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DAILY MORTALITY IN FOUR SPECIES OF NEW GUINEA ANOPHELINES

by

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INTRODUCTION

An investigation into the frequency of age groups in a population of anopheline mosquitoes may be of great practical significance. Indeed, the main objective of vector mosquito control is to reduce the life span to any length of time less than the duration of the extrinsic cycle of the parasite (e.g. *Plasmodium* species), and thus to stop transmission. An assessment of the mean expectation of life of individual mosquitoes in the population under control will certainly provide some clue as to the degree that such control is effective. In this territory mosquito control is attempted by means of residual indoor spraying.

In the New Guinea lowlands the extrinsic cycle of *Plasmodium falciparum* (in *Anopheles punctulatus*) takes 11 days, that of *P. vivax* about nine days (after data by MACKERRAS & ERCOLE, 1948a, 1948b); the cycle of *Wuchereria bancrofti* takes about 13 days (VAN DIJK, 1958). As it takes about two days after emergence from the pupa before the *punctulatus*-group anophelines are ready to take blood meals, the critical age of these mosquitoes will be in the order of 11–15 days. Concerning the recognition of age groups, MER (1932) was the first to separate nulliparous and parous females according to the size of the ampullae part of the paired oviducts. The drawback of this otherwise simple method is that it is impossible to investigate further details with the parous group.

A much more refined technique was developed by several Russian workers, especially POLOVODOVA (quoted by GILLIES, 1958). This technique, allowing for a very accurate estimation of the age of any individual, is based on the count of the mean number of corpora lutea occurring per individual ovariole. In *Anopheles maculipennis* at least, the number of corpora lutea corresponds to the number of egg batches already produced. It will be clear that, provided a sufficient number of individuals is analysed, the frequency of age groups in the population under observation can accurately be assessed by means of this technique.

In the *punctulatus*-group anophelines however, it was found impossible to make this method work and to enumerate satisfactorily the number of corpora lutea found. Perhaps the regularity as found in *maculipennis* is lacking. On the other hand, these negative results may be due to insufficient experience and skill of this investigator. It was nevertheless clear that the corpora lutea technique is very time-consuming and as such is hardly applicable in this part of New Guinea as long as the entomology is so completely a one-man job.

In the present investigation Mer's method (with several modifications see below)

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Though important as a decisive character, the size of the ampullae was not exclusively used as a criterium for parous or nulliparous. Several other qualitative characters were taken into account (*Table I*).

The combination of the characters mentioned in *Table I* in most cases permitted group-classification of an individual.

The stage of development of the eggs was noted for every specimen investigated

TABLE I

DIFFERENCES IN MATURITY OF ANOPHELINE FEMALES

Organ	*Parous*	*Nulliparous*
Ampullae	Globose or truncate.	Usually more conical, sometimes globose.
Diameter	Usually > 155 μ, mean value between 185 and 215 μ.	At most 150 μ.
Paired oviducts	Frequently rather short with a wide lumen; transverse folds may be present, epithelium well developed.	Frequently rather long and narrow; epithelium delicate.
Oviducal aperture	Apically more or less funnel shaped.	Apically narrow and cylindrical.
Ovaries	Frequently with a distinct central cavity.	Without a distinct central cavity.

(according to Christophers' classification, 1911, with some more differentiation in stage II). In some large series it was also noted whether or not the specimens had previously been fertilized.

RESULTS

Developmental stage of the ovaries in biting females. An account of the developmental stage of the ovaries in females in need of a blood meal is given in *Table II*. By

TABLE II

DEVELOPMENTAL STAGE OF THE OVARIES IN FEMALES IN NEED FOR A BLOOD MEAL

Species	*Locality*	*Developmental stage of the eggs*								*% of total in II*
		I	*eII*	*m-l II*	*II → III*	*III*	*IV*	*V*	*V+II*	
A. koliensis*	Hollandia	78	410	219	38	11	1	–	7	83
A. farauti	Merauke	21	198	171	14	21	3	1	9	86
A. amictus hilli	Merauke	9	124	65	4	3	6	8	13	88
A. bancrofti	Merauke	12	226	103	8	6	–	1	14	92
A. bancrofti	Kimaan	5	195	106	3	5	8	–	14	93

* Taken in the period between September, 1958–January, 1959.

far the greater part of the biting population have their ovaries in stage II. The correlation between ovarian development and readiness to search for a blood meal is not absolute however, as there must be other factors that influence the searching behaviour. It is interesting that some females with completely developed eggs were caught on a donor. Instead of following egg-laying as usual, the searching behaviour for obtaining a blood meal was activated before egg-laying in these females, whatever the cause.

In some stage III and IV females, traces of a previous blood meal were found in addition to freshly sucked blood. Those individuals probably needed a second blood

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fluctuations, see below) and are thus represented in *Table III* and following similar tables. All mosquitoes, apart from some taken in November 1957, were collected in one locality, i.e. a banana and cassava garden at the border of an extensive sago swamp near a row of native houses. The data on rainfall were collected at a meteorology station about 500 m away from the catching station. (See *Graph.*) As may be expected in a rather unstable species as *Anopheles koliensis*, the proportions of parous fluctuated from catch to catch, due to the irregular input of newly emerged females. An example

TABLE III

DAILY MORTALITY IN *Anopheles koliensis* AT HOLLANDIA

Period	*Total invest.*	*Total parous*	*Tot. st. II*	*Tot. st. II par.*	*Prop. parous in %*	*p*	*Daily mort. in %*	*Numb. malaria diagnoses*	*Mm rain (rain days)*
1957									
Nov. 1–14	58	28	—	—	48	0.69	31	18	27 (2)
Nov. 15–28	58	33	37	24	65	0.81	19	27	37 (3)
Nov. 29–12	297	150	263	143	54	0.74	26	25	87 (6)
Dec. 13–26	136	82	105	66	63	0.79	21	23	86 (4)
Dec. 27– 9	54	26	43	24	56	0.75	25	28	96 (10)
			448	257	57	0.75	25	24	
1958									
Jun. 1–14	68	46	61	41	67	0.82	16	22	0 (0)
Jun. 15–28	57	32	56	32	58	0.76	23	31	64 (5)
Jul. 29–12	98	68	97	68	70	0.84	16	17	118 (4)
			214	141	66	0.81	19	23	
Aug. 24– 6								80*	97 (5)
Sep. 7–20	84	62	80	61	76	0.87	13	136	28 (3)
Sep. 21– 4	176	134	145	121	83	0.91	9	69	94 (3)
Oct. 5–18	54	42	48	39	81	0.90	10	42	46 (2)
Oct. 19–1	46	32	41	30	73	0.85	15	61	27 (3)
Nov. 2–15	117	86	105	81	77	0.88	12	34	193 (6)
			419	332	80	0.89	11	68	
Nov. 16–29								24	134 (5)
Nov. 30–13	147	93	121	83	68	0.83	17	43	79 (7)
Dec. 14–27	42	25	39	25	64	0.80	20	28	58 (6)
Dec. 28–10	101	59	80	48	60	0.77	23	48	81 (8)
1959									
Jan. 11–24	129	74	113	69	61	0.78	22	67	69 (4)
			353	225	63	0.79	21	39	
Jan. 25–7	165	126	159	124	77	0.88	12	72	23 (3)
Feb. 8–20	276	151	233	150	64	0.80	20	70	109 (7)
			392	274	70	0.84	17	71	

* 66 diagnoses in period Sept. 1–6 only.
DDT routine sprayings: 28. June '57; 28. Jan. '58; 7. Sept. '58; 5. Febr. '59.

of these short term fluctuations is shown in *Table IV*, illustrating catches in November and December 1957.

In November-December, 1957, relatively low average proportions of parous were found (48–65%), compared with two-week periods; the mosquito density was low at the same time, not exceeding 5–10 per man per hour. The observations in Hollandia (which had to be interrupted for a long stay in Southern New Guinea) were resumed in June, 1958. The average proportion of parous then found was higher than before (66%); the mosquito density was still low, increasing in July up to about 20 per man

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but the *koliensis* population remained numerous (about 50 per man per hour) until the end of February when the observations had to be stopped.

The differences found in the proportion of parous in different periods are statistically significant: differences between November-December 1957 and September-October 1958, $p < 0.001$; differences between the latter period and December 1958–January 1959, $0.01 < p < 0.05$. The p values calculated from the observed data suggest two hypotheses: either they reflect the actions of a long term mechanism, acting on the population in such a way that the expectation of life for the individual mosquitoes in different periods is different, or the differences in proportions of parous in different periods will be caused by fluctuations in numbers over longer periods in the biting population. In a period of increase the input by new emerges will exceed the total adult mortality. A p calculated in such period may indicate a figure lower than the actual expectancy of life, while in periods of population decrease the reverse may be found.

The figures concerning population density, as recorded above, do not lay claim to any accuracy but they indicate a general tendency. So in a period of very low density (November-December 1957) a low value for p was found. An increase in biting density was found together with a significant increase for p (June-July $\rightarrow$ September-October 1958); a high level p was maintained in a period of some decrease in numbers (November 1958) and a decreasing p value was calculated for a period with a distinct increase in number (November-December 1958–January 1959). Subsequently p increased again in a biting population with a more or less constant high density. A clear correlation between fluctuations in proportion of parous and density was not observed. Though the effects of varying density cannot be ruled out it is very improbable that they provide an entirely satisfactory explanation.

Some support for the first hypothesis is obtained from a correlation between the p values calculated for different periods and the number of malaria diagnoses at Hollandia in corresponding periods.

In *Anopheles koliensis* the p-values fluctuated between 0.74 and 0.91. According to MACDONALD (1952b) the expectation of life in a population with a daily mortality of 10% ($p = 0.90$) is 9.49 days; stated otherwise: out of 1,000 individuals of this population, 349, 206 or 122 individuals will survive through a period of 10, 15 or 20 days. Hence under these circumstances a fair number can reach the critical, potentially dangerous, age as a vector. Similar numbers when the daily mortality is 30% ($p = 0.70$) are 28, 5 and < 1. A low level p was found correlated with malaria on a low level (November-December 1957, June-July 1958). A significant increase of p had occurred before the first week of September 1958; p was maintained on a high level until the middle of October. A sharp increase in the malaria incidence at Hollandia occurred in the first week of September, reaching a peak in the third week of that month, and subsequently decreasing. A small secundary peak was produced in the last week of October. A normal malaria level was reached again in the last week of November. The p values were found to decrease after October, reaching medium figures at the middle of December. Another increase of p at the end of January-February 1959 was found to be correlated with another increase in malaria incidence.

Apart from a decrease in daily mortality, an increase of number in the vector population might account for an increase in malaria. The malaria increase in September 1958 was positively correlated with an increase of the biting population, but an-

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infected, potentially dangerous mosquitoes with respect to transmission, will be greater than the average mortality for the whole mosquito population.

The average risk an individual runs of being poisoned by DDT, however, must be small for this *Anopheles koliensis* population.

Susceptibility tests according to the Busvine & Nash technique with help of a W.H.O. standard equipment did not reveal any sign of resistance in Hollandia. The median lethal dose for *Anopheles koliensis* was about 0.5% DDT, a figure in the same order as that found in T.P.N.G. by PETERS & STANDFAST (1958): m.l.d. for *Anopheles koliensis* 0.62% DDT. The data on the Hollandia population are shown in *Table V*.

Fluctuations in malaria incidence are in fact reported every year from Hollandia; they were also known from the pre-spraying period. Usually, there is a peak in the last month of the second quarter or in the third quarter of the year. Though the actual figures need to be handled with great care they may provide some supporting evidence for the regularity of density and daily mortality-rate fluctuations occurring in the vector population. It will be clear that fluctuations as mentioned above are of prime importance for the actual transmission of malaria occurring at any time.

TABLE VI

DAILY MORTALITY IN *Anopheles farauti* IN MERAUKE

Period	*Total invest.*	*Total parous*	*Total st. II*	*Tot. st. II par.*	*Prop. parous in %*	*p*	*Daily mort. in %*	*Mm rain (rain days)*
Feb. 1–14	148	69	128	52	41	0.64	36	50 (9)
Feb. 15–28	149	65	123	60	49	0.70	30	176 (11)
Mar. 1–14	47	23	45	21	46	0.68	32	61 (6)
Mar. 15–28	61	31	58	29	50	0.71	29	84 (10)
Apr. 29–11	33	16	33	16	50	0.71	29	79 (8)
	438	204	378	178	46	0.68	32	

Anopheles farauti (Merauke)

The figures found in a population of *Anopheles farauti* during February, March and April, 1958, are shown in *Table VI*. All specimens were caught in the same locality; the species was numerous at the time of collecting.

In the three-month period there was little variation in the proportion of parous of the different batches; an overall mortality of 32% was found. According to MACDONALD (1952b), out of 1,000 individuals 28 viz. 5 are expected to survive through a period of 10 viz. 15 days when the daily mortality is as high as 30%. Again, the indoor spraying with dieldrin does not suggest an efficient control of the population as a whole of this species. This may partly be explained by the insufficient persistence of the dieldrin under local conditions. At the time of observation (six months after the last spraying) its effect was no longer patent (VAN DEN ASSEM, 1959). On the other hand, the daily mortality at Merauke was in the same order as that found in DDT-sprayed Hollandia during an observation period immediately preceeding, and significantly higher than found in Hollandia later on, another argument for the insufficient control by DDT.

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it is improbable that the figures found were seriously influenced by a sudden input of young specimens resulting in an unrealistic high daily mortality.

Anopheles amictus hilli (Merauke)

Data on this species are represented in *Table X*. The very low daily mortality—5% daily overall—is interesting.

Anopheles amictus hilli is a rather stable species in the Merauke district during the wet season; it readily attacks man but is caught in smaller numbers than *A. farauti* or *A. bancrofti* in the same locality. It is also less inclined to fly indoors for a blood meal than are the former species. Thus, it is tempting to explain the very significant differences in mortality rates by the effects of indoor spraying. This explanation, however, does not hold true for the period under observation. The solution has probably to be sought amongst the natural factors concerning the ecology of adult life that are different for these species. At present no further information is available, it will be subject for further research. It may be noted that *Anopheles amictus hilli* with a high expectation

TABLE X

DAILY MORTALITY IN *Anopheles amictus hilli* IN MERAUKE

Period	*Total invest.*	*Total parous*	*Total st. II*	*Tot. st. II par.*	*Prop. parous in %*	*p*	*Daily mort. in %*	*Mm rain (rain days)*
Feb. 1–14	41	36	36	34	94	0.97	3	50 (9)
Feb. 15–28	95	85	90	80	89	0.94	6	176 (11)
Mar. 1–14	33	27	32	26	81	0.90	10	61 (6)
Mar. 15–28	20	18	18	17	94	0.97	3	84 (10)
Apr. 29–11	38	31	26	24	92	0.96	4	79 (8)
	227	197	202	181	90	0.95	5	

of life has no vector properties, unlike the relatively shorter living but far more numerous *Anopheles farauti*, which acts as a vector of *Plasmodium* and *Wuchereria bancrofti* in this territory.

Comparison of evening and early morning catches

METSELAAR (1957) assumed that the hours after midnight were of more importance—as regards malaria transmission—than the evening. This conclusion was based on a significantly higher sporozoite rate found in individuals collected indoors by day, compared with specimens collected in the evening in the open. A significantly higher proportion of parous individuals should thus be expected in early morning catches compared with evening catches of the same date. Data derived from ovary dissections in four anopheline species, however, do not support this view. The differences in the sporozoite rates as reported will probably not be due to a consistent difference in distribution of age groups in the biting population at different times of the night (*Table XI*).

In *Anopheles koliensis* at Hollandia the differences in numbers biting in early evening and early morning were very striking; especially just after sunset, the density was relatively high the year round.

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sis (in Hollandia, DDT sprayed), *Anopheles farauti* (in Merauke, dieldrin sprayed), *Anopheles bancrofti* (in Merauke, dieldrin sprayed and at Kimaan, unsprayed) and *Anopheles amictus hilli* (in Merauke, dieldrin sprayed).

It was decided whether or not a dissected individual was parous from the size of the ampullae of the oviducts (Mer's technique) and from several additional qualitative characters. The author was unable to make the corpora lutea technique work in the species investigated. All specimens were obtained in leg catches, except for *Anopheles bancrofti* at Kimaan, which were collected from walls of native huts.

During an observation period of three months in Merauke a daily mortality of 32% in *Anopheles farauti*, 31% in *Anopheles bancrofti* and 5% in *Anopheles amictus hilli* was found. The highly significant difference between the former two and the latter species most probably results from different factors concerning adult ecology; it was improbable that the difference was due to selective kill by the residual spraying. The daily mortality in *Anopheles bancrofti* in sprayed and unsprayed territory was not significantly different (31% viz. 36%). Both *Anopheles bancrofti* and *Anopheles amictus hilli* are rather stable species in Southern New Guinea.

An extensive series of observations was made on *Anopheles koliensis*, an unstable species of the *punctulatus*-group, in Hollandia. Short term fluctuations in the proportion parous were found from catch to catch, due to irregular input in the population of newly emerged females. Some evidence was produced for the existence of a long term mechanism, producing a significantly different daily mortality at different times of the year. The factors responsible for this latter movement are not yet clear.

In *Anopheles koliensis* a variation of 20% was found as amplitude of the long term fluctuation, the extremes being a daily mortality of 10% and 30%.

It was concluded that a daily mortality as calculated does not suggest an efficient control of the *koliensis* population as a whole. The decrease in malaria incidence after the start of a DDT house spraying campaign in Hollandia can probably be explained by the higher mortality of the house-entering mosquitoes. It must be assumed that more mosquitoes infect themselves on a gametocyt carrier indoors than in the open. No significant differences in proportions parous in samples, collected in the evening and the early morning hours of the same day, were found.

Pre-gravidity in the sense of Gillies, was not found in the species investigated. Unlike *Anopheles gambiae*, the first blood meal most probably suffices for a development of the eggs beyond stage II.

RESUMEN

Sobre la mortalidad diaria de cuatro especies de anofelinos de la Nueva Guinea. Se ha calculado la mortalidad diaria, mediante la fórmula $p^n = M$, de Macdonald, para cuatro especies de anofelinos de la Nueva Guinea, procedentes de tres localidades: *Anopheles koliensis* (en Hollandia, donde se aplicó el rociamiento con DDT), *Anopheles farauti* (en Merauke, donde se usó dieldrín), *Anopheles bancrofti* (en Merauke, con rociamiento de dieldrín, y en Kimaan, sin tratamiento con insecticidas), y *Anopheles amictus hilli* (en Merauke, con un rociamiento de dieldrín).

Se dedujó del tamaño de las ampollas de los oviductos (con la técnica de Mer), así como de varias características cualitativas, si un insecto había sido fecundado o no. El autor no logró practicar la técnica para el examen de los cuerpos lúteos en las especies examinadas. Todos los especímenes han sido obtenidos, permitiéndoles picar

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CULEX FATIGANS FROM NEW-GUINEA AND *AEDES POLYNESIENSIS* FROM SAMOA AS INTERMEDIATE HOSTS OF *WUCHERERIA BANCROFTI* (PERIODIC FORM)

by

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INTRODUCTION

This paper deals with laboratory experiments carried out to investigate the ability of two species of mosquitoes to act as an intermediate host of the periodic form of *Wuchereria bancrofti*. The species of mosquitoes were:

(1) A laboratory-bred strain of *Culex fatigans* originally coming from Sorong in West New-Guinea.

(2) A laboratory-bred strain of *Aëdes polynesiensis* originally coming from Samoa.

Fig. 1. $1\frac{1}{2}$ days after infective feed in *A. polynesiensis*.

The mosquitoes were infected by feeding them on a microfilariae carrier who had acquired filarial infection in Paramaribo, Suriname.

Our investigations were concerned with two subjects:

(1) In New-Guinea the microfilariae of *W. bancrofti* exhibit a nocturnal periodici-

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