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MALARIA BUREAU REPORTS.

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PREFACE.

This volume continues the publication of data collected by the Malaria Bureau, and contains a record of the observations made during the last three years on the distribution of anophelines.

The first report is concerned with the distribution of anophelines in a localised area, and is followed by summaries of the results obtained during the last three years, first on the subject of distribution in general, and secondly on the species of anophelines that were found in domestic receptacles of water. The second report deals with an outbreak of malaria at the mouth of a river and the distribution of anophelines in relation thereto; it is followed by notes on the distribution of anophelines in mangrove.

It is relevant to note here that a new method for studying distribution has been introduced in this volume. The method is described on page 7, and the results of its application to the data collected during the last three years are summarised on page 23. It is hoped that this method will produce wider and more useful results than the methods generally used at present.

Certain alterations in the names of the species have become necessary since the first volume was issued in November, 1919. These are described on page 1, and should be studied before the reports are read. It is necessary to use these new names in order that the records may be uniform and comparable with the records of workers in other countries, but the disappearance of such familiar terms as "rossi" and "sinensis" is regrettable and will probably cause some confusion until the new names become familiar.

During my absence on leave the work of the Bureau is being carried on single-handed by Dr. W. A. Lamborn. I hope that his observations will be published in a future volume. The present volume, however, I am solely responsible for.

The material for this publication was prepared while I was enjoying the hospitality of the Lister Institute of Preventive Medicine, and every facility was placed freely at my disposal by the staff of that Institution. I am also much indebted to Dr. G. A. K. Marshall and Mr. F. W. Edwards, of the British Museum, for help and advice on several occasions.

H. P. HACKER.

LONDON,

November, 1920.

NOTES TO PREFACE.

In view of the detailed nature of the tables of contents and synopses of the reports it has been considered that an index to this volume is superfluous.

The experiments on the mode of action of oil, which were mentioned on page 60 of Volume I., have not yet been completed. The publication of their results has, therefore, been deferred to a later volume.

CONTENTS.



	PAGE
CHANGES IN NOMENCLATURE OF SPECIES	1

REPORT I.—

ON THE DISTRIBUTION OF ANOPHELINES IN THE BRICKFIELDS ROAD AREA OF KUALA LUMPUR, NOVEMBER, 1917	3
--	---

IA.—NOTES ON THE ASSOCIATION METHOD AS APPLIED TO A LARGER MASS OF DATA, NOVEMBER, 1920	12
--	----

IB.—NOTES ON ARTIFICIAL RECEPTACLES OF WATER AS BREEDING PLACES OF ANOPHELINES, NOVEMBER, 1920	25
--	----

REPORT II.—

ON AN OUTBREAK OF MALARIA ON THE BANKS OF THE PERAK RIVER, JANUARY, 1918	29
---	----

IIA.—NOTES ON THE RELATION OF ANOPHELINE BREEDING TO MANGROVE, JANUARY, 1918	45
---	----

TABLES.

RELATING TO THE BRICKFIELDS ROAD AREA OF KUALA LUMPUR.

	PAGE
A.—Relative frequency of the species found	5
B.—Distribution of species	<i>facing page</i> 6
C.—Association values of the species found	9

RELATING TO THE OUTBREAK OF MALARIA ON THE BANKS OF THE PERAK RIVER.

D.—Extent of the outbreak	31
E.—Species found during the investigation	32
F.—Differentiation between the larvae of <i>A. kochi</i> , <i>A. tessellatus</i> and <i>A. aurirostris</i>	33
G.—Distribution of species	35
H.—Relation of the incidence of the outbreak to rainfall	37
Comparison of species found in December, 1917, with those found in January, 1918:—	
J.—On Bagan Datoh Estate (Lower Division)	38
K.—In the garden of the Bagan Datoh Rest-house	39
L.—At the native-owned plantation half a mile down river from Bagan Datoh	39

PLANS.

1.—Brickfields Road Area	<i>facing page</i> 6
2.—Mouth of Perak River	30

DIAGRAMS.

1.—Curves showing relation of the incidence of the outbreak of malaria to rainfall <i>facing page</i> 37	
2.—Diagram showing possible relationship between mangrove and anopheline breeding	47

CHANGES IN NOMENCLATURE OF SPECIES.

The variety of *A. rossi*, which is the common one in the Malay States, and which was previously called *A. rossi* var. *indefinitus*, has been shown ⁽¹⁾ to be the species described as *A. vagus* by Dönitz in 1902. The latter name will be used therefore in this volume and in future reports.

The Indian type, previously called *A. rossi* (Giles), has been regarded by Edwards ⁽²⁾ as identical with the *A. subpictus* of Grassi. In the Malay States there is a variety with a larva that differs from that of *A. vagus*, and resembles the larva of the Indian type. The adult hatching from this kind of larva has a distal black band on the female palps, which tends to be broader than the corresponding band of *A. vagus*. This variety was called *A. rossi* (Giles) in the first volume, but Edwards has examined some of the specimens and considers that they cannot properly be called *A. subpictus* (*rossi*, Giles), first, because the black band is not sufficiently broad, and, secondly, because it is not sufficiently uniform in the different specimens. Barber, who first differentiated ⁽³⁾ this type in the Malay States, showed these two facts in a long series of measurements of the palpal bands, and also suggested that owing to the difference in habitat the type might not be the same as the Indian form. The observations of Barber on its breeding places are confirmed by the evidence brought forward in this volume, and the variety has therefore been distinguished from the Indian form by calling it—in accordance with a suggestion from Mr. Edwards—*A. subpictus* var. *malayensis*.

Specimens which were identified as the *A. hunteri* of Strickland have been examined by Edwards, and he finds that they correspond with Leicester's specimens of *A. separatus*, although they do not agree exactly with his description. The latter name has therefore been applied to these specimens.

Mr. Edwards has also shown reasons ⁽⁴⁾ for altering the name of *A. sinensis* to *A. hyrcanus*, and accordingly the latter name has been used in this volume.

These alterations may be summarised as follows for easy reference:—

A. rossi var. *indefinitus* (Ludlow) becomes *A. vagus* (Dönitz).

A. rossi (Giles) becomes *A. subpictus* (Grassi).

Variety wrongly called *A. rossi* (Giles) in the first volume becomes *A. subpictus* var. *malayensis*, nov.

A. hunteri (Strickland) becomes *A. separatus* (Leicester).

A. sinensis (Wiedemann) becomes *A. hyrcanus* (Pallas).

⁽¹⁾ Swellengrebel, N.H., and Swellengrebel—de Graaf, J.M.H., 1920. Bull. Entom. Res., Vol. XI, p. 89.
See also Christophers, S. R., 1916. Ind. Journ. Med. Res., Vol. III, p. 477.

⁽²⁾ Edwards, F. W., 1920. Bull. Entom. Res., Vol. X., Pt. II, p. 129.

⁽³⁾ Barber, M. A., 1917. Philippine Journ. Science, Vol. XIII, Sect. B., pp. 1-46.

⁽⁴⁾ Edwards, F. W., 1920. Bull. Entom. Res., Vol. X., Pt. II, p. 129.

REPORT I.

ON THE DISTRIBUTION OF ANOPHELINES IN THE BRICKFIELDS ROAD AREA OF KUALA LUMPUR, NOVEMBER, 1917.

SYNOPSIS.

- 1.—Reason for the investigation.
- 2.—Date of the mosquito survey.
- 3.—Number of observations—the basis of the report.
- 4.—Species of anophelines found.
- 5.—Description of the area examined.
- 6.—Typical breeding places of the species found.
- 7.—A new method for studying the typical distribution of species.
- 8.—The grouping of the species found into representative faunae.
- 9.—Summary and conclusions.

IA.—NOTES ON THE ASSOCIATION METHOD AS APPLIED TO A LARGER MASS OF DATA, NOVEMBER, 1920.

SYNOPSIS.

- 1.—Amount of data available for calculating association values.
- 2.—Association values of the various species.
- 3.—Summary and conclusions.

IB.—NOTES ON ARTIFICIAL RECEPTACLES OF WATER AS BREEDING PLACES FOR ANOPHELINES, NOVEMBER, 1920.

SYNOPSIS.

- 1.—Problem stated.
- 2.—Observations.
- 3.—Discussion of observations.
- 4.—Conclusions.

REPORT I.

ON THE DISTRIBUTION OF ANOPHELINES IN THE
BRICKFIELDS ROAD AREA OF KUALA LUMPUR, NOVEMBER, 1917.

1.—REASON FOR THE INVESTIGATION.

A request was received from the Executive Engineer, Malaria Advisory Board, for information on the mosquito breeding properties of this swampy area, with a view to deciding whether the eradication of the swamps was advisable from the point of view of their being a possible source of malarial infection. Moreover, the Medical Officer in charge of the General Hospital had reported that a large number of the cases of malaria treated at that hospital came from this area, and had suggested that a mosquito survey of the area might be useful in finding the source from which these cases had been infected.

2.—DATE OF THE MOSQUITO SURVEY.

The field work was done during October and November, 1917. During this period the weather was on the whole drier than usual, the rainfall for October being 5.97 inches, and for November 7.13 inches, whereas the average monthly rainfall for the whole year was 8.82 inches⁽¹⁾. The rainfall, however, was very unevenly distributed, and on two occasions as much as 2.76 inches and 2.56 inches fell in 24 hours. These heavy rainfalls caused the flooding of certain parts of the area, and so may have altered to some extent the usual distribution of species.

3.—NUMBER OF OBSERVATIONS—THE BASIS OF THE REPORT.

The report is based on the examination of 513 breeding places found in the area, and on the identification of 5,205 larvae and 779 adults collected from them. This total of 5,984 specimens contained representatives of 9 different species.

After the species of the larvae had been determined by microscopic examination, 540 of them developed into adults, and in no case was there a discrepancy between the identification of the adult insect and the previous identification of its larva, although 8 species out of the 9 found were dealt with. It is submitted that this absence of discrepancy shows the accuracy and value of previous work on the identification of larvae, since the larval characters used were those described by Stanton⁽²⁾ and Strickland⁽³⁾; and, also, that it forms a test of the reliance that may be placed on the identification of species throughout this report, in spite of the fact that it is based mainly on the examination of larvae and not of adults.

(1) General Hospital Records.

(2) Stanton, A. T., 1915. Bull. Entom. Res., Vol. VI., Pt. II., pp. 159-172.

(3) Strickland, C., 1915. Short Key to the Identification of the Larvae of the Common Anopheline Mosquitos of the Malay Peninsula, Central Survey Office, Kuala Lumpur, F.M.S.

4.—SPECIES OF ANOPHELINES FOUND.

The following table shows the species found, together with their relative frequency.

TABLE A.

Species.	TIMES FOUND.		TOTAL SPECIMENS FOUND.	
	Number.	Percentage.	Number.	Percentage.
<i>A. barbirostris</i>	282	39.6	2,025	33.8
<i>A. hyrcanus</i>	123	17.3	335	5.6
<i>A. kochi</i>	129	18.1	902	15.1
<i>A. vagus</i>	106	14.9	2,511	42.0
<i>A. subpictus</i> var. <i>malayensis</i>	2	0.3	3	0.05
<i>A. tessellatus</i>	33	4.6	113	1.9
<i>A. aconitus</i>	16	2.2	56	0.9
<i>A. fuliginosus</i>	14	2.0	21	0.4
<i>A. maculatus</i>	8	1.1	18	0.3
TOTALS	713	100.1%	5,984	100.05%

It will be seen from the table that the species commonly regarded as the most dangerous, namely, *A. maculatus*, *A. fuliginosus* and *A. aconitus*, were relatively uncommon. It must be realised, however, that, with the exception of *A. tessellatus*, about which no records are available, all the species mentioned have been infected with malaria; and until the relative pathogenicity of each species has been determined as quantitatively as possible, the full value of work on distribution of anophelines will not be obtained.

Of the four species so predominant that they may be regarded as the characteristic species of the neighbourhood, viz., *A. barbirostris*, *A. hyrcanus*, *A. kochi* and *A. vagus*, as a rule the two former breed in large reedy pools or ponds, and the two latter in small open pools or puddles. A great disparity is seen between the frequency with which these species were obtained and the number of specimens obtained; thus, the large pool breeders were found in 56.9 per cent. of the breeding places and supplied only 39.4 per cent. of the specimens, whereas the small pool breeders were found only in 33 per cent. of the places, but supplied 57.1 per cent. of the specimens. The more widely distributed species therefore supplied fewer specimens than the more localised species. This suggests that there is a concentration of the less widely distributed species in the places which happen to be suitable as breeding places, although the fact that it is easier to collect specimens from a small open pool may

have some effect in causing this disparity. Further quantitative field work is called for since the extent to which such a concentration may occur has a bearing on all questions of distribution, and especially on the importance of isolated breeding places which differ from the general nature of their surroundings.

5.—DESCRIPTION OF THE AREA EXAMINED AND ITS ANOPHELINE DISTRIBUTION.

The area examined is shown on the accompanying plan. It consisted ⁽¹⁾ of open swampy land lying between the railway line and the river, and for the purpose of description has been divided into 18 districts, each being a small unit having fairly definite and uniform characteristics. Each of these districts is marked by a reference letter on the plan, and the corresponding Table B shows a short description of each district and the details of the species found therein, together with a remark as to whether the large pool breeders or the small pool breeders predominated in the district.

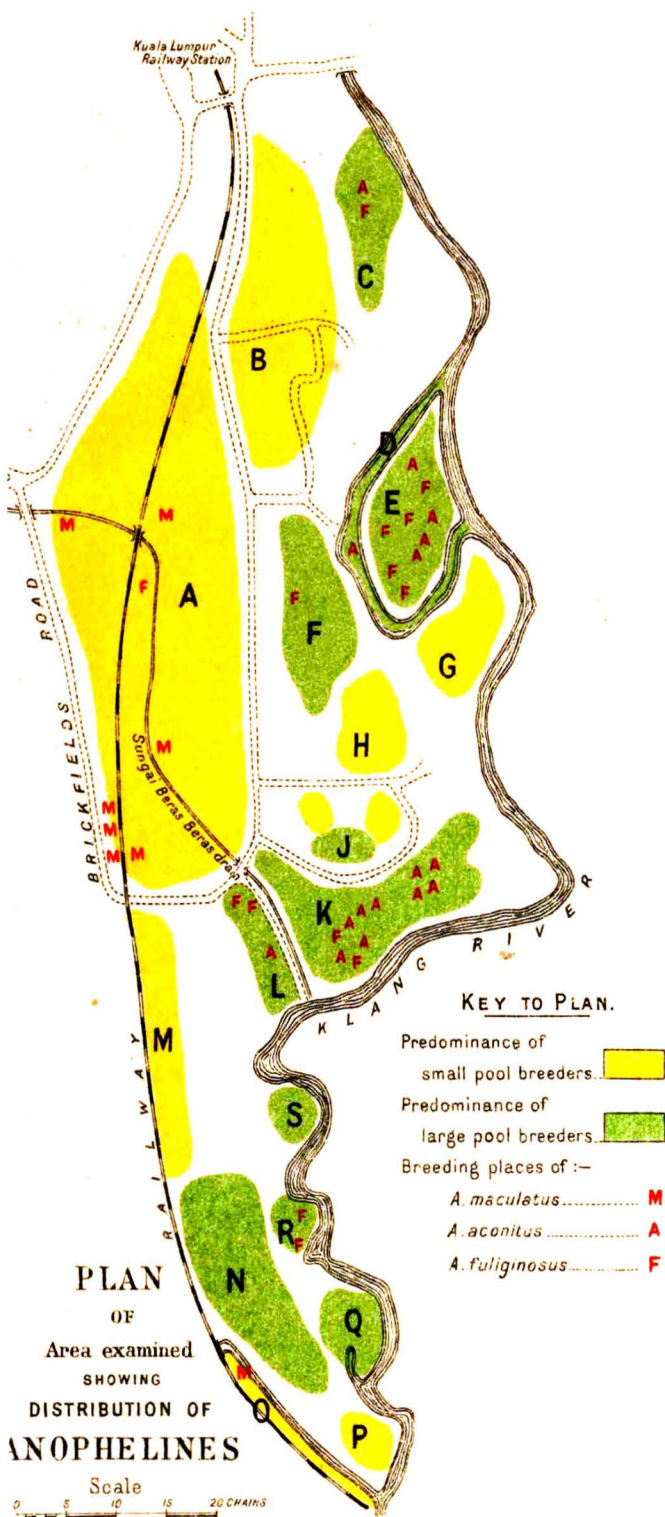
There were open grassy swamps alongside the railway (Districts A, M, O and P), characterised by the predominance of the small pool breeders, *A. kochi* and *A. vagus*. These were the only species found in the occasional pools amongst the houses of District B and in the Chinese village (District G). In the duck ponds near the Chinese village (District H) *A. vagus* was the characteristic species.

There were deeper swamps along the course of the river and in its bends and backwaters, for example, Districts Q, R, S and K. These were characterised by the predominance of the large pool breeders, *A. barbirostris* and *A. hyrcanus*. District D was a swamp in the old bed of the river, and District E a deep swamp encircled by the original bend in the course of the river and the new straight cut into which the stream had been diverted. District N was a wide swampy ravine stretching across the area from the railway and draining into a backwater of the river. District J was a swamp in which filling had been commenced at its upper and lower ends, leaving a swamp in the centre; the distribution of anophelines was more equally balanced here, the small pool breeders being found in the filled portion, and the large pool breeders in the central swamp.

The districts in which the small pool breeders predominated are coloured yellow on the plan, the districts characterised by large pool breeders are coloured green, and the places at which the accredited carriers of malaria were found are shown by red letters.

It is seen from the plan that *A. maculatus* was found only in those districts in which the small pool breeders predominated, whereas the other carriers, *A. aconitus* and *A. fuliginosus*, were closely associated with the large pool breeders.

(¹) Since the date of this survey a large part of the area has been filled in and utilised for railway sidings and houses.



Reference Letter of District.	DESCRIPTION OF THE DISTRICT.
A	Open grassy swamps with a few large pools. Some seepage water from the railway embankment and along the course of the Sungei Beras drain
B	Area characterised by houses with occasional small pools.
C	Swamp in old river bed.
D	Stagnant water in old river bed with reedy edges and also small muddy pools
E	Swamp encircled by old river bed and containing large open pools.
F	Swamp on bank of old river bed with large pools.
G	Chinese village on rather high ground.
H	Large muddy pools used as duck ponds.
J	Area partly "filled" leaving a swamp in the centre.
K	Extensive swamp with secondary growth and large fish ponds.
L	Swampy strip along the Klang Road as far as the river.
M	Pools and drains along the railway.
N	Swampy ravine with secondary growth of vegetation and three large reedy pools.
O	Open area between railway embankment and a small sandy stream.
P	Open grassy swamp with many buffalo wallows and hoof marks.
Q	Swamp around a backwater of the river.
R	Swamp in bend of the river.
S	Swamp in bend of the river.

6.—TYPICAL BREEDING PLACES OF THE SPECIES FOUND.

Although an experienced collector is able to prophesy from the nature of the breeding place, with some degree of accuracy, what species will be found therein, it is quite impossible to classify the characteristics of breeding places so as to constitute a description of the typical breeding place of each species. Even when the descriptions of the 513 breeding places dealt with in this report are analysed and classified, complete confusion results as a consequence of the large number of categories to be considered, many of which will not be found mentioned in each description, and all of which will be subject to the error involved in describing characters which cannot be exactly measured, such as the amount of shade, the amount of mud, or the rate of flow of water; characters which themselves vary from time to time. Dr. Strickland's elaborate attempt to co-ordinate and classify the observations of five years (1) shows clearly the limitations of this method as a means for determining the typical breeding place of each species, and therefore another method of co-ordinating the facts relating to distribution has been introduced into this survey.

7.—A NEW METHOD FOR STUDYING THE TYPICAL DISTRIBUTION OF SPECIES.

This method is based on the numerical frequency with which the various species are found together in nature, and thereby shows which species are most commonly found in the same breeding place in association with any given species. The number of times a species is found alone, added to the number of times it is found in association in the same breeding place with each of the other species, gives the total number of "association units" available for that species. It is necessary for the purpose of comparison to express the component parts of this number as percentages, and it is permissible to do so if the total number of association units, on which this series of percentages is based, is also shown. A convenient term for the percentage association of one species with each of the other species is "association value."

The method has the advantage that it does not depend on personal descriptions of individual breeding places, but is built up quite mechanically from the records of the species found in a given number of breeding places.

The objects for which this method of recording the results has been introduced may be enumerated as follows:—

(1) If each species has a typical breeding place, then those species found most commonly together would form a group or fauna based on a common preference for a certain type of breeding place. Each fauna could then be correlated to the type of country it inhabits by a knowledge of the usual breeding places of its commoner members; and thus an ecology of the Malayan anophelines could be built up.

(1) Unpublished report which can be referred to at the Bureau.

(2) Any marked inter-association between two species belonging to different faunae would indicate that the cause for this inter-association should be looked for in the breeding habits of the two species.

(3) The association values of a species, based on all previous observations, will form a convenient measure of its distribution, and will replace general impressions of the usual distribution of that species as a standard with which its distribution in a newly investigated area may be compared. The association values of the species calculated on the results obtained from the newly investigated area will form a measure of its distribution in that area, and can be compared readily with the standard figures based on all previous observations.

If the values for the new area differ markedly from the standard figures, this fact will indicate that some local condition in the area must be sought for as the cause of the difference.⁽¹⁾

(4) The association values of a species will show what other species may be expected in its breeding place. They may be of use, therefore, for estimating the probability of dangerous species being present when only a relatively innocuous one has been found, but the figures must become very large and must be based on a very wide experience before such a use of them would be justified.

8.—THE GROUPING OF THE SPECIES FOUND INTO REPRESENTATIVE FAUNAE.

When the association values are calculated for the nine species found in the present survey, three of the species are distinguished from the rest by having the association value for themselves higher than the association value for any other species. In other words, they were more frequently found by themselves, while all the other species were more often found in association with one of these three species than alone. These three species may be regarded, therefore, as types with typical breeding places, whereas the others have not such typical breeding places, but favour breeding places similar to one or another of the type species.

One of these type species is *A. barbirostris*, and the following species show a higher association value for it than for any other species, viz., *A. hyrcanus*, *A. fuliginosus*, *A. aconitus* and *A. subpictus* var. *malayensis*. *A. barbirostris* is usually found in large open reedy pools, and therefore this group may be regarded as characteristic of such places.

Another of the types is *A. kochi*, a species preferring small open grassy pools. The fact that the highest association value of *A. tessellatus* is for *A. kochi* groups these two species together.

The third type species is *A. vagus*, which is usually found in open muddy pools, and the species with the highest association value for it is *A. maculatus*.

⁽¹⁾ The figures from the new area will be added in their turn to the previous figures to form a new standard for comparison with new work. The divergence which they showed will be swamped by the greater mass of "normal observations," but such divergence will have some effect on the association values, which will then reflect a wider experience.

The following table shows the association values of the various species, arranged in the groups defined above, and is followed by remarks on the significance of the figures for each species in the order shown in the table :—

TABLE C.

SPECIES.	Total association units on which the association values are calculated.	ASSOCIATION VALUES.								
		<i>A. barbirostris.</i>	<i>A. hyrcanus.</i>	<i>A. fuliginosus.</i>	<i>A. aconitus.</i>	<i>A. subpictus var. malayensis.</i>	<i>A. kochi.</i>	<i>A. tessellatus.</i>	<i>A. vagus.</i>	<i>A. maculatus.</i>
<i>A. barbirostris</i>	260	39*	29	5.5	5	1	7.5	1.5	12	0
<i>A. hyrcanus</i>	159	48*	15	4.5	4.5	0	12	5.5	10.5	0
<i>A. fuliginosus</i>	27	52*	26	0	7	4	0	0	11	0
<i>A. aconitus</i>	25	52*	28	8	4	0	4	0	4	0
<i>A. subpictus var. malayensis</i>	4	50*	0	25	0	0	0	0	25	0
<i>A. kochi</i>	146	13	13	0	1	0	49*	10	12	1
<i>A. tessellatus</i>	42	9.5	21	0	0	0	35.5*	31	2	0
<i>A. vagus</i>	124	24	14	2.5	1	1	14.5	1	39.5*	3
<i>A. maculatus</i>	9	0*	0	0	0	0	22.2	0	44.4*	33.3

The maximum association value for each species is marked by an asterisk.

A. barbirostris.—This is the type species of a group since it occurs most commonly by itself (39 per cent.). The fact that it is found usually in large open pools with reedy edges, stamps the species grouped with it in the table as belonging to an open swamp fauna, preferring large collections of water to breed in.

Its chief associate is *A. hyrcanus* (29 per cent.), and the recognised malaria carriers associated with it are *A. fuliginosus* (5.5 per cent.) and *A. aconitus* (5 per cent.). This means that having found *A. barbirostris* the chances are about 1 in 3½ that *A. hyrcanus* would be present in the same place, and about 1 in 20 that either *A. fuliginosus* or *A. aconitus* would be present. These probabilities are calculated only on the results of the present survey, and are applicable only to the area under investigation. When the figures become very large and contain results from all kinds of country, it is hoped that the probabilities will have a wider application.

The association values for the small pool breeders, *A. vagus* (12 per cent.) and *A. kochi* (7.5 per cent.), are rather higher than the values which might be expected from a general impression of the breeding places of those species. This unexpectedly high association is probably due to the flooding of parts of the area referred to previously (1), and to the effect of the heavy rainfalls in washing out larvae from small pools into the larger swamps. If this is so, these association values will differ from those of other areas, and the difference will be due to local conditions in the

area at present being studied. When the method is applied universally to a large series of data the effect of local variations will be overshadowed by the mass of normal observations, the figures for each species will form a measure of the usual distribution, and so replace general impressions as the standard of what to expect. A case such as this would then form a divergence from the usual association values, and thereby show that some local condition must be sought for as the cause of the divergence.

Perhaps the most striking fact in the table is that *A. barbirostris* and all the members of its group show no association with *A. maculatus*. The method is capable, therefore, of expressing the facts that have already been seen in the study of the distribution of *A. maculatus* throughout the various districts of the area. See page 6 and the corresponding plan.

A. hyrcanus.—Its highest association is with *A. barbirostris* (48 per cent.) and its next highest association is with itself (15 per cent.).

Its value for *A. kochi* (12 per cent.), the typical small grassy pool breeder, is higher than that of *A. barbirostris* for *A. kochi*, although the value for *A. vagus* (10.5 per cent.) is about the same in the two species. This tends to confirm a general impression that *A. hyrcanus* prefers rather smaller collections of water than those used by *A. barbirostris*, although it breeds in very similar places. Its association values for the carriers *A. fuliginosus* (4.5 per cent.) and *A. aconitus* (4.5 per cent.) are about the same as those of *A. barbirostris* for these species.

A. fuliginosus.—It is very closely associated with *A. barbirostris* (52 per cent.), and its next association value is with *A. hyrcanus* (26 per cent.). As the table shows, *A. fuliginosus* was never found by itself, and since 78 per cent. of its associations are with these two species it undoubtedly belongs to the open swampy pool fauna.

A. aconitus.—Its association values are very similar to those of *A. fuliginosus*, 80 per cent. of them being with *A. barbirostris* and *A. hyrcanus*.

A. subpictus var. *malayensis*.—Although the figures for this species are small they show a very typical association. In marked contrast with its close relative *A. vagus*, which prefers small pools, it is usually found in large pools with reedy edges. Of its four associations, two are with *A. barbirostris* (50 per cent.) and one each with *A. fuliginosus* (25 per cent.) and *A. vagus* (25 per cent.).

A. kochi.—*A. kochi* is the type species of the second group, because it is found more commonly (49.1 per cent.) by itself than with any other species; and this is probably due to its being the species most typical of the small grassy pools which it prefers for breeding purposes. It has associations with the swamp breeders, *A. barbirostris* (13 per cent.) and *A. hyrcanus* (13 per cent.), and with the other typical small pool breeder, *A. vagus* (12 per cent.). It is associated with *A. tessellatus* (10 per cent.); and was found with *A. maculatus* (1 per cent.), the species most dependent on light open places for breeding in.

A. tessellatus.—*A. tessellatus* has its highest association value for *A. kochi* (35.5 per cent.), and is therefore grouped with it in this series of observations. But

the value for itself is almost as high (31 per cent.), which indicates that it tends to have a breeding place of its own type. This is because it often inhabits pools with decaying leaves in swamp jungle or secondary growth, a fact that is shown by its never having been found in association with *A. maculatus*.⁽¹⁾

A. vagus.—*A. vagus* is the type of the third group, since its highest association value (39.5 per cent.) is for itself. This is probably due to its being the species found typically in small muddy pools and puddles. It is associated fairly frequently (14.5 per cent.) with *A. kochi*, the small grassy pool breeder. Three per cent. of its associations are with *A. maculatus*, and this is because some of the pools in which it breeds are in ditches and at the foot of banks, and are therefore freshened by the seepage of ground-water, which renders the places suitable for *A. maculatus*.

A. maculatus.—Its highest association value (44.4 per cent.) is for *A. vagus*, but it is found by itself almost as frequently (33.3 per cent.). This indicates that although found in close association with the small muddy pool breeder it has a fairly distinct habitat, namely, fresh spring or seepage water. It is also found in association with *A. kochi* (22.2 per cent.), the small grassy pool breeder. In this series of figures it has no association with any of the large pool breeders,⁽²⁾ nor with *A. tessellatus* the shade breeder.

9.—SUMMARY AND CONCLUSIONS.

(a) A method for grouping the species found into representative faunae has been introduced, and will be applied to further results as a method for elucidating problems of distribution.

(b) The most widely distributed species in the area were the large pool breeders, *A. barbirostris* and *A. hyrcanus*; and the recognised malaria carriers, *A. fuliginosus* and *A. aconitus*, although not numerous at the time of examination, have been shown to be closely associated with these species by a preference for similar breeding places. It is possible, therefore, that, under other conditions the carriers may become much more numerous.

(c) The small pool breeders, *A. vagus* and *A. kochi*, were not so widely distributed, but supplied proportionately more specimens. The recognised carrier *A. maculatus* has been shown to occur in the same kind of places as these species, and in more continuous wet weather would probably be more numerous, owing to the increase in seepage water and small pools in the districts characterised by the predominance of small pool breeders. The weather at the time of the examination was unfavourable for the breeding of *A. maculatus* since, in dry weather with occasional heavy rainfalls, many of the springs would be dry and those containing water would be flushed out by the intermittent heavy rainfalls.

(d) An adequate cause of the malaria cases admitted to hospital from this area has been found therefore, and the reclamation of these swamps is justified as a measure for malaria prevention, apart from other considerations, such as the enhanced value of the land and the removal of a large source of general mosquito nuisance.

⁽¹⁾ But see later results on p. 19.

⁽²⁾ But see later results on p. 15.

IA.—NOTES ON THE ASSOCIATION METHOD AS APPLIED TO A LARGER MASS OF DATA, NOVEMBER, 1920.

I.—AMOUNT OF DATA AVAILABLE FOR CALCULATING ASSOCIATION VALUES.

The method of studying the association of species, which was introduced ⁽¹⁾ for the purpose of dealing with the facts relating to distribution in the report on the Brickfields Road Area, has been used for co-ordinating the data obtained by three years' collecting. The figures that have accumulated will now be recorded so that the general trend of the results may be seen, and that the validity of the method may be criticised.

The data available up to the present is based on the examination of 4,049 breeding places and the identification of 39,605 specimens. As batches of data became available they were plotted on a table which was similar in form to the one on page 9, but which was left open, so that at any period the number of times a given species had been found in association with each of the other species could be seen at a glance.

The identification of species depended mainly on the examination of larvae, and there are unfortunately certain pairs of species in which it is impossible to say with certainty to which of the two species a given larva belongs, unless it is allowed to develop into an adult. These pairs are:—

A. ludlowi and *A. subpictus* var. *malayensis*.

A. maculatus and *A. karwari*.

A. umbrosus and *A. separatus*.

A. novumbrosus and *A. albotacniatus* var. *montanus*.

It is possible, however, by various means, such as by noting the habitat, or the species of adults hatching from neighbouring breeding places, or minor points in the structure of the larva, to say that the larva probably belongs to one of the pair rather than to the other.

Therefore, records referring to any of these species were entered on the table in two columns. A record in which the identification was certain, because it had been confirmed by the hatching of adults, was entered in one column, while a record in which the identification was only probable was entered in the other. In this account of the results only the certain records will be used for calculating association values; but the probable series of figures is available, and may be of use in indicating probable associations which are not shown in the restricted series of figures.

Moreover, results of collections made merely to obtain material for examination or experiment have been rigidly excluded from the table, and only results based on individual breeding places examined in field survey work have been entered. After these reductions have been made the data remaining for discussion consists of 4,076 association units.

(1) See page 7.

2.—THE ASSOCIATION VALUES OF THE VARIOUS SPECIES.

When the method is applied to this larger mass of data the fact that a species is found most commonly alone does not bear the importance given to it in the first description of the method (1). No less than fifteen out of the nineteen species were found more commonly by themselves than with any other species (2); so that some other method of grouping must be looked for.

When the commonest associate of each species is studied a grouping appears, and three pairs of species are seen to be closely inter-associated in that each is the commonest associate of the other.

These pairs are:—

- A. kochi* and *A. vagus*,
A. barbirostris and *A. hyrcanus*,
A. aitkeni and *A. leucosphyrus*,

and each pair forms a centre around which the other species can be grouped in the following manner:—

GROUP I.—SMALL POOL FAUNA.

Central pair: *A. kochi* and *A. vagus*.

Members: *A. maculatus* is most often associated with *A. kochi*, and as *A. karwari* is most commonly found with *A. maculatus*, it also comes into this group. *A. ludlowi* is associated with *A. vagus*.

GROUP II.—SWAMP FAUNA.

Central pair: *A. barbirostris* and *A. hyrcanus*.

Members: *A. acomitus* is mainly associated with *A. barbirostris*, and *A. fuliginosus*, *A. tessellatus* and *A. separatus* with *A. hyrcanus*. *A. umbrosus* and *A. subpictus* var. *malayensis* show the same association value for each of the central members of this group.

GROUP III.—JUNGLE FAUNA.

Central pair: *A. aitkeni* and *A. leucosphyrus*.

Members: *A. albotaeniatus* var. *montanus* and *A. novumbrosus* show *A. aitkeni* as their chief associate.

GROUP IV.—SPECIES WITH NO ASSOCIATIONS.

Members: *A. aurostris* and *A. asiaticus*.

The association values of each member of these groups will now be given and the significance of the figures will be discussed.

(1) See page 8.

(2) As this may be due to the inclusion of results based on breeding places from which only a few larvae were examined, marks have been made on the table to indicate when 5 or more larvae were examined, and when 10 or more were examined. In the future, therefore, a series of results will be available based only on breeding places from which at least 5, or at least 10 larvae, have been examined. At present the three series show very similar results, and the total figures based on all examinations have been taken. The disadvantage of the other two series is that they show no figures for species commonly found sparsely distributed; and the disadvantage of using the total series of figures may be readily overcome by always attempting to obtain at least 10 specimens from each breeding place.

THE ASSOCIATION VALUES

GROUP I.—SMALL POOL FAUNA.

A. kochi.

Total number of association units	544
Found by itself	279	times or	51.3 per cent.
" with <i>A. vagus</i>	93	" "	17.1	"
" " <i>A. hyrcanus</i>	78	" "	14.3	"
" " <i>A. barbirostris</i>	30	" "	5.5	"
" " <i>A. tessellatus</i>	26	" "	4.8	"
" " <i>A. maculatus</i>	19	" "	3.5	"
" " <i>A. karwari</i>	10	" "	1.8	"
" " <i>A. aconitus</i>	3	" "	0.6	"
" " <i>A. leucosphyrus</i>	3	" "	0.6	"
" " <i>A. aitkeni</i>	2	" "	0.4	"
" " <i>A. fuliginosus</i>	1	" "	0.2	"
Totals	544	100.1	"	"

The figures show that *A. kochi* has a fairly wide distribution, but 68.4 per cent. of the associations are with itself and *A. vagus*, typical small pool breeders.

Among the swamp breeders its association with *A. hyrcanus* is greater than its association with *A. barbirostris*, and it has only a low association with jungle breeders.

A. vagus.

Total number of association units	816
Found by itself	514	times or	63.0 per cent.
" with <i>A. kochi</i>	93	" "	11.4	"
" " <i>A. hyrcanus</i>	84	" "	10.3	"
" " <i>A. barbirostris</i>	64	" "	7.8	"
" " <i>A. fuliginosus</i>	18	" "	2.2	"
" " <i>A. maculatus</i>	15	" "	1.8	"
" " <i>A. karwari</i>	10	" "	1.2	"
" " <i>A. aconitus</i>	6	" "	0.7	"
" " <i>A. tessellatus</i>	5	" "	0.6	"
" " <i>A. subpictus</i> var. <i>malayensis</i>	3	" "	0.4	"
" " <i>A. ludlowi</i>	3	" "	0.4	"
" " <i>A. umbrosus</i>	1	" "	0.1	"
Totals	816	99.9	"	"

A. vagus occurs 74.4 per cent. times alone or in association with *A. kochi*, which stamps it as a small pool breeder. It resembles *A. kochi* in showing a greater association with *A. hyrcanus* than with *A. barbirostris*, which tends to show that *A. hyrcanus*

with which these two species are found together in open ravine country as at Gemas (1). It also confirms the fact that *A. hyrcanus* breeds in smaller pools than *A. barbirostris*.

Its association with jungle species depends on the fact that it and *A. hyrcanus* enter very rapidly into a ravine when the jungle is felled (2). This is an example of one of the uses of the method, namely, that an inter-association between members of two faunae shows that the cause must be sought for in the breeding habits of the two species involved.

A. harwari.

Total number of association units	87
Found by itself	45 times	or 51.7 per cent.	
" with <i>A. maculatus</i>	15	" "	17.2	"
" " <i>A. vagus</i>	10	" "	11.5	"
" " <i>A. kochi</i>	10	" "	11.5	"
" " <i>A. hyrcanus</i>	5	" "	5.7	"
" " <i>A. tessellatus</i>	1	" "	1.2	"
" " <i>A. fuliginosus</i>	1	" "	1.2	"
			87		100	"

Its associations with *A. maculatus*, *A. vagus*, and *A. kochi* amply justify its presence in this group of small pool breeders.

A. ludlowi.

Total number of association units	17
Found by itself	14 times	or 82.4 per cent.	
" with <i>A. vagus</i>	3	" "	17.6	"
			17		100	"

The figures are too small to be of much value, but the frequency with which *A. ludlowi* is found alone depends on its specialisation for salt water. The fact that it is found in association with *A. vagus* shows that it breeds in similar places, namely, open muddy pools, and also that *A. vagus* can breed in brackish water.

When the figures become larger it is quite possible that *A. ludlowi* will separate itself off from this fauna and will form the typical species of a salt water fauna.

(1) See Vol. I., pages 52 and 59.

(2) See Vol. I., page 28.

GROUP II.—SWAMP FAUNA.

A. barbirostris.

Total number of association units	570
Found by itself	229	times or	40.2 per cent.
" with <i>A. hyrcanus</i>	169	" "	29.6 "
" " <i>A. vagus</i>	64	" "	11.2 "
" " <i>A. fuliginosus</i>	34	" "	6.0 "
" " <i>A. kochi</i>	30	" "	5.3 "
" " <i>A. aconitus</i>	27	" "	4.7 "
" " <i>A. tessellatus</i>	5	" "	0.9 "
" " <i>A. umbrosus</i>	5	" "	0.9 "
" " <i>A. maculatus</i>	3	" "	0.5 "
" " <i>A. subpictus</i> var. <i>malayensis</i>	3	" "	0.5 "
" " <i>A. leucosphyrus</i>	1	" "	0.2 "
Totals	570	100	" "

This series of figures corresponds fairly closely with a general impression of its breeding places. The low association value for *A. subpictus* var. *malayensis* depends merely on the rarity of that species, and is another example of the weakness of the method referred to in the remarks on *A. vagus* (¹). When, however, the figures for *A. subpictus* var. *malayensis*, on page 19, are examined, it is seen to be closely associated with *A. barbirostris* and to belong very obviously to this group.

In the case of *A. maculatus*, however, the low association value is a correct one as may be seen by referring to its figures on page 15.

A. hyrcanus.

Total number of association units	973
Found by itself	487	times or	50.1 per cent.
" with <i>A. barbirostris</i>	169	" "	17.4 "
" " <i>A. vagus</i>	84	" "	8.6 "
" " <i>A. kochi</i>	78	" "	8.0 "
" " <i>A. fuliginosus</i>	51	" "	5.2 "
" " <i>A. tessellatus</i>	46	" "	4.7 "
" " <i>A. aconitus</i>	19	" "	2.0 "
" " <i>A. maculatus</i>	16	" "	1.6 "
" " <i>A. aitkeni</i>	7	" "	0.7 "
" " <i>A. umbrosus</i>	5	" "	0.5 "
" " <i>A. karwari</i>	5	" "	0.5 "
" " <i>A. subpictus</i> var. <i>malayensis</i>	3	" "	0.3 "
" " <i>A. separatus</i>	2	" "	0.2 "
" " <i>A. leucosphyrus</i>	1	" "	0.1 "
Totals	973	99.9	" "

This series is very similar to that of *A. barbirostris*. Its association with *A. maculatus* is higher, however, for the reason shown in the remarks on the association of *A. maculatus* (see page 15).

(¹) See p. 15.

A. aconitus...

Total number of association units	78
Found with <i>A. barbirostris</i>	27	times or	34.6	per cent.
" " <i>A. hyrcanus</i>	19	" "	24.4	"
" " <i>A. fuliginosus</i>	10	" "	12.8	"
" by itself	8	" "	10.3	"
" with <i>A. vagus</i>	6	" "	7.7	"
" " <i>A. maculatus</i>	3	" "	3.8	"
" " <i>A. kochi</i>	3	" "	3.8	"
" " <i>A. subpictus</i> var.						
<i>malayensis</i>			2	" "	2.6	"
Totals	78		100	"

This series of figures shows that *A. aconitus* has a close association with the central species of this group, and no association with any of the members of Group III., jungle breeders.

A. fuliginosus.

Total number of association units	129
Found with <i>A. hyrcanus</i>	51	times or	39.5	per cent.
" " <i>A. barbirostris</i>	34	" "	26.4	"
" " <i>A. vagus</i>	18	" "	14.0	"
" by itself	10	" "	7.7	"
" with <i>A. aconitus</i>	10	" "	7.7	"
" " <i>A. subpictus</i> var. <i>malayensis</i>			3	" "	2.3	"
" " <i>A. karwari</i>	1	" "	0.8	"
" " <i>A. kochi</i>	1	" "	0.8	"
" " <i>A. tessellatus</i>	1	" "	0.8	"
Totals	129		100	"

The association of *A. fuliginosus* is very similar to that of *A. aconitus*, and shows the same absence of jungle breeders. These two recognised carriers of malaria, therefore, seem dependent on open sunlit places for breeding in, and this confirms a previous observation (1) on the intermixing of faunae that occurs at the edge of jungle.

(1) Vol. I., page 36.

A. subpictus var. *malayensis*.

Total number of association units	14
Found with <i>A. barbirostris</i>	3	times or	21.4	per cent.
" " <i>A. hyrcanus</i>	3	" "	21.4	"
" " <i>A. fuliginosus</i>	3	" "	21.4	"
" " <i>A. vagus</i>	3	" "	21.4	"
" " <i>A. aconitus</i>	2	" "	14.3	"
Totals	14		99.9	"

Although the figures are small they show that *A. subpictus* var. *malayensis* is very closely associated with the species of the swamp fauna and that it has never been found alone. In both these respects it shows a marked contrast to its near relative *A. vagus*, which is a typical small pool breeder.

A. tessellatus.

Total number of association units	152
Found by itself	51	times or	33.5	per cent.
" with <i>A. hyrcanus</i>	46	" "	30.2	"
" " <i>A. kochi</i>	26	" "	17.1	"
" " <i>A. leucosphyrus</i>	12	" "	7.9	"
" " <i>A. vagus</i>	5	" "	3.3	"
" " <i>A. barbirostris</i>	5	" "	3.3	"
" " <i>A. aitkeni</i>	2	" "	1.3	"
" " <i>A. umbrosus</i>	2	" "	1.3	"
" " <i>A. fuliginosus</i>	1	" "	0.7	"
" " <i>A. karwari</i>	1	" "	0.7	"
" " <i>A. maculatus</i>	1	" "	0.7	"
Totals	152		100	"

The figures show a very wide and general association—with swamp breeders, with small pool breeders, and with jungle breeders. This seems to show that *A. tessellatus* is not dependent on any special type of breeding-place. It has frequently been found in quite dense jungle, where there is no grass, but only decaying leaves in the pools; and the figures show clearly that it may be found in quite open places.

A. separatus.

Total number of association units	13
Found by itself	11 times or 84.6 per cent.		
„ with <i>A. hyrcanus</i>	2	„ „ 15.4	„	
			—	—		
Totals	...	13		100	„	
		—		—		

The figures are too small to be of real value, but they tend to confirm an impression that *A. separatus* prefers the open swamp just outside jungle, in contradistinction to its close relative *A. umbrosus*, which breeds typically in swampy jungle.

A. umbrosus.

Total number of association units	49
Found by itself	36 times or 73.5 per cent.		
„ with <i>A. barbirostris</i>	5	„ „ 10.2	„	
„ „ <i>A. hyrcanus</i>	5	„ „ 10.2	„	
„ „ <i>A. tessellatus</i>	2	„ „ 4.1	„	
„ „ <i>A. vagus</i>	1	„ „ 2.0	„	
			—	—		
Totals	...	49		100	„	
		—		—		

The large majority of the associations of *A. umbrosus* are with itself, because it is the typical species of low-lying swampy jungle (¹), but it comes into this group, because 10.2 per cent. of its associations are with *A. barbirostris* and with *A. hyrcanus*. It appears that at the present stage the method has led to the association of a swamp fauna and a hill jungle fauna; it may lead at a later stage to a differentiation of the swamp fauna into an open swamp and a jungle swamp fauna.

(¹) Vol. I., page 36.

GROUP III.—JUNGLE FAUNA.

A. aitkeni.

Total number of association units	220
Found by itself	154	times	or 70.0	per cent.
" with <i>A. leucosphyrus</i>	...	36	"	"	16.4	"
" " <i>A. maculatus</i>	...	12	"	"	5.4	"
" " <i>A. hyrcanus</i>	...	7	"	"	3.2	"
" " <i>A. albotaeniatus</i> var. <i>montanus</i>	...	6	"	"	2.7	"
" " <i>A. kochi</i>	...	2	"	"	0.9	"
" " <i>A. tessellatus</i>	...	2	"	"	0.9	"
" " <i>A. novumbrosus</i>	...	1	"	"	0.5	"
Totals	...	220		100		"

Its association with *A. maculatus* and *A. hyrcanus* depends on the rapidity with which those species enter a ravine when the jungle is felled (1). The association of *A. aitkeni* with *A. leucosphyrus* and *A. albotaeniatus* var. *montanus* confirms the previous observation (2) that these species are usually found in hilly jungle ravines, while *A. umbrosus* is characteristic of low-lying swampy jungle. Indeed it is possible that as the figures accumulate this group will form a small jungle pool fauna, that the swamp fauna will differentiate into open swamp and jungle swamp faunae, and that there will be two similar main groups (*i.e.*, reading the following table either down or across).

Open small pools or open hilly country.*	Jungle small pools or hilly jungle.
Open swamp or open low-lying country.	Jungle swamp or low-lying jungle.

* Small pools are characteristic of hilly country, swamp of low-lying. Hence the alternatives in the table.

(1) Vol. I, page 28.

(2) Vol. I, page 36.

A. leucosphyrus.

Total number of association units	103
• Found by itself	44	times or 42.7	per cent.
" with <i>A. aitkeni</i>	36	"	" 35.0	"
" " <i>A. tessellatus</i>	12	"	" 11.6	"
" " <i>A. albotaeniatus</i> var. <i>montanus</i>	3	"	" 2.9	"
" " <i>A. kochi</i>	3	"	" 2.9	"
" " <i>A. maculatus</i>	2	"	" 1.9	"
" " <i>A. novumbrosus</i>	1	"	" 1.0	"
" " <i>A. barbirostris</i>	1	"	" 1.0	"
" " <i>A. hyrcanus</i>	1	"	" 1.0	"
Totals	...	103	100	..		

It is very closely associated with *A. aitkeni*, and moreover its list of associates is similar to that of *A. aitkeni*.

A. albotaeniatus var. *montanus.*

Total number of association units	12
Found with <i>A. aitkeni</i>	6	times or 50.0	per cent.	
" " <i>A. leucosphyrus</i>	3	"	" 25.0	"
" by itself	2	"	" 16.7	"
" with <i>A. maculatus</i>	1	"	" 8.3	"
Totals	...	12	100	..		

Its close association with *A. aitkeni* and *A. leucosphyrus* justifies its position in this group.

A. novumbrosus.

Total number of association units	4
Found by itself	2	times or 50.0	per cent.	
" with <i>A. aitkeni</i>	1	"	" 25.0	"
" " <i>A. leucosphyrus</i>	1	"	" 25.0	"
Totals	...	4	100	..		

Although the figures are very small indeed, they express the experience that *A. novumbrosus* has usually been found in jungle-covered ravines.

GROUP IV.—SPECIES WITH NO ASSOCIATIONS.

A. aurostris.

Total number of association units	2
Found by itself	2 times	or 100 per cent.	

This species shows no associations in the restricted series of data, but it has also been found six times in association with larvae which were probably ⁽¹⁾ *A. umbrosus* but possibly *A. separatus*. When more data have been collected, therefore, it will probably be found to group itself with *A. umbrosus*, in agreement with the fact that it was originally found ⁽²⁾ in jungle pools by Dr. Watson.

A. asiaticus.

Total number of association units	39
Found by itself	39 times	or 100 per cent.	

This species shows no association with any other species, since it has been found only in bamboos, a specialised breeding place in which no other anopheline has been found.

3.—SUMMARY AND CONCLUSIONS.

(a) The method generally used for studying distribution is to collect descriptions of the breeding places of a species, in the hope that a description of the typical breeding place of that species may be evolved. Apart from the confusion that arises from the number of characters which require description, this method is defective, in the first place since it depends on personal impressions, and in the second place since these personal impressions are based on natural characters which vary from time to time. The method introduced in this volume is based on the number of times a given species is found with each of the other species in a certain number of breeding places. It is not based on personal impressions.

(b) A peculiar merit of this method is that it enables past observations, of whatever dimensions, to be epitomised in a series of figures (the association values) showing the relative distribution of any given species.

(c) This epitome can be used as a standard for estimating the probability of other species being found in company with any given species. When, therefore, any one species is found, it will be possible to prophesy what other species may be expected to be present in the same place. To prophesy with any degree of accuracy, the observations must be based on the widest experience, both in respect of number of observations and types of locality.

(d) This epitome can also be used as a standard by which the distribution of any species can be measured in any area. The practical value of such a standard is that if the distribution in a newly investigated area does not conform to the standard, local causes must be sought for.

⁽¹⁾ Page 12.

⁽²⁾ Watson, M., 1910. *Annals Trop. Med.*, Vol. IV., pp. 251-3.

(e) The epitome, however, merely reflects the past experience relating to the various species, and is much affected by the number of observations on each species. For example, on the few occasions a rare species is found a certain species might always be present in its breeding place. Yet the association values of the common species would not show this close association merely because of the rarity of the associate; but the association values of the rare species would show the association.

The association values, therefore, do not necessarily place the species in the best order for grouping into faunae; and since this was one of the objects for which the method was introduced, the failure to do so constitutes a weakness of the method.

(f) The ideal way of overcoming this weakness would be to increase the data on the species with few records, until the basis of data for each species is of the same order of magnitude, but this is obviously impossible when rare species are being dealt with. At the present stage of the investigation the weakness may be overcome by taking into consideration the association values of both species in considering the relationship between them. Later, however, when the figures become adequate a simple formula to express both sides of the relationship may be found.

(g) In spite of the weakness of the method as a means for grouping the species into faunae, referred to in sections (e) and (f), the figures, even as they are expressed at the present stage of the investigation, show that the species may be grouped in the following manner, and it is of interest to note that this grouping agrees closely with what is generally known about the breeding places of the species.

Group I, depending on a common preference for small open pools or open hilly country:—*A. kochi*, *A. vagus*, *A. maculatus*, *A. karwari* and *A. ludlowi*. The last of these species may separate itself from this group as the chief member of a salt water fauna when further data have been collected.

Group II, depending on a preference for large swampy pools or low-lying country:—*A. barbirostris*, *A. hyrcanus*, *A. aconitus*, *A. fuliginosus*, *A. subpictus* var. *malayensis*, *A. separatus*, *A. tessellatus*, and *A. umbrosus* and probably *A. aurorostris*. The last three may separate themselves later into a jungle swamp fauna, leaving the rest of the group as an open swamp fauna.

Group III, depending on a preference for jungle, probably hilly jungle:—*A. aitheni*, *A. leucosphyrus*, *A. albotaeniatus* var. *montanus* and *A. novumbrosus*.

Group IV, species with highly specialised breeding places:—*A. asiaticus*.

(h) The fact that such a grouping does occur shows that the various species are to some extent selective in their breeding places. The fact that the groups are not sharply delimited shows that the species are not strictly limited in their choice of breeding place, but have a certain amount of adaptability.

(i) When the figures show a marked inter-association between members of two different faunae, such as that seen between *A. maculatus* and *A. aitheni* on page 16, this will show that the cause must be sought for in the breeding habits of the two species.

(j) This method of recording the results of routine work on distribution shows possibilities of usefulness, therefore, and will be continued as a method of summarising the data on distribution.

IB.—NOTES ON ARTIFICIAL RECEPTACLES OF WATER AS BREEDING PLACES OF ANOPHELINES, NOVEMBER, 1920.

1.—PROBLEM STATED.

In the study of the distribution of anophelines it is important to determine the extent to which the various species are able to breed in domestic or artificial collections of water, such as disused tins and bottles, roof gutters or rainwater tanks. In the Malay States it is extremely rare to find anopheline larvae in such places, and each species shows a preference for certain natural conditions. The question arises, however, whether anophelines will be able to adapt themselves to breed under domestic conditions when their natural breeding places are destroyed by anti-malarial measures, or whether they will have to go further afield in search of places more suitable for them to breed in.

A request for information on this question has been received from the Principal Medical Officer, and therefore it is proposed to review the relevant data which have accumulated during the past three years.

2.—OBSERVATIONS.

No record of the many negative examinations has been kept, except in the case of wells, and therefore it is difficult to estimate the rarity with which anopheline larvae were found in artificial collections of water. Some idea of the rarity with which they were found in such places may be obtained, however, by realising that the observations described here were spread over a period of three years, that over 4,000 natural breeding places were discovered during the same period, that during 1918 and 1919 special attention was paid to the examination of disused tins and bottles, and that special expeditions were undertaken to native rubber plantations to find latex-collecting cups containing water in which larvae might be present.

The positive findings may be recorded under the following headings:—

(a) *Disused Domestic Receptacles.*

Anophelines were found only 14 times in such places.

In 11 cases the receptacle was an old tin and the species present were *A. maculatus* (8 times), *A. kochi* (3 times), *A. vagus* (once), and *A. leucosphyrus* (once).

In two cases the receptacle was an earthenware jar and the species found were *A. kochi* in one and *A. vagus* in the other.

On one occasion *A. vagus* was found in a split coco-nut shell.

In every case the receptacle had been abandoned for a long time and often it contained earth, decaying leaves, algae and even seedling plants, so that it had come to resemble a natural pool. Many of the tins had been eaten through by rust and the water was in actual contact with the soil.

(b) Rubber latex collecting cups.

On one occasion anophelines were found in a group of six latex cups and one tin, situated within 200 yards of one another, in a native-owned plantation which was not being tapped and in which most of the cups had been left standing, so that they contained water. The species present in each case was *A. leucosphyrus*.

These cups had been left untouched for a considerable time, since many of them had earth, debris, and seedling plants in them.

(c) Concrete water tanks.

Anophelines were found in these places on seven occasions. The species were *A. kochi* (6 times), *A. barbirostris* (twice), *A. vagus* (once) and *A. hyrcanus* (once).

In all these cases the tanks had been neglected, the water was green with algae, there was floating debris, and in some cases water plants were present.

(d) Wells.

By means of an apparatus devised to remove the surface film of water without disturbing it, forty-six wells were examined to ascertain whether anopheline larvae were present. The wells examined may be classified as follows:—

Concrete	10
Brick	15
Wood lined	5
Earth wells with over 3 feet to water level	8
Earth wells with under 3 feet to water level	8

Anopheline larvae were obtained in only two of these wells, both of which belonged to the last class in the list. One of them was two feet, and the other one foot deep, and they were, therefore, more like bathing-pools than wells. *A. kochi* was found in the former and *A. barbirostris* and *A. kochi* in the latter.

The edges of the shallower well were covered with vegetation and both of them closely resembled natural pools of water.

3.—DISCUSSION OF THE OBSERVATIONS.

The fact that the receptacles in which anopheline breeding was found had been neglected for a long time, and therefore had become similar to natural collections of water, is important. An attempt to obtain anopheline larvae by exposing earthenware jars containing water near a prolific breeding place of *A. maculatus*, failed; and even after the breeding place had been destroyed, no larvae appeared in the artificial collection of water.

It would appear, therefore, that only when the conditions in the artificial receptacle come to resemble those of a natural pool, is there much likelihood of the receptacle becoming a breeding place of anophelines.

Each species of anopheline prefers a certain kind of breeding place, although it is not strictly limited to it. This fact emerges from the study ⁽¹⁾ of the species which are commonly found associated together in the same breeding place, and is clearly illustrated by the following observation on the shade-loving species *A. umbrosus*. Adult specimens of *A. umbrosus* were found in Dr. Pou's house at Sungei Lembing, although there was no jungle within half a mile of the house. A search of the surrounding country was made, therefore, to determine whether this species was breeding in the open, or whether it had come from the distant jungle. The larvae were found in two pools in the shade of an old mining tunnel, which fact shows the preference of this species for shade of some kind. The dependence of the various species on a certain type of breeding place is shown also by the kind of anophelines found in artificial breeding places. Thus, in the tins the species found most commonly were *A. maculatus*, *A. kochi* and *A. vagus*, all small pool breeders; whereas the large pool breeders, *A. barbirostris* and *A. hyrcanus*, were found only in the concrete tanks and wells. Again *A. leucosphyrus*, which is usually found in jungle, was the species found in a tin under the shade of a hedge, and in the group of latex cups in the shade of a rubber plantation.

The anophelines seem therefore to wait until the artificial receptacles of water have approximated to natural conditions before they use them as breeding places, and it would appear that the choice exercised in the matter of natural breeding places is similarly exercised in the case of artificial breeding places. The latter statement might suggest that the fact of the place being an artificial one was a matter of no importance to the anopheline; but on the other hand, when an artificial place is used for breeding purposes some cause can often be suggested as to why it has been used. Thus at Gemas ⁽²⁾ *A. maculatus* was found in a kerosene tin when the weather was very dry, and when in consequence the number of natural places suitable for this species was limited; at Perhentian Tinggi ⁽³⁾ two tins containing *A. kochi* and one tin containing *A. maculatus* were found in a ravine recently filled, in which many natural breeding places had been destroyed; and in the Agricultural Department, Kuala Lumpur, an area in which most of the natural places had been destroyed by sub-soil drainage, *A. maculatus* was found in a disused latex coagulating pan.

Again, out of the many latex cups examined only six were found containing larvae. These were in close proximity to one another and all contained the same species, *A. leucosphyrus*. There were natural pools in the vicinity apparently suitable for this species, but they did not contain larvae. These six cases therefore were probably due to some common cause which was not detected.

This frequency with which a cause can be suggested when anophelines are found to be breeding in artificial receptacles tends to prove that the phenomenon is partly one of adaptation to adverse conditions, and that it might become more frequent as anti-malarial measures become more stringent.

(1) See Conclusion 4, page 24.

(2) Vol. I., page 34.

(3) Vol. I., page 69.

4.—CONCLUSIONS.

(a) That anopheline larvae are found occasionally in artificial receptacles of water and more commonly so when the external conditions are unfavourable for their breeding.

(b) That age and disuse render artificial receptacles more suitable for the breeding of anophelines.

(c) That the care of domestic collections of water and the clearing up of disused tins and bottles are justified, therefore, on the ground of malaria prevention, apart from their value as methods for reducing the general mosquito nuisance.

REPORT II.

ON AN OUTBREAK OF MALARIA ON THE BANKS OF
THE PERAK RIVER, JANUARY, 1918.

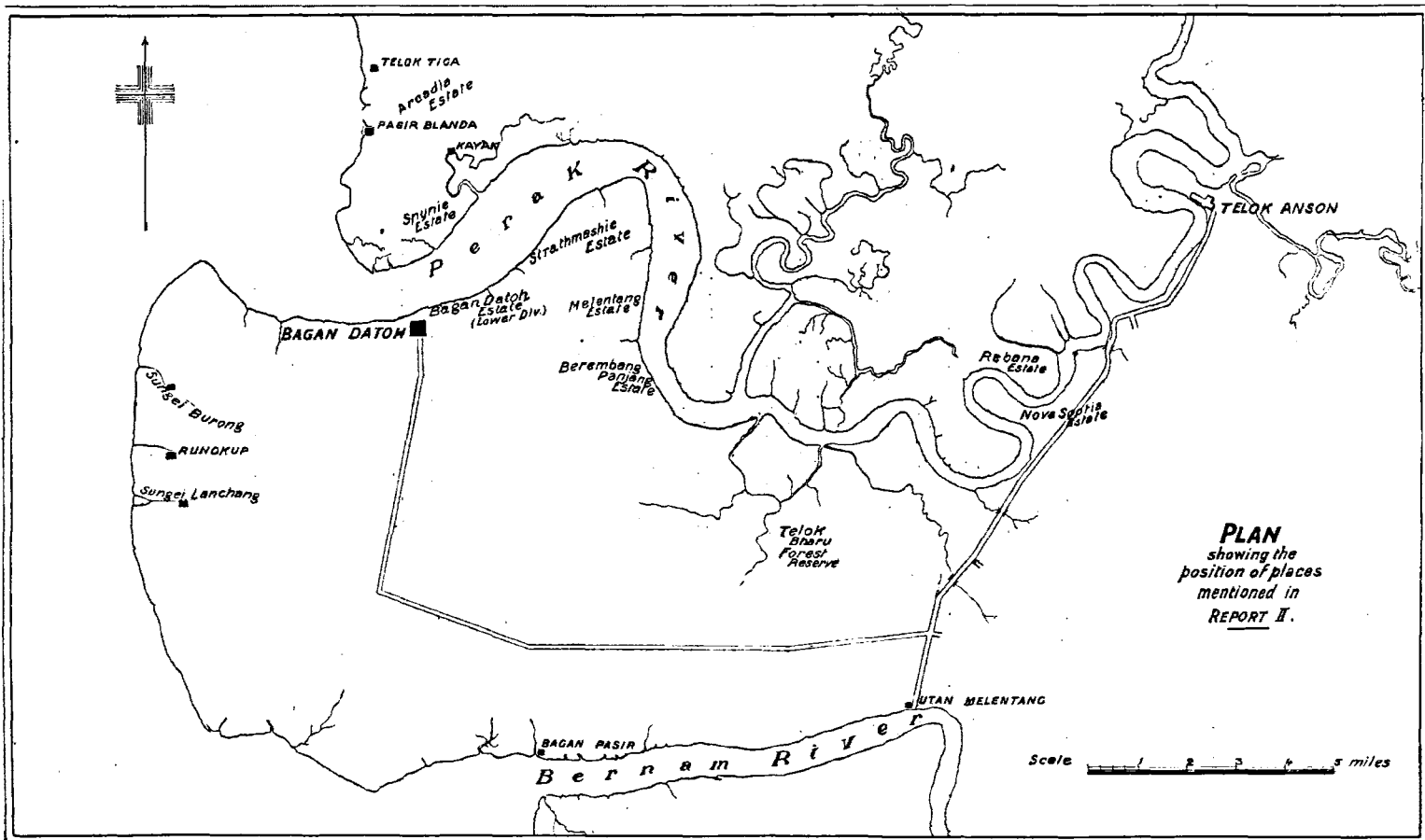
SYNOPSIS.

- 1.—Reason for the investigation.
- 2.—General description of the area affected by the outbreak.
- 3.—Extent of the outbreak.
- 4.—Species of anophelines found.
- 5.—Malaria carrying properties of the species found.
- 6.—Distribution of anophelines throughout the affected area.
- 7.—Relation of the distribution of *A. ludlowi* to the affected area.
- 8.—Relation of the distribution of *A. umbrosus* to the affected area.
- 9.—Relation of the outbreak to the rainfall.
- 10.—Relation of the distribution of *A. ludlowi* to rainfall.
- 11.—Dissection of adult mosquitos caught in houses.
- 12.—Causation of the outbreak.
 - a.—Reasons in favour of regarding *A. ludlowi* as the chief causative agent.
 - b.—Reasons against regarding *A. umbrosus* as the chief cause of the outbreak.
 - c.—Possibility of more than one species being active in causing the outbreak.
- 13.—Suggestions for the reduction of malaria in the area.
 - a.—Methods directed against infection of mosquitos.
 - b.—Methods for reducing anopheline breeding.
 - (1)—Methods directed mainly against *A. ludlowi*.
 - (2)—Methods directed mainly against *A. umbrosus*.

IIA.—NOTES ON THE RELATION OF ANOPHELINE BREEDING
TO MANGROVE, JANUARY, 1918.

SYNOPSIS.

- 1.—Problem stated.
- 2.—Observations.
- 3.—Conclusions.



PLAN
 showing the
 position of places
 mentioned in
REPORT II.

REPORT II.

ON AN OUTBREAK OF MALARIA ON THE BANKS OF
THE PERAK RIVER, JANUARY, 1918.

1.—REASON FOR THE INVESTIGATION.

The work was done at the request of the Senior Health Officer, because the district which had previously been regarded as a fairly healthy one, even for newcomers and Tamil labourers, had been visited by a rather heavy outbreak of malaria.

2.—GENERAL DESCRIPTION OF THE AREA AFFECTED BY THE OUTBREAK.

The area affected by the outbreak is shown on the accompanying sketch plan, on which all the places mentioned are marked.

The country is a flat alluvial plain at the mouth of the Perak River, bounded by mangrove swamp and containing some patches of virgin jungle typical of low-lying country. Rubber is not cultivated much below Rebana Estate (about 13 miles in a straight line from Bagan Datoh), the main cultivation of the district being coco-nut.

The coco-nut estates are intersected by large drains, which are controlled by water gates and used extensively for the water transport of coco-nuts.

3.—EXTENT OF THE OUTBREAK.

Of the virulence of the outbreak there can be little doubt, as no less than three out of the party of five men engaged in this investigation became infected, and the work had to be stopped on this account before it was completed.

The limit to which the outbreak extended up the river can be seen from the following Table D, which shows the health statistics for recent years of estates on the river bank at varying distances from its mouth. The percentage average monthly admissions to hospital have been taken, as they give a better idea of the incidence of the outbreak than the figures for malaria admissions, because the standard of diagnosis of malaria was found to be variable on the different estates and was hardly ever founded on microscopic examination of the blood.

TABLE D.

Estate.	DISTANCE FROM BAGAN DATOH		Average Population.	PERCENTAGE ADMISSION TO HOSPITAL.				
	By River.	In direct line.		1913.	1914.	1915.	1916.	1917.
Bagan Datoh	¼ mile.	¼ mile.	663	5.12	—	10.8	9.8	21.4
Spynie ...	{ Opposite bank but a little higher up river. }		285.3	—	—	1.1	4.7	11.6
Strathmashie	3 miles.	3 miles.	654.9	8.81	11.93	5.0	9.6	14.3
Melentang ...	9 "	4½ "	373.7	—	—	7.3	7.3	10.6
Rebana ...	23 "	13 "	1,038	—	5.91	4.5	4.3	5.5

It will be seen from the table that the effect of the outbreak diminishes in a very regular way in going from Bagan Datoh up river, and that Rebana Estate escaped infection.

That this is approximately a correct estimation of the limit of the outbreak is confirmed by the following series of spleen rates taken by Dr. Orme personally, during the period June to October, 1917.

Bagan Datoh Estate (Lower Division)	∴	66.1 per cent.	in 59 children.
Spynie Estate		88.67	„ „ 53 „
Strathmashie Estate		96.96	„ „ 33 „
Melentang Estate		80.0	„ „ 20 „
Berembang Panjang Estate (next estate to Melentang, about a mile further up-river, no statistics available)	6.97	„ „ 43 „
Rebana Estate. (No spleen rates taken, but Nova Scotia, the estate on the opposite side of the river)...	5.29	„ „ 208 „

Thus, from the table and the spleen rates, it may be concluded that the outbreak was most severe at the mouth of the river, that it extended as far up-river as Strathmashie and Melentang, but did not extend further. Rebana Estate may be regarded as definitely outside the area affected by the outbreak, and it may therefore be used as a healthy control for comparing the species of anophelines found there with those found on the affected estates.

4.—SPECIES OF ANOPHELINES FOUND.

The following table shows the species found and their relative frequency in a general survey of the area. It is followed by notes on the morphology of certain larvae which presented difficulties in identification.

TABLE E.

Species.	Times found.	Number of specimens.
<i>A. ludlowi</i>	80	390
<i>A. vagus</i>	105	479
<i>A. kochi</i>	5	7
<i>A. tessellatus</i>	20	33
<i>A. aurostris</i> (see note A.)	8	13
<i>A. leucosphyrus</i> , Type 1	1	1
<i>A. leucosphyrus</i> , Type 2 (see note B.)	2	3
<i>A. hyrcanus</i>	101	351
Umbrosus-like larvae (see note C.)... ..	145	645
TOTAL	467	1,922

NOTE A. *A. aurostris*. The adult of this species was described ⁽¹⁾ by Dr. Watson and the larva is now described for the first time ⁽²⁾.

⁽¹⁾ Watson, M., 1910. *Annals Trop. Med.*, Vol. IV., pp. 251-3.

⁽²⁾ Dr. Stanton kindly identified the adults and examined some of the larvae.

Its larva was always found in brackish pools at the roots of mangrove and nipah palms, and was recognised as a new type before adults were bred out. The adult differs from *A. tessellatus* chiefly in the absence of spotting on the legs, and might be regarded merely as a variety of *A. tessellatus* if it were not for the constant difference between the larvae, which is a reason for regarding it as a separate species.

The larvae of *A. aurostris*, *A. tessellatus* and *A. kochi* show an interesting gradation in the shape and arrangement of their hairs and may be differentiated by attention to the points shown in the following table.

TABLE F.

Feature.	<i>A. kochi</i> .	<i>A. tessellatus</i> .	<i>A. aurostris</i> .
Internal anterior clypeal hairs.	Long, fine with a few lateral branches.	Short and thick with lateral branches.	Short and thick with lateral branches.
Position of internal anterior clypeal hairs.	Wide apart.	Wide apart.	Almost as close together as in a myzorhyncus larva.
Inner thoracic hairs.	Well branched, like that of <i>A. vagus</i> .	Simple branching i.e. 5 or 6 branches.	Very simple branching i.e. 2 or 3 branches.

The palmate hairs also show a gradation in their complexity, those of *A. kochi* being the most differentiated and those of *A. aurostris* being the most simple.

NOTE B. *A. leucosphyrus*. Two adults, bred out from pupae which were obtained in pools at the roots of mangrove, differed from the type of *A. leucosphyrus* usually seen, in the extreme narrowness of the palpal bands, which were merely specks on the black palps. The apical band, usually quite broad, was represented by a few white scales, and the whole palp seemed even shorter than it is in the common form. The commoner variety was also obtained, but in fresher water 13 miles up the river. One larva was obtained which was probably of the rarer variety, since it was found in brackish pools, but no difference was detected between it and the common types of *A. leucosphyrus* larva.

There seems therefore not sufficient reason to separate these two types into species.

NOTE C. Umbrosus-like larvae.

Of 68 adults developing from umbrosus-like larvae, 22 proved to be *A. umbrosus* and 46 of them were labelled as *A. hunteri* (Strickland). Mr. Edwards has compared some of these specimens with Leicester's specimens of *A. separatus*, and regards them as belonging to the same species. The latter name has therefore been applied to them. The adult females of *A. separatus* varied very greatly. Some had an apical white band on the palps, which itself varied considerably in breadth, while others had two or three white bands. All umbrosus-like larvae hatching out with white on the palps were classified as *A. separatus*.

The distinction between the larvae of *A. umbrosus* and *A. separatus* (*hunteri*) which was pointed out by Dr. Strickland⁽¹⁾ was that the posterior clypeal hairs of *A. separatus* are simple, whereas those of *A. umbrosus* are forked. This was confirmed in the large majority of the specimens in this series, but occasionally an *A. separatus* larva had forked posterior hairs. This distinction is useless in field work; first, because it is very difficult to establish even if the larva is killed and prepared, since the larvae in both species are usually very dark coloured, and secondly, because it is not an invariable distinction.

It was found to be possible to distinguish *A. separatus* larvae from those of *A. umbrosus* by looking out for a combination of the following two characters which are present in the former.

(1) Low bifurcation of the external anterior clypeal hairs; that is, the hairs divide low down into two main branches, each of which then divides up in the manner characteristic of *A. umbrosus*, and

(2) A bushy character of the antennal hair.

Both characters must be present, because some larvae which were thought to be *A. separatus*, owing to the presence of bifurcation alone, bred out as *A. umbrosus*; but the identification as *A. separatus* of a larva possessing a combination of those two characters was always found to be correct.

5.—MALARIA CARRYING PROPERTIES OF THE SPECIES FOUND.

Little is known as yet about the relative pathogenicity of the various species. It is probable that no anopheline is free from suspicion, but *A. ludlowi* and *A. umbrosus* are the two species on the list which are usually recognised as bad carriers. Dr. Barber's results⁽²⁾ obtained in the Malay Peninsula show that *A. ludlowi* is much more susceptible to experimental malaria than *A. umbrosus*, thus:

Zygotes in midgut.

<i>A. ludlowi</i> ...	42 infected out of 69	...	60.9 per cent.
<i>A. umbrosus</i> ...	25 ,, ,, 143	...	17.5 ,,

Sporozoites in salivary glands, dissected 10 days after feeding.

<i>A. ludlowi</i> ...	3 out of 23	...	13.0 per cent.
<i>A. umbrosus</i> ...	2 ,, ,, 26	...	7.7 ,,

A. separatus, which is very closely related to *A. umbrosus*, was infected once in eight experiments.

Recent work⁽³⁾ in the Dutch East Indies also points to *A. ludlowi* being more dangerous than *A. umbrosus* in the Malayan Islands, but caution must be used in applying results from the islands to the conditions on the mainland and vice versa.

6.—DISTRIBUTION OF ANOPHELINES THROUGHOUT THE AFFECTED AREA.

In view of the remarkable change in the species, observed during the six weeks spent in the district and reported later⁽⁴⁾, the importance of knowing the conditions

(1) Strickland, C., 1916. Ind. Journ. Med. Res., Vol. IV., pages 263-270.

(2) Barber, M. A., 1917. Philippine Journal of Science, Vol. XIII., Sect. B., page 10.

(3) Swellengrebel, N. H., and Swellengrebel—de Graaf, J. M. H., 1920. Parasitology, Vol. XII., pages 180-198.

(4) See pages 38-40.

existing at one and the same time throughout the area examined is at once evident. This simultaneous survey of a large area is impossible for a staff of five men, but the results in Table G were all obtained in the first 11 days of the work—from December 11th to the 21st, 1917—during an attempt to discover quickly what species were present, and their relative distribution with regard to the estates shown in Table D. As the chief object of the work was to determine the relative distribution of *A. ludlowi* and *A. umbrosus*, the search was divided into two parts: (1) open pools and drains round cooly lines (1) and houses, which are the likely sources of *A. ludlowi*, and (2) the nearest strip of jungle or secondary growth, which is the probable haunt of *A. umbrosus*. This division is also retained in the following table.

TABLE G.

Locality.	Open Pools and Drains round Cooly Lines and Houses.			Nearest Strip of Jungle or secondary growth.		
	Species.	Times found.	Remarks.	Species.	Times found.	Remarks.
Bagan Datoh Village.	<i>A. ludlowi</i>	7	—	The nearest jungle was on Bagan Datoh Estate (<i>see</i> below).		
Bagan Datoh Estate (Lower Division).	<i>A. ludlowi</i>	17	—	umbrosus	7 (U 2)	Strip of mangrove jungle along river.
	<i>A. vagus</i>	7				
Spynie Estate ...	<i>A. ludlowi</i>	4	Pool covered with coconut leaves.	umbrosus	8 (U 1)	Mangrove jungle with nipah palms.
	umbrosus	1		<i>A. ludlowi</i>	2	
Strathmashie, Estate.	<i>A. ludlowi</i>	6	—	<i>A. leucosphyrus</i> (Type 2)	2	Drains on edge of mangrove.
	<i>A. vagus</i>	8		<i>A. aurostris</i>	2	
Melentang Estate	<i>A. hyrcanus</i>	3	Reedy drain.	umbrosus	4 (U 2)	—
	umbrosus	1 (U 1)				
Berembang Panjang Estate	<i>A. hyrcanus</i>	17	—	umbrosus	3 (U 2)	Secondary jungle beside river.
	<i>A. tessellatus</i>	7		<i>A. hyrcanus</i>	3	
Rebana Estate ...	<i>A. vagus</i>	36	—	umbrosus	10	—
	<i>A. kochi</i>	5				
	<i>A. hyrcanus</i>	4				
	<i>A. leucosphyrus</i> (Type 1)	1				
	<i>A. tessellatus</i>	1				

Umbrosus refers to umbrosus-like larvae. When the actual species was determined, by the hatching out of adults, to be either *A. umbrosus* or *A. separatus*, this is shown by the symbol U and S respectively. The number of adults on which the identification depends is shown adjacent, thus (S 3) means that from the places in question three adults of *A. separatus* were bred out. These symbols will be used throughout the rest of the report when umbrosus-like larvae are mentioned.

7.—RELATION OF THE DISTRIBUTION OF *A. ludlowi* TO THE AREA AFFECTED.

It will be seen by comparing Table G with Table D that the area occupied by *A. ludlowi* is included within the area affected by the outbreak, but that it is not

(1) Cooly lines = Labourers' barracks.

so extensive. Thus *A. ludlowi* was not obtained higher up the river than Strathmashie, while Table D shows that Melentang, the next estate up-river, was affected by the outbreak. Its frequency falls off in passing up-river in very much the same way that the severity of the outbreak did, thus:—

Bagan Datoh village	7 out of 7 places	=	100 per cent.
Bagan Datoh Estate (Lower Division)	17	„	24	„	= 70.8 „
Spynie Estate	4	„	5 „ = 80 „
Strathmashie Estate	6	„	14 „ = 42.9 „
Melentang Estate (probably affected, see spleen rates)	0	„	4 „
Berembang Panjang Estate (probably not affected)	0	„	24 „

On the healthy control, Rebana Estate, which was very thoroughly examined, it was not found in 47 breeding places, and here a typically fresh water fauna was present consisting of *A. vagus*, *A. kochi*, *A. tessellatus*, *A. leucosphyrus*, *A. hyrcanus*, *A. umbrosus* and *A. separatus*.

8.—RELATION OF THE DISTRIBUTION OF *A. umbrosus* TO THE AREA AFFECTED.

Table G shows that umbrosus-like larvae were found universally throughout the district in their appropriate breeding places, that is, jungle or secondary growth; and they were also commonly found outside their usual breeding places, in open drains and pools. Their distribution was very much wider than the area affected by the outbreak, and they were found ten times on Rebana, the estate up-river which was not affected by the outbreak.

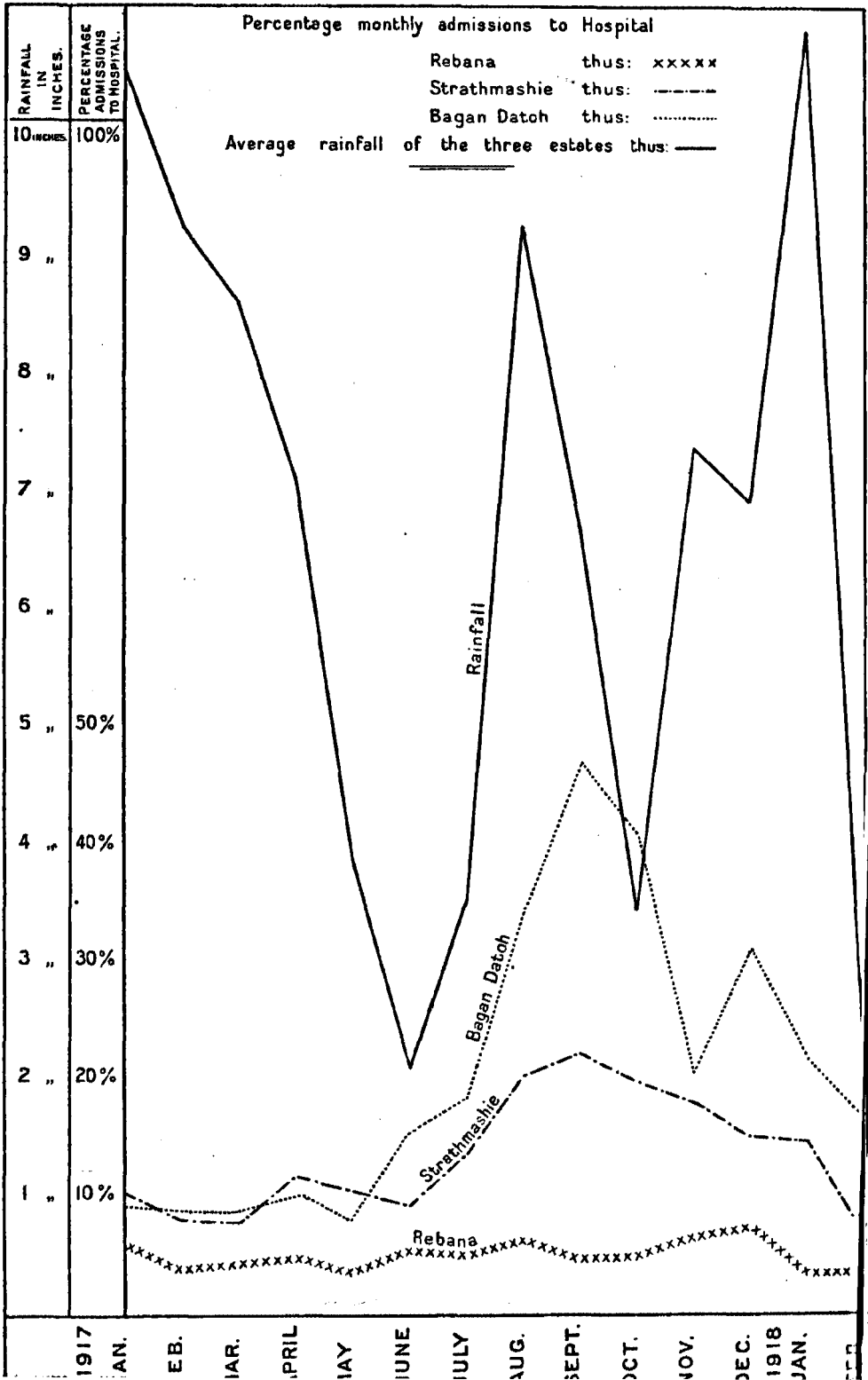
They were also found at the following places which are in the district, but which have no bearing on a study of the outbreak on the banks of the river:—

- Telok Tiga village (3 times, U₂).
- Arcadia Estate (47 times, U₃, S₂₄).
- Kayan village (47 times, U₂₄, S₆).
- Pasir Blanda village (twice, U₁).
- Sungei Burong village (3 times).
- Rungkup village (twice).
- Sungei Lanchang village (once).
- Bagan Pasir village (once, S₁).
- Utan Melentang village (7 times, U₂).
- Telok Bharu Forest Reserve (once).

9.—RELATION OF THE OUTBREAK TO RAINFALL.

Preceding the outbreak there had been three months of very dry weather in May, June and July. There was a consensus of opinion amongst the planters that there had never been such a prolonged period of dry weather since the district was opened, and this opinion is confirmed by the records. Not until 1913 is reached is a similar period found, when the monthly rainfalls for July to September were 4.37, 1.47, and 3.48 inches respectively, which is nearly as severe a drought as that experienced in 1917.

CURVES SHOWING RELATION OF OUTBREAK TO RAINFALL



The relation of the outbreak to this period of dry weather may be studied from the monthly rainfalls and the percentage monthly admissions to hospital on the various estates. These figures are shown in the following table.

TABLE H.
(1) RAINFALL IN INCHES FOR 1917.

Estate.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.
Bagan Datoh ...	10.73	8.62	9.20	5.34	5.17	1.22	1.13	6.81	8.09	5.90	12.48	6.79
Spynie ...	10.46	6.39	6.35	9.49	5.30	2.42	1.02	8.46	7.22	4.79	10.12	3.62
Strathmashie ...	9.80	6.96	9.16	7.29	4.24	1.40	2.87	10.64	8.39	3.20	9.06	4.51
Melentang ...	9.88	7.06	6.79	8.26	3.79	1.55	3.28	10.81	8.28	4.03	6.85	not obtained
Rebana ...	12.14	13.76	9.93	5.82	3.62	3.44	4.43	6.80	3.46	3.21	6.44	7.0
Dry weather.												
(2) PERCENTAGE MONTHLY ADMISSION TO HOSPITAL FOR 1917.												
Bagan Datoh ...	9.2	8.5	8.9	10.1	8.2	15.5†	18.6	34.6	47.5	41.2	20.5	31.1
Spynie ...	6.2	0.9	4.7	0.0	5.3	7.4	14.7†	12.5	14.4	20.5	22.7	17.9
Strathmashie ...	10.7	8.2	7.8	11.5	11.0	9.8	13.9†	20.3	22.9	20.1	18.1	15.9
Melentang...	13.0	12.2	10.6	7.7	8.4	9.7	11.5†	11.5	13.4	12.0	7.5	9.4
Rebana ...	5.4	3.6	4.2	4.6	3.7	5.5	5.1	6.7	5.5	5.8	6.95	7.7

The start of the outbreak is marked by †.

The table shows that the dry weather was experienced generally all over the district during May, June and July. The outbreak started at Bagan Datoh in June, at Spynie in July and at Strathmashie slightly in July but much more markedly in August. The outbreak is also seen to decrease from September, after the relatively high rainfalls of August and September. This relationship between the outbreak and the rainfall probably has an important bearing on the causation of the outbreak and is therefore demonstrated graphically by comparing the admission rates of three estates with the average rainfall of those three estates in the form of curves.

The curves are built up from the figures in Table H, but the estates are chosen to show the relationship best; they are:—

Bagan Datoh Estate—mouth of the river.

Strathmashie Estate—3 miles up-river.

Rebana Estate—13 miles from the mouth.

All the points previously referred to are seen on the chart:—

- (1) Rebana is not affected by the outbreak.
- (2) Bagan Datoh was affected earlier and more severely than Strathmashie.
- (3) The fall in the rainfall curve precedes the rise in the outbreak curves by two or three months.
- (4) The outbreak begins to decrease a month after the high rainfall of August.
- (5) The second smaller rise on the Bagan Datoh curve seems to be connected with the second fall in the rainfall curve, and is only represented by a flattening of the Strathmashie curve.

10.—RELATION OF THE DISTRIBUTION OF *A. ludlowi* TO RAINFALL.

During the latter part of the work, certain places in the Rest-house garden at Bagan Datoh, which had previously contained *A. ludlowi*, were found to contain mainly *A. vagus*. This led to a re-survey of Bagan Datoh Estate (Lower Division) and the native-owned plantations below Bagan Datoh. There had been much rain between the two surveys, and in comparing the rainfalls it is fortunate that accurate rainfall statistics are available—those of Mr. Gillespie, the manager of Bagan Datoh Estate, who takes a personal interest in these records.

Below is shown in tabular form the results of examining identical breeding places in December, 1917, and January, 1918. The rainfall for ten days previous to an examination is given as being the period during which the larvae taken at that examination would have been affected by the rainfall.

TABLE J.

Comparison of the species found in December, 1917, with those found in January, 1918, on Bagan Datoh Estate (Lower Division).

Breeding Place.	Species found on December 13th, the rainfall during the previous 10 days being 1'17 inches.	Species found on January 16th, the rainfall during the previous 10 days being 7'28 inches.
Grassy edge of big drain used for transporting coco-nuts near manager's bungalow.	<i>A. ludlowi</i>	<i>A. hyrcanus</i> .
Similar place in big drain	<i>A. ludlowi</i> . <i>A. vagus</i> .	<i>A. umbrosus</i> . <i>A. tessellatus</i> .
Small stagnant drain in plantation near office ...	<i>A. ludlowi</i> . <i>A. vagus</i> .	<i>A. vagus</i> . <i>A. ludlowi</i> .
Small reedy drain near cattle shed	<i>A. ludlowi</i> . <i>A. vagus</i> .	<i>A. vagus</i> .
Small pool near wire fence of manager's bungalow	<i>A. ludlowi</i> .	No larvae.
Pool in grass between office and manager's bungalow.	<i>A. ludlowi</i> .	<i>A. vagus</i> . <i>A. umbrosus</i> .
Muddy pool near office	<i>A. ludlowi</i> .	<i>A. vagus</i> .
Small open pool in plantation near office ...	<i>A. ludlowi</i> .	<i>A. vagus</i> .
Big muddy pool with stones and decaying leaves	<i>A. ludlowi</i> . <i>A. vagus</i> .	<i>A. vagus</i> .
Large muddy pool near cattle shed	<i>A. ludlowi</i> . <i>A. vagus</i> .	<i>A. vagus</i> .
Pool edged with grass near cattle shed ...	<i>A. ludlowi</i> .	<i>A. vagus</i> .
Large muddy pool in plantation near office ...	<i>A. ludlowi</i> .	<i>A. vagus</i> .
Muddy pool near cattle shed	<i>A. ludlowi</i> .	<i>A. vagus</i> .
Cattle well in plantation	<i>A. ludlowi</i> .	<i>A. vagus</i> .
Muddy pool near office	<i>A. ludlowi</i> .	<i>A. vagus</i> .
Open pool in swamp in plantation	<i>A. ludlowi</i> .	<i>A. vagus</i> .
Stagnant drain near office	<i>A. vagus</i> .	No larvae.

From the table it will be seen that out of the seventeen places, *A. ludlowi* was found in sixteen on December 13th, but on January 16th, it was found only in one place. In this solitary place only one larva of *A. ludlowi* was found. It was a full-grown larva, and although it was examined within four hours of collecting, it was dead and covered by a mass of algae and unicellular organisms.

TABLE K.

Comparison of the species found on December 11th with those found on January 12th in the Rest-house garden at Bagan Datoh village.

Breeding place.	Species found on December 11th, the rainfall during the previous 10 days being 1.17 inches.	Species found on January 12th, the rainfall during the previous 10 days being 3.71 inches.
Pool near a heap of stones	<i>A. ludlowi.</i>	<i>A. vagus.</i>
Pool in grass	<i>A. ludlowi.</i>	<i>A. vagus.</i>
Pool in grass with clear water... ..	<i>A. ludlowi.</i>	<i>A. vagus</i> <i>A. ludlowi.</i>

Here again the remarkable transition of species has occurred. The identification of *A. ludlowi* in January was based on a single specimen, whereas in December the identification of *A. ludlowi* was based on 30 specimens.

TABLE L.

Comparison of the species found on December 13th with those found on January 16th at the native-owned plantation half a mile down river from Bagan Datoh.

Breeding Places.	Species found on December 13th, the rainfall during the previous 10 days being 1.17 inches.	Species found on January 16th, the rainfall during the previous 10 days being 7.28 inches.
Pool edged with grass near house	<i>A. ludlowi.</i>	<i>A. ludlowi.</i>
Pool edged with grass near house	<i>A. ludlowi.</i>	<i>A. ludlowi.</i>
Pool with decayed leaves	<i>A. ludlowi.</i>	<i>A. ludlowi.</i>
Pool in swamp with decayed leaves	<i>A. ludlowi.</i>	<i>A. ludlowi.</i> <i>A. hyrcanus.</i>

Here the transformation of species shown in Tables J and K did not occur. *A. ludlowi* was found in January in the same places as it was found in December. It was as plentiful also, for 29 specimens were obtained in December and 31 in January.

The best data on this subject were obtained on the south bank of the river, but the following observations show that a similar change was taking place on the north bank:—

Telok Tiga.—On December 18th, 22 out of 26 specimens (84.6 per cent.) were *A. ludlowi*, and on December 31st, only 10 specimens out of 25 (40 per cent.) were *A. ludlowi*, and the species which had taken its place was *A. vagus*.

Spynie Estate.—On December 19th, 6 places breeding *A. ludlowi* with 39 specimens were obtained; on January 8th only one place was found, and only one full-grown larva and one pupa of *A. ludlowi* were found in it.

These results show that on Bagan Datoh Estate (Lower Division) and at Bagan Datoh village *A. ludlowi* was breeding extensively in December, 1917, when there

was little rain; but that it was rarely found in January, 1918, when there was more rain, its place being taken by other species.

At the native-owned plantation, which was nearer the mouth of the river than Ragan Datoh, this change in fauna did not occur, and *A. ludlowi* was found just as frequently when there was much rain as when there was little.

11.—DISSECTION OF ADULT MOSQUITOS CAUGHT IN HOUSES.

By catching large numbers of adult insects and determining the proportion of each species which showed malarial parasites, it was hoped to obtain evidence as to which species was causing the outbreak. The work was interrupted by illness at this point, as was also the investigation of the salinity of the waters.

Twenty-one adults were caught and 13 of them were females. No infected specimen was obtained.

The species caught were as follows:—

- A. vagus*, 4 females and 8 males.
- A. hyrcanus*, 5 females.
- A. ludlowi*, 2 females.
- A. umbrosus*, 2 females.

12.—CAUSATION OF THE OUTBREAK.

(a) REASONS IN FAVOUR OF REGARDING *A. ludlowi* AS THE CHIEF CAUSATIVE AGENT.

These may be tabulated as shown below.

Outbreak.	<i>A. ludlowi</i> .
(1) The area affected was at the mouth of the river.	(1) This is the habitat of <i>A. ludlowi</i> .
(2) Did not extend further than 9 miles up river.	(2) The distribution of <i>A. ludlowi</i> was included in this area but was not quite so extensive.
(3) Two estates (1) just outside the affected area were examined as controls.	(3) <i>A. ludlowi</i> was not found on these estates.
(4) The outbreak started earlier near the mouth of the river than higher up river.	(4) This suggests that the causative agent spread up river from its mouth and therefore was probably <i>A. ludlowi</i> .
(5) The severity of the outbreak diminished in going up river from its mouth.	(5) The frequency of <i>A. ludlowi</i> fell off in going up river in just the same way.
(6) The outbreak occurred after an unusually severe period of dry weather lasting 3 months.	(6) This by concentrating the ground water up river might render its salinity suitable for <i>A. ludlowi</i> to breed in.
(7) The outbreak ceased with the onset of rains.	(7) In rainy weather <i>A. ludlowi</i> was observed to disappear from pools in which it was found in dry weather.
(8) —	(8) <i>A. ludlowi</i> is the species which is probably the most susceptible to malaria among the anophelines found.

(1) Rebana and Berembang Panjang.

(b) REASONS AGAINST *A. umbrosus* BEING THE CHIEF CAUSE OF THE OUTBREAK.

These are as follows:—

(1) Its universal distribution throughout the district which bore no special relation to the area affected by the outbreak.

(2) The frequency with which it was found on Rebana Estate, which escaped the outbreak entirely.

(3) The effect of rainfall. Little data was obtained with regard to *A. umbrosus*, but it was found on two occasions to have replaced *A. ludlowi* when the water had been freshened by abundant rain, whereas the outbreak ceased with the increase of rainfall.

(4) Its low susceptibility to malaria as compared with *A. ludlowi*.

(c) POSSIBILITY OF MORE THAN ONE SPECIES BEING ACTIVE AS A CAUSE OF THE OUTBREAK.

This question must always be kept in mind, as when an outbreak is started by a susceptible species like *A. ludlowi*, the proportion of infective persons may rise to a point at which relatively insusceptible species are likely to become infected. If in these circumstances the relatively insusceptible *A. umbrosus* were to become infected, considerable trouble might arise, as the latter insect is a large strong species with probably a longer flight than the smaller species, and almost certainly a longer life. The evidence presented in this report shows no indication that any species other than *A. ludlowi* was responsible for the outbreak, but other data are available which seem to suggest, on epidemiological grounds, that *A. umbrosus* may become infected, and carry on an outbreak in the manner suggested above.

From the reasons discussed above it may be concluded:

(1) that *A. ludlowi* was the main cause of the outbreak;

(2) that it was enabled to travel up-river by the increase of the salinity of the water during the dry weather;

(3) that the onset of the rains made the water unsuitable for *A. ludlowi* to breed in and thereby stopped the outbreak.

Much important work remains to be done on the question of the salinity required by *A. ludlowi* in its breeding places, and on the distribution of *A. ludlowi* in relation to rainfall. The relative functions of *A. ludlowi* and *A. umbrosus* in the causation of malaria in the coast districts is also a promising field of investigation.

13.—SUGGESTIONS FOR THE REDUCTION OF MALARIA IN THE DISTRICT.

(a) METHODS DIRECTED AGAINST INFECTION OF MOSQUITOS.

The mosquitos, although they can be reduced very greatly, will probably always be present in the district, and endeavour should be directed to lessening the chances

of their becoming infected and therefore dangerous. The chief measures available are :—

(1) *Stopping local recruiting of labour.*

Labourers arriving direct from India are remarkably free from malarial infection for 1,726 arrivals at Port Swettenham gave a spleen rate of only 3.5 per cent. (1). Locally recruited labourers frequently come from estates on which malaria is rife and are a source of danger when brought into a healthy district. In road or railway building the use of local gangs of labourers is inevitable, but an estate with a more stable labour force would be well advised to draw its labour from a source, as far as possible, free from malarial infection.

(2) *Stopping migration of labour.*

On two of the estates one division was generally considered much healthier than the other, and it was customary to move a labourer convalescent from malaria to the healthy division to recuperate. This should be stopped, as there is a grave danger of spreading the disease to the healthier division. Malaria is often more infective during convalescence than in the acute stage of the disease.

(3) *The sterilisation of gamete carriers.*

Malaria may wisely be regarded as an infectious disease, and the carriers of infection may be isolated, treated, and so prevented from being a danger to the community. Dr. Orme, the Health Officer of the district, is a strong advocate (2) of this method of attacking malaria, and it is being carried out to some extent in the area.

The method depends on periodical blood examination of the population for the detection of gamete carriers. These are taken to hospital, carefully prevented from infecting any mosquitos by being kept under nets, and are treated until their blood is free from gametes. At the same time all fever patients are vigorously treated, and are not discharged from hospital until the blood is free from gametes.

This method is very sound ; but even under the most careful watching, some gamete carriers are sure to escape notice ; and if suitable mosquitos are prevalent, there is just as much likelihood of infection occurring, as if there were few mosquitos and many gamete carriers to infect them. The method in question, therefore, is by itself inadequate, and should be combined with measures for the reduction of mosquitos. A disease with such a complex origin as that of malaria should be attacked in as many ways as possible. Single methods are doomed to partial success.

(b) METHODS FOR REDUCING ANOPHELINE BREEDING.

It may be impossible wholly to eliminate anopheline breeding in this district, because both *A. ludlowi* and *A. umbrosus* were obtained from the large main drains which are essential for the cheap transport of coco-nuts ; but it would be possible to reduce the number very considerably. And by halving the number of mosquitos the chances against one becoming infected become very much more than doubled.

(1) Report of Uncinariasis Commission to the Orient, 1915-17. International Health Board, New York, page 66.

(2) Orme, W. B. 1918. Indian Med. Gaz. Vol. LIII, No. 8, pp. 292-7.

Any work that is attempted should be thorough. A clean sweep of all breeding places within 10 chains of the cooly lines is more valuable than a half-hearted attempt at dealing with the usually recommended 40 chains circle. A breeding place overlooked near the lines is quite sufficient to undo the benefit of extensive labour at a distance.

By intelligent searching for breeding places much expense would be saved. A labourer trained to recognise anopheline larvae, and incited to work by a high wage or a bonus, will very likely save his salary in a short time by directing attention to dangerous places.

(1) *Methods directed mainly against A. ludlowi.*

The evidence given in this report indicates that *A. ludlowi* is the species to be chiefly guarded against, and that precautions should be redoubled in dry weather.

(a) *In small open pools.*—*A. ludlowi* was most frequently found in these places. The pools were shallow and due to irregularities in the ground; they were often old crab holes or places where trees had been cut out. Of course, no extensive fillings can be undertaken in such flat country, but with a little ingenuity much could be done to obliterate these pools. If the pools cannot be got rid of they should be well oiled.

(b) *Small drains.*—*A. ludlowi* was frequently found in the small superficial drains in the plantations. The wisest plan would be to reduce the number of these drains by deepening the large drains. Where this cannot be done, they should be kept scrupulously clean from weeds and debris and watched carefully. If breeding is found they should be oiled.

(c) *Large drains.*—These are indispensable, but they should be cleaned, and freed from all overhanging vegetation, which affords protection to larvae. No floating debris should be allowed to collect in them, and they should be periodically flushed out by means of the water gates. Here again oiling might be carried out, but against this there is the local opinion that oil would have a bad effect on the coco-nuts.

(d) *Borrow pits along foot-paths.*—*A. ludlowi* was found in these places. Contractors should be prohibited from digging pits to obtain the material required for building bunds and foot-paths. The material should be obtained from ditches which should be properly graded and provided with suitable outlets. Where these pits exist attempts might be made to convert them into drains.

(e) *Pits for bathing and cattle water supply.*—These should be closed wherever possible. Where their retention is necessary, they should be kept clean and absolutely free from debris. They might be screened with advantage. Unauthorised digging for water should be stopped.

(f) When the limits of salinity required by *A. ludlowi* have been determined, it may be possible to devise some method of either diminishing or increasing the salinity so as to render the water unsuitable for *A. ludlowi*. It may also be possible to introduce an easy hydrometer test for indicating when the water is especially dangerous, and therefore when anti-mosquito measures should be carried out most vigorously.

(2) *Methods directed mainly against A. umbrosus.*

Measures against this species may well be undertaken, as it is a proved carrier of malaria, but these measures will probably render the country more favourable to *A. ludlowi* to breed in. Therefore, as *A. ludlowi* seems to be the more dangerous species, *A. umbrosus* should not be attacked, unless full precautions against *A. ludlowi* can also be undertaken.

(a) *Jungle and secondary growth.*—The surroundings of cooly lines should be drained and cleared to a distance of not less than 40 chains, or the lines should be removed to a cleared area.

(b) *Drains overgrown with vegetation.*—These should be kept quite clean, weeded, and open to the light. They should be oiled if necessary.

(c) *Peaty drains.*—Umbrosus-like larvae were found very frequently in these drains. They should be kept clean from vegetation and debris. When a drain collects water from jungle the possibility of short-circuiting it on the edge of the estate should be considered. This would prevent larvae of *A. umbrosus*, washed out from the jungle, from being carried down into the centre of the estate.

IIA.—NOTES ON THE RELATION OF ANOPHELINE BREEDING TO MANGROVE.

1.—PROBLEM STATED.

During the work at Bagan Datoh which forms the subject of the main report, the large amount of mangrove swamp in the district made it necessary to determine the extent to which anophelines bred in such places, and the opportunity was taken to study the distribution of anophelines in mangrove in the light of Dr. Strickland's observations ⁽¹⁾ on this subject.

2.—OBSERVATIONS.

On the sea coast the observations fully confirm those of Dr. Strickland as to the absence of anopheline breeding in the mangrove. The following places were examined, proceeding from North to South.

Telok Tiga.

In the mangrove at the mouth of the river nothing was found until the village situated a quarter of a mile up the small river was reached, when *A. ludlowi* (eight places) and *A. umbrosus* (three places, U₂) were found.

Pasir Blanda.

A very thorough search on two occasions revealed no breeding place in the mangrove nor in the open pools round the village near the mouth of the river. The first breeding places encountered going inland were situated about half a mile from the coast, where *A. ludlowi* (one place), *A. umbrosus* (two places, U₁) and *A. hyrcanus* (two places) were found in pools alongside the bridle path.

Sungei Burong.

Over 12 man-hours were spent searching the mangrove at the mouth of the river without result. Anophelines were not met with until the village situated about three-quarters of a mile inland was reached, when *A. hyrcanus* (six places) and umbrosus-like larvae (three places) were found.

Rungku.

Here again a search at the mouth of the river produced no results, and the first breeding place was met with some half a mile inland, where umbrosus-like larvae were obtained in two places. In the village itself, about three-quarters of a mile inland, *A. hyrcanus* was found in 17 places.

Sungei Lanchang.

About six man-hours were spent searching the mangrove without result. At the village about a mile inland *A. ludlowi* (two places), *A. umbrosus* (one place U₁) and *A. hyrcanus* (seven places) were found.

⁽¹⁾ Unpublished report which can be referred to at the Bureau. Pages 466-171.

To summarise these observations: on the sea coast a belt of mangrove was found to be free from anophelines. The width of this belt varied from a quarter of a mile at Telok Tiga to one mile at Sungei Lanchang, and after this belt was crossed the species found were *A. ludlowi*, *A. umbrosus* and *A. hyrcanus*.

When the mangrove within the mouth of the river was examined, a very different condition of affairs was found, however, and anophelines were encountered right inside the mangrove. The following are the observations on this subject:

Chinese village, half a mile below Bagan Datoh.

In pools at the roots of mangrove and ferns, and in drains in dense shade, *A. umbrosus* was found six times (U1).

Bagan Datoh Estate (Lower Division).

In a strip of mangrove with scattered nipah palms about half a mile above Bagan Datoh, *A. umbrosus* was found seven times (U2).

Spynie Estate.

In mangrove jungle with nipah palms the following species were found:

- A. umbrosus* (eight times U1).
- A. ludlowi* (twice).
- A. leucosphyrus*, type 2 (twice).
- A. aurostris* (twice).

Kayan Village.

Where the mangrove had been cleared and nipah palms had been planted, *A. umbrosus* (23 times, U24) and *A. aurostris* (six times) were found.

Strathmashie Estate.

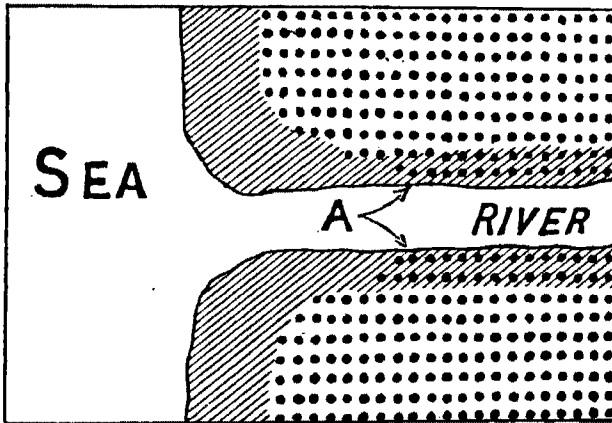
In a drain on the edge of mangrove *umbrosus*-like larvae were found twice.

Thus in contrast with their absence from the mangrove on the sea coast, anophelines were frequently found in the mangrove on the river banks. The species found were *A. umbrosus* (most commonly), *A. ludlowi*, *A. leucosphyrus*, type 2, and *A. aurostris*. All the adults bred out from *umbrosus*-like larvae obtained in mangrove proved to be *A. umbrosus*. *A. separatus* was not obtained in mangrove, although at Kayan it was obtained plentifully a quarter of a mile from the nipah plantation. The larvae were usually found in pools at the roots of mangrove and nipah palms, in drains, and in small streams flowing through the jungle. Larvae were not obtained from the narrow dark tortuous crab-holes, although the water was sucked out from them and examined, but they were occasionally found in the conical basin-like pools made by crabs.

3.—CONCLUSIONS.

The foregoing observations suggest that it is probably not the question of mangrove by itself that governs the presence or absence of anophelines, but the question of the salinity of the water. The observations, so far as they go, indicate that the belt of

mangrove and the belt of anopheline distribution cut each other in the estuary of the river at a certain point A, as shown in the diagram below, and this point possibly depends on the salinity of the water above and below it.



Stippled area indicates anopheline distribution.

Shaded area indicates belt of mangrove.

A indicates point at which anophelines appear in the mangrove.

Further work is needed on this subject and should be controlled by the examination of the salinity of the water from each breeding place.