of arboviruses and the results are reported here. Since this study was completed further work in South Africa has further incriminated *Ae. circumluteolus* as a vector of the important Rift Valley fever virus (Jupp et al. 1983, McIntosh et al. 1983). Additionally, Linthicum et al. (1985) in Kenya have obtained evidence that a feral population of another member of the same *Neomelaniconion* subgenus, *Ae. lineatopennis* (Ludlow) (= *Ae. mcintoshi*; Huang 1985), acts as a reservoir vector of Rift Valley fever virus by transovarial transmission.

Because only one isolate of Pongola virus and one of Germiston virus were made from the 41,600 female *Ae. circumluteolus* collected in our bionomic study, we were unable to relate viral infection data to the bionomic data collected. The diminished infectivity could have been caused by a major change in blood-feeding patterns by this species – from cattle to antelope species – as a result of the transformation of the Reserve into a wild life sanctuary which was complete by mid-1967 just before the study began (McIntosh et al. 1972a). Alternatively there could have been a fall off in the number of available susceptible vertebrate hosts.

STUDY AREA

The geographical position, topography and climate of the Ndumu game reserve has already been described (Worth et al. 1961). The area of northern Natal in which it is located – Tongaland – is now part of the region known as Kwazulu. Nearly half of the 10,000 hectare game reserve lies within the flood plain of the Usutu and Pongola rivers which meet at the northeastern corner of the reserve. The Usutu River forms the reserve's northern boundary and the border with Mozambique, while the Pongola River runs parallel and close to its eastern boundary. Thirteen sites were chosen for collecting mosquitoes resting in ground vegetation. Ten of these were just within or on the edge of the flood plain of the Usutu river, while 3 sites were much drier and located outside the flood plain. During periodic floodings of the rivers various low lying areas of the reserve suddenly become inundated and the water level in certain pans rose. The mosquito collection sites situated at or near the flood plain were close to the areas where larvae of *Ae. circumluteolus* developed. Each site measured 743 sq. meters and had ground cover shaded by trees throughout the year. At Ndumu rain falls usually in the summer from November through March. The rest of the year is much drier, particularly June through August when there is little or no rain.

METHODS

Mosquito collecting: The portable "hand suction trap" made by us for collecting mosquitoes off ground vegetation was similar to the trap of De Freitas et al. (1966). It consisted of a "Perspex" cylinder 18 cm long and 15 cm in diameter housing a 12 volt, 8 watt, DC electric motor with a 3-bladed rubber propeller. In our design the mosquitoes were aspirated directly through the fan into a 20 cm^3 collecting cage. About 10% of the catch was damaged at high mosquito densities. The inlet was covered by wire mesh screen comprised of squares measuring 13 × 13 mm to prevent debris and very large insects being aspirated. The operator of a trap carried a satchel on his back containing the 12 volt motor cycle battery, held the trap by its upwards curving handle in his right hand and a short stick equipped with a metal hook in his left hand. He combed one-half of the area of a collecting site parting the vegetation with his stick while keeping the inlet of the cylinder about 10 cm off the ground. Monthly visits were made to Ndumu from August 1967 through December 1968 except in November 1968 when weather conditions in northern Kwazulu prevented access to the Ndumu area. Each visit usually consisted of 6 collecting days: each morning 2 men collected for half an hour at each of four sites so that each of the sites 1-12 was sampled twice. Site 13, the forest site, was used only from February 1968 and was not sampled as frequently as the other sites.

Examination of mosquitoes: Mosquitoes were brought back alive to the field laboratory on the day of collection, killed with hydrogen cyanide and examined microscopically. The specimens of *Ae. circumluteolus* were extracted from each collection, the number of males and females counted and females classified as to their metabolic state. The undamaged females were separated into 3 classes: "gravid," "blood-engorged" and "empty." Mosquitoes with enlarged ovaries and no detectable unassimilated blood were recorded as "gravid," specimens with any detectable blood whether the ovaries were partly developed or not were recorded as "blood-engorged," while all other females were regarded as "empty." This last category included females with an undistended abdomen, those where it was fluid-filled and those where it may have contained fatty material. A small proportion of females were unclassified owing to damage – this number was recorded. Blood-meals from a proportion of the engorged mosquitoes in each collection were stored on filter paper and later tested for vertebrate host origin by the precipitin test according to the method used by Anderson (1967). During the period February through October 1968 mosquitoes other than *Ae. circumluteolus* were also identified, usually from half of the collections.

Analysis of results, rainfall and river flow: The monthly population index used for *Ae. circumluteolus* was the number of mosquitoes collected per trap hour expressed as Williams' modification of the geometric mean (Mw) (Haddow 1960). It was calculated from the total number of mosquitoes collected, including those damaged, and the total number of trap hours during each visit to Ndumu. The proportion of each category of females was expressed as the percentage of the total collection of female mosquitoes during each month and the number damaged was not included. Rainfall was recorded at the general store just outside the game reserve. Monthly averages are shown in Fig. 1. Flow rates for the Pongola River were recorded in a gauging station 22.5 km upstream from the gorge at Josini where a dam has subsequently been built. Flow rates for the Usutu were recorded at a station 22.5 km upstream from the confluence with the Pongola River. A flow greater than 2,500 cusecs (70, 8 m^3/sec) was regarded as indicative of the river being in flood on the advice of hydrologists in the Department of Water Affairs.

RESULTS

Monthly fluctuations in population level of Ae. circumluteolus*:* Table 1 shows the numbers of male and female mosquitoes collected monthly over the 16 months of the study, together with the corresponding Williams' means. The same figures are expressed graphically in Fig. 1 together with the monthly rainfall. The histogram in Fig. 2 illustrates the relationship between population density and river flooding (Usutu and/or Pongola rivers) in time. The winter and spring months, June to October 1968, viz the period when *Ae. circumluteolus* was nearly inactive is omitted from this figure.

Table 2 illustrates productivity of the 13 trapping sites when expressed in terms of the average catch of all *Ae. circumluteolus* mosquitoes per man hour over the whole study period.

Seasonal pattern of blood-feeding and egg production: Figure 3 shows the relative proportions

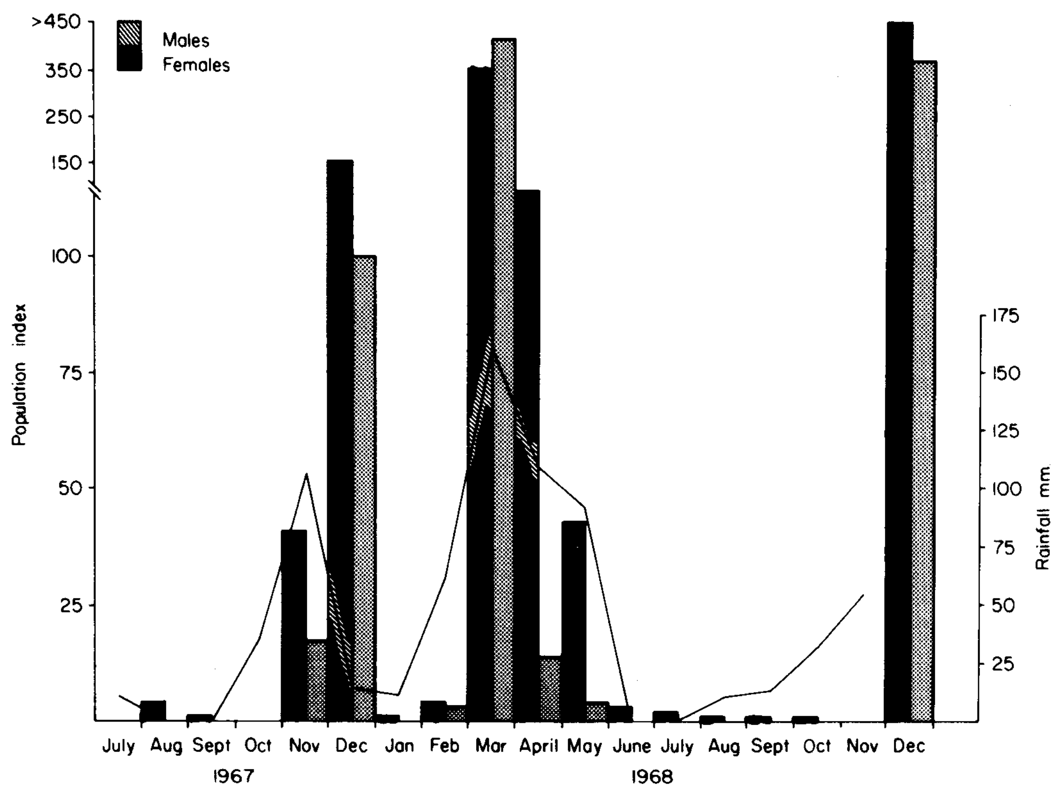

Fig. 1. Monthly fluctuations in rainfall and population level of *Aedes circumluteolus* at Ndumu: mean catch per trap hour expressed as Williams' mean.

Table 1. Monthly fluctuations in population level of *Aedes circumluteolus** at Ndumu, Kwazulu, South Africa.

	No. trap hrs	Females No. mosqs	Females Mw/trap⁺ hr	Males No. mosqs	Males Mw/trap⁺ hr
Aug. 1967	16	102	4	3	<1
Sept.	24	23	1	1	<1
Oct.	24	4	<1	0	0
Nov.	24	1,290	41	700	17
Dec.	22	7,377	154	7,230	100
Jan. 1968	24	37	1	6	<1
Feb.	26	319	4	293	3
Mar.	24	16,151	354	28,457	416
April	25	3,776	114	669	14
May	25	1,601	43	200	4
June	25	222	3	13	<1
July	16	43	2	0	0
Aug.	13	11	1	0	0
Sept.	25	25	1	11	<1
Oct.	21	22	1	0	0
Dec.	25	19,971	561	17,857	371

* The numbers in this table and in Fig. 1 include the damaged but identifiable mosquitoes.
⁺ Williams' modification of the mean.

of gravid, blood-engorged and empty individuals in each month's catch of *Ae. circumluteolus*. Engorged and empty mosquitoes occurred in every catch and gravid insects as well except in October 1967 and August 1968 when catches were at their lowest (4 and 11 mosquitoes, respectively). In those summer months when the male catch was about the same as the female catch, i.e., December 1967 and February and December 1968, or greater as in March 1968, only 2–9% of female mosquitoes were gravid. During these months the catches were almost certainly composed predominantly of newly emerged mosquitoes which had not yet developed eggs. Such mosquitoes would account for the large proportion of "empty mosquitoes" present during these 4 months, i.e., 57–86% of the total catches.

Precipitin test: Blood meals from 1,018 *Ae. circumluteolus* tested by the precipitin test revealed that 96% were of antelope (Bovidae) origin.

Prevalence of other mosquito species: Culex species were always present in the collections with *Culex (Culex) neavei* Theobald, *Cx. (Cux.) zombaensis* Theobald, *Cx. (Eumelanomyia) insignis* (Carter) and *Cx. (Cux.) antennatus* (Becker) being the commonest species in that order. The population densities of these mosquitoes all fell to low levels during the dry winter months except *Cx. neavei* which usually persisted in significant numbers. During the dry

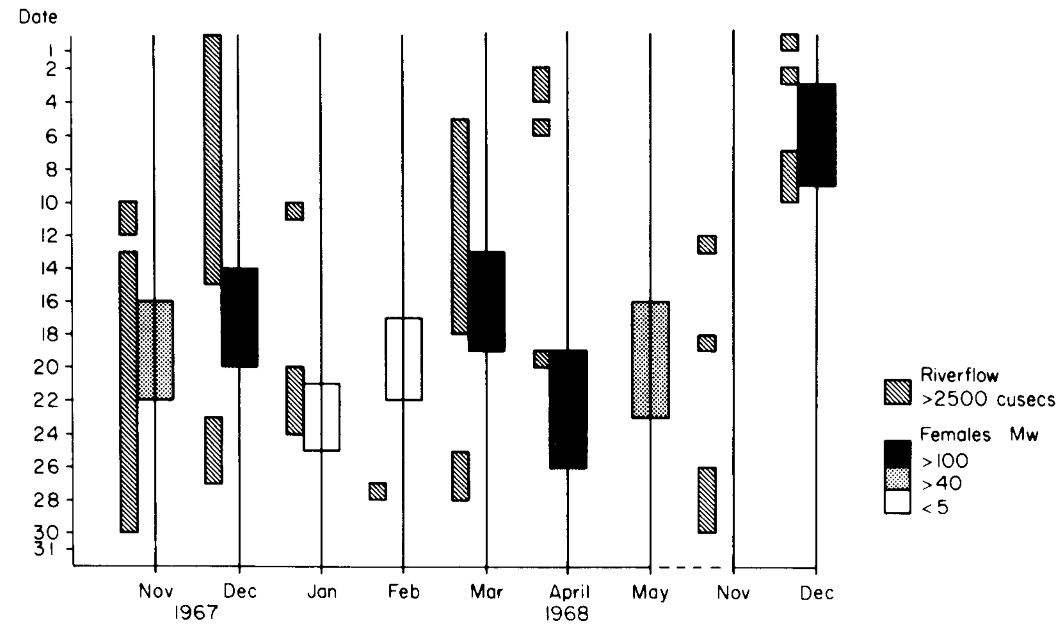

Fig. 2. Density of *Aedes circumluteolus* at Ndumu related to flow of Pongola and/or Usutu rivers: June–October 1968 data are omitted.

Table 2. Average catch of *Aedes circumluteolus** per man hour according to location of sites at Ndumu.

Site	Location	No. mosquitoes
10	Near floodplain	738
4, 5 and 7	Near floodplain	425–492
6, 9 and 11	Near floodplain	216–290
2, 3 and 13	Intermediate	134–186
1, 8 and 12	Outside floodplain	71–103

* All mosquitoes - males, females and damaged.

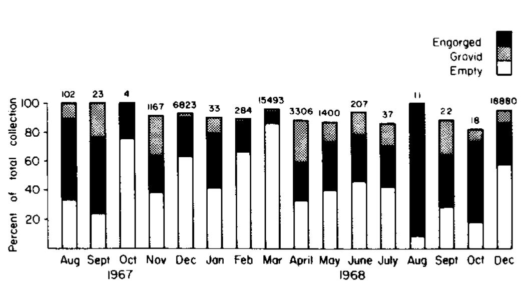

Fig. 3. Seasonal pattern of blood-feeding and egg production of *Aedes circumluteolus* at Ndumu; numbers above columns are total numbers of females collected.

months, June through October 1968, aedine mosquitoes nearly disappeared from the catches except *Ae. (Aedimorphus) albocephalus* (Theobald) and *Ae. circumluteolus*. In March and December 1968 when densities of *Ae. circumluteolus* were at their highest numbers of all other species of mosquitoes collected in the traps were extremely low, possibly because they had been displaced from their usual resting places.

DISCUSSION

The only local rainfall at Ndumu which could have had any significant influence on the hatching of the eggs of *Ae. circumluteolus* and their subsequent development into adults is rain which was concentrated on one day or several consecutive days prior to a collecting trip carried out during the warmer months (mean monthly temperature 22.2–27.8°C). Such rainfalls only occurred on October 24–27, 1967 (34.5 mm), November 17–22 (81.5 mm), March 7, 1968 (133 mm) and April 19 (62 mm). Although Fig. 1 shows there is some relationship between monthly rainfall and population indices of females and males, the relationship is by no means consistent, suggesting there must be another cause for some of the population peaks. Study of Fig. 2 reveals that high population levels were mainly related to the degree of previous river flooding expressed in terms of river flow. During the period of the study the Usutu River was more frequently in flood than the Pongola River. As shown in Table 2 resting sites where the highest densities of *Ae. circumluteolus* were collected were those closest to areas in the reserve most frequently flooded by the Usutu River (sites 4–7 and 9–11). Sites with intermediate catches (2, 3 and 13) were further away from such inundated areas, while sites 1, 8 and 12 located in dry areas of the reserve gave the smallest catches. Each monthly collecting trip shown in Fig. 2 is examined with regard to its relationship to rainfall and river flooding.

The modest population level which occurred in November 1967 can only have been caused by a combination of the previous 34.5 mm rain of October 24–27 and river flooding which began in November 6 days before the start of mosquito collecting but which continued throughout the 6 day trip. However, in the laboratory we have found that at an air temperature of 25°C this mosquito requires a minimum of 7 days from egg hatching to adult emergence. Nieschulz et al. (1934) also reported a similar developmental time from observation on *Ae. circumluteolus* in the field. This means that mosquitoes present in the catches on the first day or second day of this trip probably originated from larval sites provided by local rain rather than river flooding, while the latter could have accounted for mosquitoes collected over the remaining 4 days. The high density of December 1967 is readily explained by river flooding which commenced 14 days before the start of collecting. The 82 mm rain recorded from November 17 to 22 may have also contributed to this mosquito population. Low populations in January and February 1968 followed months of low rainfall; there was no river flooding in February while flooding in January occurred too late. The population peak in March with more males than females, was clearly due to extensive river flooding which started 8 days before the start of collecting and the 133 mm of local rain which fell in a single day, March 7. On this trip the number of mosquitoes taken increased daily from the first until the last collecting days indicating emergence was occurring over the collecting period. In April the density of males dropped while females remained at a fairly high level, indicating that little emergence was still taking place. As there was limited river flooding in April it is most likely that the majority of the females taken in the catches were survivors from mosquitoes which had emerged the previous month. The modest density in May is explained by the absence of river flooding since April 19. The 62 mm recorded on this date, a month before collecting began, may have produced a limited number of mosquitoes of which a proportion were still alive. River flooding which started on November 27 is the only explanation for the December 1968 population level which had the highest density of females and second highest density of males in the study.

On 3 occasions larvae of *Ae. circumluteolus* were collected in inundated areas adjacent to pans within the flood plain of the Usutu River. Several attempts to find larvae in temporary rain water pools away from the flood plain were all unsuccessful. This also indicates that river flooding rather than local rain *per se* is important to cause the hatching of dormant eggs.

Aedes circumluteolus therefore fits the category of a typical floodwater *Aedes*, whose eggs hatch mainly because of riverine flooding in the Tongaland area of northern Kwazulu, with subsequent peaks in adult population density. This compares with other floodwater mosquitoes, such as *Ae. (Ochlerotatus) juppi* McIntosh and *Ae. (Neomelaniconion) unidentatus* McIntosh occurring on the South African Highveld (T. P. Gargan, R. J. Novak and P. G. Jupp, unpublished data) and *Ae. (Aedimorphus) vexans* and *Ae. (Ochlerotatus) sticticus*, Meigen occurring in the Columbia River Valley in North America (Gjullin et al. 1950). Our unit has not encountered *Ae. circumluteolus* in other parts of northern Kwazulu at such high levels as those reached at Ndumu. However, it has been collected at river margins or marshes after rain in smaller numbers. It appears that local rainfall needs to be heavy and concentrated to cause similar surface flooding to that associated with riverine flooding. The observations of Nieschultz et al. (1934) are in accord with this.

During the dry winter and spring months (June to October) the mosquito dropped to very low levels but never completely disappeared. Male mosquitoes were either absent or fewer than females. It appears that during this dry period a very small proportion of the total population is surviving for long periods; the females collected from August to October usually appeared old. Theoretically if virus infection rates in *Ae. circumluteolus* were high enough the small residual winter-spring population could include infected mosquitoes which would carry a virus through the dry season at Ndumu. However, the infection rates in summer populations have always been low. (Worth et al. 1961, McIntosh et al. 1972a). It seems more likely that these viruses overwinter in the egg stage and survive by transovarial transmission, particularly in view of the work done by Linthicum et al. (1985).

The 2 months when gravid mosquitoes did not feature in the collections are not thought to indicate a cessation of ovarian development, especially as blood-engorged mosquitoes were collected on both occasions. Rather they were probably due to the small size of the sample of mosquitoes available for examination. The results indicate that blood-feeding and ovarian development occur at Ndumu throughout the year and that there is no reduction of these activities during the winter. The almost exclusive preference for antelope blood shown by *Ae. circumluteolus* at Ndumu in 1967–68 differs from the preferences shown in a study prior to 1964 (Paterson et al. 1964). In that study the mosquito was shown to have a wider host range, feeding on man and his domestic animals besides antelope. However, the new host preference probably reflects the change which took place in the Ndumu reserve by mid-1967 when the natives and their animals were moved out (McIntosh et al. 1972a).

As concerns the other main species of mosquito collected in the study, it appears that *Ae. albocephalus* is the only other ground-breeding *Aedes* in which small numbers of adults occur throughout the winter in the reserve. The paucity of *Cx. (Cux) poicilipes* (Theobald) in these resting collections as compared to the frequency at which it has been sampled in bait traps at Ndumu (McIntosh et al. 1972b) indicates this species may rest elsewhere, possibly above ground level.

Since the study was conducted, the Pongola River has been dammed. This has prevented it from flooding as frequently as before including where it passes through the Ndumu game reserve. This development appears to have caused a reduction in population densities of *Ae. circumluteolus* in the reserve and presumably in the whole of Tongaland judging from the infrequency at which the species has been collected during malarial vector surveillance and during collections for arboviral vector research.

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