FIELDING

NOTES ON THE BIONOMICS OF *STEGOMYIA FASCIATA*, FABR.

(PART I)

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INTRODUCTION

Since the discovery of the rôle of *Stegomyia fasciata* in the transmission of yellow fever, much attention has been devoted to the study of the life history and habits of this mosquito in Europe, Africa and America.

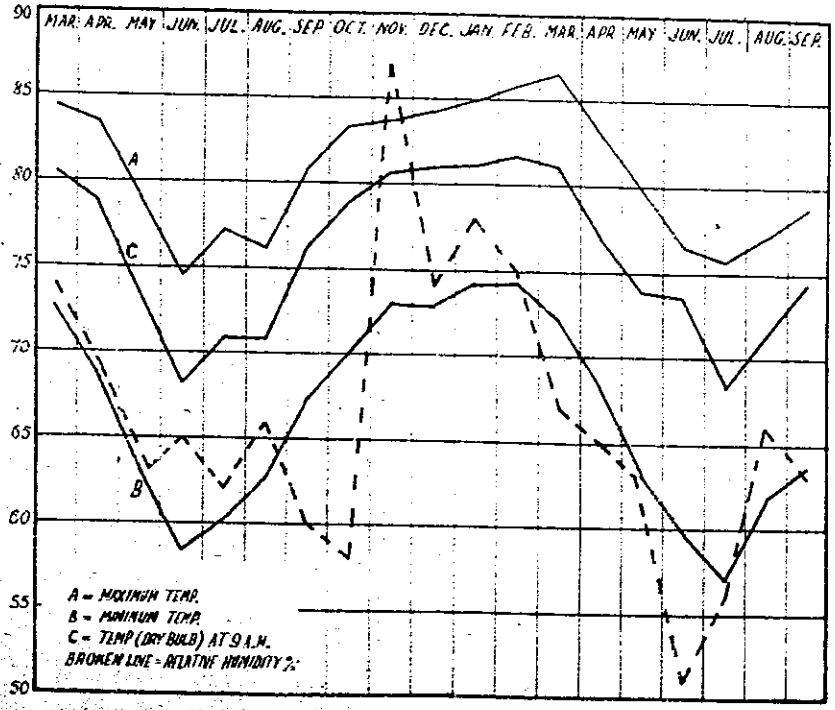
Notwithstanding the vital importance of such knowledge to Australia, no research work of any importance, other than Taylor's inquiry into the distribution of the species in the coastal towns of Queensland, has been undertaken in Australia, either to confirm or to supplement the result of investigations carried out elsewhere by Finlay, Goeldi, MacGregor, Carrol, Reed, Newstead, Boyce, Bacot and many other workers, to whom we are indebted almost entirely for our knowledge of the bionomics of this widely distributed species.

The necessity for breeding large numbers of *Stegomyia fasciata* to meet the requirements of this Institute appeared a favourable opportunity to undertake some of this hitherto neglected work. Although very incomplete, it has been considered advisable to record the results of these observations and experiments at this stage, rather than to defer their publication until further contemplated experiments have been undertaken.

It will be observed that where similar information has been sought I have followed very closely the methods and technique of Bacot (1916), whose paper has been freely consulted as being by far the most complete and comprehensive of its kind yet published.

The various stages of *Stegomyia fasciata* have been so fully and accurately described by earlier writers that it is quite unnecessary to attempt to add anything to their descriptions, nor can new facts be added regarding the seasonal prevalence and distribution of this mosquito in Townsville to the published records of Taylor (1915).

The accompanying chart shows the temperatures and humidity readings taken in the Institute grounds during the period in which the experiments were carried out.



METHODS OF KEEPING AND FEEDING ADULT MOSQUITOES

In these experiments the adult mosquitoes were kept in small cages, as commonly used, measuring 12 by 8 by 7 inches, and consisting of a light wooden frame covered with cheese cloth on three sides and one end, and zinc on the bottom. The remaining end was fitted with a calico sleeve sufficiently large to permit of the passage of the arm and necessary water containers. A glass panel occupied one-third of one side of each cage, but it was considered to be of no

advantage when light fabric was used for screening the remainder of the cage. The mosquitoes were offered food daily, the following substances being tried at different times, viz., blood, banana, peptone and sugar solution, milk and sugar, etc. After some initial trials blood was found to be the most satisfactory for the females and banana for the males, these foods being used subsequently to the exclusion of all others.

Feeding experiments with small animals, such as guinea-pigs, rats and puppies, were, as a rule unsuccessful, even when the animal was left in the mosquito cage overnight. The most satisfactory method of feeding with blood, and the one adopted throughout the following experiments, was found to be the presentation of the arm and hand each morning for a period of from ten to twenty minutes according to circumstances, it being noticed, for example, that during hot and humid weather the mosquitoes fed more readily than in cool weather.

In some current experiments, in which it is desired to feed *Stegomyia* on dog's blood, successful results are obtained by the following method :—One of the cages previously referred to is placed upon the floor and the dog's head thrust into it, the animal being kept in a recumbent position and the sleeve of the cage secured behind its head with one hand whilst the other presses the body to the floor to prevent the animal rising. To ensure successful feeding it is found necessary to shave or clip the hair on the face and ears. Other methods of feeding upon dogs were tried, but found to be unsatisfactory.

1. EGGS

The maximum number of eggs laid by a fertilised captive female

From a long series of observations it has been found that the average egg production of a fertilised captive female fed on blood is about seven hundred and fifty, covering a laying period of from forty to seventy-two days. None of my records cover a longer period of productiveness, although females have been kept in captivity up to a maximum of ninety-three days. It was found impossible, however, owing to absence from Townsville, to continue

blood feeding after the sixty-sixth day, banana being substituted for blood, with the invariable result that egg production ceased on the change of food.

The following record may be cited as typical of many illustrating the rate of egg production. A female, bred for five generations in the laboratory, was segregated with two males and fed exclusively on human blood. The first feed was taken four days after her emergence, and the first batch of eggs laid three days later. During the first thirty-one days of her life she fed eight times and laid four hundred and thirty-seven eggs. During the succeeding thirty-one days she fed six times and laid two hundred and sixty eggs. Two more feeds were taken and fifty-five eggs laid during the following ten days. In all she fed sixteen times and laid seven hundred and fifty-two eggs during the seventy-two days of her life. On the day following the laying of the last batch of eggs she was accidentally destroyed. A complete record of these eggs was not kept to determine the percentage of fertility, but a certain proportion of the batches were kept under observation and found to produce between 80 per cent. and 90 per cent. of larvae.

The selection of situation for oviposition

(*a*) Females in captivity

The receptacles usually provided in the cages for oviposition were dishes 3 inches to 4 inches in diameter, and contained about ½-inch to 1 inch of water. The favourite position of the ovipositing female appears to be resting on the sides of the receptacle with the apex of the abdomen just touching the water. Very often eggs are laid on the surface film of the water as the female moves about on it, but occasionally as many as fifteen eggs are laid in a batch. A considerable proportion of the eggs laid near the margin of the receptacles are drawn against the sides by capillary attraction, and become stranded as the water dries. Under more natural conditions these doubtless form the bulk of the resistant eggs which lie dormant until the supply of water is replenished. When wet filter paper is provided the eggs are usually scattered over its surface, but occasionally they are laid so close together as to overlap.

(*b*) Wild females

It has been frequently noticed that almost any vessel containing water and left in or near the Institute building would attract the females of *Stegomyia* and offer a suitable place for oviposition.

In view of the opinion held by some other observers that these mosquitoes show a decided preference for certain positions and certain rooms, the investigations detailed in Experiment No. 1 were undertaken with the object of confirming, or otherwise, the results of my casual observations.

EXPERIMENT I

No.	Position	Nature of Receptacle	Contents	*Stegomyia* eggs laid	Remarks
1	Workroom on floor ...	Enamel bucket ...	Tap water	+	
2	Workroom on bench ...	Earthenware sink ...	Tap water	+	
3	Workroom on bench ...	Pickle bottle	Tap water and sea water equal parts	+	
4	Workroom on bench ...	Pickle bottle	Rain water	+	
5	Workroom on bench ...	Tin used as water bath	Rain water	+	Also *Culex* sp.
6	Hallway	Glvd. iron cistern 12 ft. from floor	Rain water	+	
7	Bathroom on bench ...	Glvd. tin	Tap water	+	
8	Bathroom on floor ...	Earthenware jar ...	Tap water	+	Also *Culex fatigans*
9	Water Closet	Cast iron cistern not used for some time	Tap water	+	
10	Sterilising room on bench	Enamel bowl	Tap water and dirty tubes	+	
11	Ster. room under bench	Earthenware jar ...	Tap water and dirty slides	+	Also *Culex fatigans* and *C. vitiens*
12	Animal house on shelf 8 ft. from floor	Glvd. iron dish ...	Tap water	+	
13	Animal house on shelf 8 ft. from floor	Enamel bowl	Tap water	+	
14	Under roof of Institute	Glvd. iron dish ...	Tap water	−	
15	Under roof of Institute	Glvd. iron dish ...	Tap water		
16	Under roof of Institute	Enamel bowl	Tap water		

The investigations on the whole tend to show that in Townsville at any rate *Stegomyia* do not appear to show a preference for certain rooms or situations, but will oviposit in almost any receptacle containing water. This finding is noteworthy in view of the fact

that Bacot's experiments gave entirely different results. In a set of twelve receptacles under observation he found *Stegomyia* ovipositing in only one—a card cream jar in the kitchen. It must be noticed, however, that he found *Stegomyia* ovipositing in such positions as the following: in safe stands in gallery, in a tin bowl in yard and in tins and wooden tub in the mosquito house.

Baits to attract pregnant females

Experiments to ascertain if ovipositing females prefer contaminated water to clear

The following experiments (see Experiment II were carried out in a cage containing a large number of *Stegomyia*, the females of which were given opportunities of feeding regularly on human blood. The glass dishes each contained 200 c.c. tap water, in addition to the organic matter, and were placed as far apart as the size of the cage would permit. Except in the case of leaves, which stood for forty-eight hours, none of the cultures were permitted to stand for more than twenty-four hours before being introduced into the cage. One trial was carried on a second day owing to the small number of eggs laid on the first.

Bacot, who carried out similar experiments, remarks: 'While the evidence of selection . . . appears definite, it is very far from being the unanimous unvarying character that might be expected from a deep-seated instinct coupled with larval needs. On the contrary, it carries with it a certain suggestion of bias on the part of the females in reference to their own tastes largely parallel, but not necessarily identical, with provision for their offspring. . . .'

It appears to me that these remarks might be fittingly applied to the results of my experiments. It might be observed, however, that sugar and water appeared to be the most attractive bait, yet it has been shown that sugar gave unfavourable results as a larvae food.

Experiment II

No.	Contents of dish	No. of eggs laid	Percentage of total	Remarks
	(1st Series)			
1	Tap water	30	8·6	No smell
2	Tap water plus 0·5 grm. dried mango leaf, standing for 2 days	30	8·6	Did not smell strongly
3	Tap water plus 0·5 grm. fresh fowl faeces	120	34·3	Did not smell strongly
4	Tap water plus 0·5 grm. freshly killed cockroach	170	48·5	Smelled strongly
	Total eggs laid	350		
	(2nd Series)			
1	Tap water	40	6·66	
2	Tap water plus 0·5 grm. dry pawpaw leaf allowed to decompose for 2 days	20	3·34	Did not smell strongly
3	Tap water plus 0·5 grm. dried cockroach	186	31·00	Slight smell
4	Tap water plus 1 cc. ox bile	160	26·66	Fairly strong smell
5	Tap water plus 0·5 grm. Sodium Taurocholate	194	32·34	Fairly strong smell
	Total eggs laid	600		
	(3rd Series) 1st Count			
1	Tap water	14	21·5	On account of the few eggs laid the dishes were placed in the cage again on the following day and a second count was made 48 hours later.
2	Tap water plus 0·5 horse faeces ...	20	30·8	
3	Tap water plus 1 cc. urine 3 days old ...	31	47·7	
	Total eggs laid	65		
	(3rd Series) 2nd Count			
1	Tap water	120	24·0	Did not smell
2	Tap water plus 0·5 horse faeces ...	90	18·0	Smelling
3	Tap water plus 1 cc. urine 3 days old ...	290	58·0	Smelling strongly
	Total eggs laid ...	500		
	(4th Series)			
1	Rain water ...	43	20·8	At the conclusion of all dishes had eggs in them
2	Tap water plus 0·5 grm. powdered rice	46	19·3	Slight smell
3	Tap water plus 0·5 grm. sugar ...	27	60·0	Slight smell
	Total eggs laid ...	216		

Table I contains a summary of this experiment (Experiment II)

Table 1.

Contents of Receptacles	Percentage of the total eggs laid in each series
Tap water and pawpaw leaf ...	3·74
Tap water (2nd series) ...	6·66
Tap water (1st series) ...	8·6
Tap water and mango leaf ...	8·6
Tap water and horse faeces (2nd count) ...	18·2
Tap water and powdered rice ...	19·0
Rain water ...	22·5
Tap water (1st count) ...	21·5
Tap water (2nd count) ...	24·0
Tap water and ox bile ...	26·66
Tap water and horse faeces (1st count) ...	30·8
Tap water and dry cockroach ...	31·0
Tap water and sodium taurocholate ...	32·34
Tap water and fowl faeces ...	34·3
Tap water and urine (1st count) ...	47·7
Tap water and fresh cockroach ...	48·5
Tap water and urine (2nd count) ...	58·0
Tap water and sugar ...	60·5

Experiments to determine whether Stegomyia *would oviposit on sea water*

For this purpose two buckets, each containing 4 litres of water, were exposed in a room where *Stegomyia* were fairly numerous. Bucket 'A' contained sea water drawn from the harbour, and bucket 'B' tap water from the town service pipes. The sea water was exposed continuously for a period of two months (19th April to 19th June), during which time no eggs were laid on it, whilst the other containing tap water was exposed for one month (19th May to 19th June) and was frequently visited by both *Stegomyia* and *Culex*

fatigans for oviposition. In all, four hundred and ninety-one eggs of *Stegomyia* were laid.

A further series of experiments were then carried out to determine the amount of dilution necessary before *Stegomyia* could be induced to oviposit upon sea water mixed with rain water. For this purpose jars containing various percentages of sea water, from 10 per cent. to undiluted sea water, were exposed in one of the stock-breeding cages containing numerous females, which fed regularly on human blood. As each jar was found to contain eggs it was removed from the cage, and that portion of the experiment was considered finished. In the case of the jars containing 80 per cent. to 100 per cent. sea water, the vessels were kept under observation for a considerable period and until there was no further possibility of oviposition taking place. The results are shown in Table 2.

Table 2.

Number of eggs of *Stegomyia fasciata* laid on different percentages of sea water.

Percentage of sea water in rain water	Number of eggs laid	Remarks
10	?	Numerous eggs were laid but were not counted
20	?	
30	12	
40	43	
50	24	
60	28	
70	20	
80	nil	
90	nil	
100	nil	

Hatching of Eggs of Stegomyia

The period elapsing between the deposition of the eggs and the emergence of the young larvae is extremely variable. In the routine of breeding *Stegomyia* in this Institute, it has been found that the

great majority of eggs laid overnight in the small dishes of water provided for the purpose hatched during the second and third day following in summer, and during the third and fourth day in winter. A certain proportion of eggs of some batches, however, do not hatch within this period, but do so after a further period of immersion, this period being, as I have ascertained, so long as four and a half months. Had further observations been made in this direction, it is very probable that the period of delayed hatching of continuously immersed eggs would have been found to be sometimes longer, since Bacot has shown that under these conditions hatching may be delayed from two to five months.

The influence of periods of drying upon the hatching of eggs

Theobald (1903) first drew attention to the fact that eggs of *Stegomyia fasciata* are capable of withstanding the effects of long periods of dessication. Since then, several others have published the results of experiments to ascertain the maximum period of viability of these eggs under various conditions of dry storage, and to elucidate the problems concerning the factors which bring about their subsequent hatching. Bacot believes that the incubation period of the eggs in Freetown is from thirty to forty hours, but that hatching of any given batch may be distributed over a lengthy period if subjected to periods of drying. The whole of the eggs of a batch may hatch as soon as they are replaced in water, or a proportion of them only may do so. Others may resist the first or second immersion and yield to a subsequent one. The principal factors which act as stimuli to hatching appear to be temperature and humidity. As he points out, a response to cooling would well serve the needs of the species in allowing of the fullest advantage being taken of the facilities for breeding in small temporary accumulations of rain water.

With the object of confirming the above observations under local conditions a number of experiments (III, IV, V) were carried out, which will be found to accord generally with the conclusions arrived at by earlier investigators.

Experiments to show the effects on eggs of drying and replacement of water

Experiment III

Twenty eggs laid overnight were placed in a tube $1\frac{1}{2}$ inches in diameter, containing 15 c.c. of tap water, which completely evaporated and was replaced on the days shown.

Days	3	5	7	8	11	14	15	20	21	Total
Number of eggs hatched	2	4	...	9	2	...	1	...	...	18
Days when found dry (indicated –)	...	...	–	...	...	–	...	–	...	...
Days when water replaced (indicated +)	...	...	...	+	...	...	+	...	+	...

Thus eighteen eggs (90 per cent.) hatched in fifteen days and after two dryings; two eggs (10 per cent.) were found to be collapsed when examined on 24th day.

Experiment IV

Twenty-five eggs laid overnight were placed in a petri dish 3 inches in diameter, containing 10 c.c. of tap water, which completely evaporated and was replaced on the days shown.

Days	2	3	4	5	6	8	10	11	12	15	18
Number of eggs hatched	3	8	...	...	5	1	...	...	...	...	...
Days when found dry (indicated –)	...	...	–	...	...	–	–	...	–	–	–
Days when water replaced (indicated +)	...	...	...	+	...	+	+	...	+	+	+

Experiment IV (*continued*).

Days	20	21	23	26	28	29	32	33	34	Total
Number of eggs hatched	...	7	...	...	...	...	...	...	...	...
Days when found dry (indicated –)	–	...	–	–	–	–	–	–	–	...
Days when water replaced (indicated +)	...	+	...	...	...	...	...	...	...	...

Thus 100 per cent. of the eggs hatched in thirty-four days and after ten dryings and ten replacements of water.

Experiment V

Twenty-eight eggs laid overnight were placed in a petri dish 4 inches in diameter, containing 15 c.c. of tap water, which completely evaporated and was replaced on the days shown.

Days	2	3	4	5	7	9	11	13	16	17	20	22
Number of eggs hatched	6		13									
Days when found dry (indicated –)		–		–	–	–	–	–	–		–	–
Days when water replaced (indicated +)		+		+	+	+	–	+		+	+	

Experiment V (*continued*)

Days	23	25	26	27	29	31	32	34	38	Total
Number of eggs hatched							2		3	25
Days when found dry (indicated –)		–			–			–	–	
Days when water replaced (indicated +)	+		+			+		+	+	

Thus twenty-five eggs (89·2 per cent.) hatched in thirty-eight days and after thirteen dryings and replacements of water. The remaining three eggs were found to be uncapped on the thirty-ninth day, but no larvae were seen. Assuming that these three eggs were uncapped by larvae, 100 per cent. of the eggs in this experiment may be said to have hatched.

The viability of eggs after long periods of dry storage

The longest period during which the eggs of *Stegomyia fasciata* are known to have remained viable in dry storage appears to be two hundred and sixty-two days (Bacot (1916)). For his experiment, Bacot collected eggs laid in Sierra Leone in January, from whence they were sent to England and immersed on 20th October. Within two hours of immersion the eggs hatched freely.

The possibility of the change of climate and environment influencing the period of viability, as shown by Bacot's experiment, suggested the desirability of carrying out some experiments wholly in Townsville. In one test (Experiment VI) eggs were kept in a viable condition in dry storage for a period of two hundred and [illegible] days. In two other tests eggs retained their viability for

over two hundred days (216 and 218), and in five other tests for over one hundred and fifty days (198, 172, 169, 163, 154). The eggs used in Experiment VI were taken from the stock-breeding cages and were known, from other experiments, to be of normal fertility. Those in 'A' were stored on a shelf in the laboratory, excepting in the case of 'A' 7, which was placed between the iron roof and the ceiling of an upstairs room of the Institute building. The eggs used in 'B' were stored on a layer of sand on the roof of an out-building, where they were protected from the rain by a flat sheet of iron resting on four legs about 18 inches high. Since Bacot and others have shown that in order to withstand long periods of drying eggs must pass through a preliminary incubation period in contact with water, all the eggs used in 'A' and 'B' were incubated as shown in Experiment VI.

Experiment VI

A. Indoors.

No.	No. of eggs laid	Date of laying	Maximum possible time in contact with water before storage	Nature of storage receptacles	Date of immersion	No. of eggs hatched after immersion	Viable for days	Percentage of eggs hatched
			Hours.					
1	24	30.4.17	25	Muslin covered petri dish	28.8.17	14	120	58·3
2	25	1.5.17	27	Muslin covered petri dish	28.8.17	2	119	8·0
3	154	5.5.17	33	Muslin covered tube ...	28.8.17	32	114	20·8
4	68	8.5.17	35	Glass covered petri dish	28.10.17	33	172	48·5
5	92	13.5.17	40	Glass covered petri dish	28.10.17	40	167	43·5
6	61	23.5.17	91	Glass covered petri dish	28.12.17	32	216	52·4
7	200 (approx.)	29.4.17	113	Glass covered petri dish	11.1.18	2	257	Approx. 1

B. Outdoors.

No.	No. of eggs laid	Date of laying	Maximum possible time in contact with water before storage	Nature of storage receptacles	Date of immersion	No. of eggs hatched after immersion	Viable for days	Percentage of eggs hatched
1	41	7.4.17	43	Muslin covered tube	28.8.17	5	144	12·2
2	108	4.5.17	31	Muslin covered tube	28.8.17	2	116	1·8
3	70	17.5.17	31	Gelatine capsule	28.10.17	29	163	41·4
4	44	23.5.17	115	Muslin covered tube	28.10.17	2	154	4·5
5	64	2.6.17	115	Glass covered dish	11.1.18	1	222	1·5
6	110	23.6.17	115	Muslin covered dish	11.1.18	1	202	0·9

In a further series of experiments to determine the maximum period of viability after long periods of drying, thirty-two batches of eggs, numbering from thirty to one thousand, were stored for periods varying from three hundred and ninety-one days to five hundred and seventy-nine days and then subjected to stimuli to induce hatching. In all of these experiments negative results were obtained.

Resistance of eggs to drying over Calcium chloride

A number of experiments were carried out to determine the effect of Calcium chloride on eggs. Apparently this method of drying was too severe. The results are shown below:—

Eggs dried for 7 days ... 80 to 90 per cent. of the eggs hatched.
" " " 19 " ... 8 to 15 per cent. " " "
" " " 26 " ... None hatched.

Stimuli to the hatching of resistant eggs

Cooling of resistant eggs as stimulus to hatching

Bacot has shown that a rapid fall of a few degrees in temperature acts as a decided stimulus to the hatching of eggs which have been dried and have resisted subsequent immersion, or which have resisted the influence of the addition of fresh water.

In Experiment VII will be found the results of investigations which appear to fully confirm the first of these findings, although in these the eggs were not first subjected to periods of wetting and drying. In this experiment eggs were collected from the stock-breeding cages and kept in water until placed in the ice chest. Those eggs which failed to hatch within three days were considered to be 'overdue,' this 'overdue' period being shown below. Just before cooling, the eggs were placed in a dish containing 200 c.c. of tap water and removed to the ice chest, where they remained from two to forty-eight hours as shown, the temperature falling from about 80° F. to between 51·8° and 57·2° F.

EXPERIMENT VII

Expt.	Eggs laid	Cooled	Days Overdue	No. of eggs	No. hatched within 2 hours	No. hatched within 24 hours	No. hatched within 48 hours	Percentage hatched
1	25.3.17	3.4.17	6	6	6	...	...	100·0
2	13.4.17	24.4.17	8	16	10	...	...	62·5
3	17.4.17	2.5.17	12	6	2	...	...	33·3
4	4.5.17	26.5.17	19	35	8	5	13	76·0
5	8.6.17	27.6.17	16	24	14	...	...	58·3
6	13.6.17	27.6.17	11	8	6	...	...	75·0
7	19.6.17	27.6.17	5	168	45	...	...	41·7
8	2.1.18	18.1.18	13	25	14	4	1	76·0

Thus in this experiment of two hundred and twenty-eight eggs which failed to hatch during continuous immersion in water for periods varying from five to nineteen days longer than is generally required by non-resistant eggs, one hundred and twenty-eight were induced to hatch after periods of cooling ranging from two to forty-eight hours. Of these one hundred and twenty-eight eggs, one hundred and five hatched within two hours of being placed in the ice chest, nine within twenty-four hours, and fourteen within forty-eight hours.

The effect of lysol on resistant eggs

In certain experiments carried out at this Institute, it was found necessary to sterilize the eggs of *Stegomyia*, lysol being selected as a suitable agent. It was noted that after a short period of immersion in the disinfectant, resistant eggs hatched out soon after being submerged in water. This finding suggested the following experiment (VIII).

The eggs used were from the stock-breeding cages, and proved to be of average fertility. They remained in or on water from the time of laying until immersed for thirty seconds in lysol (of the strength retailed by the manufacturers), the overdue period being reckoned from the third day after laying to the date of immersion.

in the disinfectant. After treatment in lysol, the eggs were drained on filter paper and then immersed in water, the controls remaining in water throughout. The results of this treatment are shown in Experiment VIII.

EXPERIMENT VIII

No. of eggs treated	No. of control eggs	Date of treatment	No. of days overdue in hatching	No. of eggs hatched inside 2 hours.		No. of eggs hatched in 24 hours		Percentage Hatched after lysol
				Treated	Control	Treated	Control	
13	3	22.5.17	11	7	0	5	0	93·3
2	3	28.5.17	7	2	0	...	0	100·0
2	3	28.5.17	7	2	0	...	0	100·0
5	3	28.5.17	7	2	1	2	0	80·0
3	...	28.5.17	6	2	...	1	...	100·0
3	...	29.5.17	8	2	...	1	...	100·0
7	1	4.6.17	7	7	0	...	1	100·0
5	7	5.6.17	8	3	0	2	0	100·0
2	5	7.6.17	10	2	0	...	1	100·0
4	...	8.6.17	11	2	...	2	...	100·0
6	6	11.6.17	14	3	0	...	0	50·0
3	3	14.6.17	17	3	0	...	0	100·0
3	...	15.6.17	18	1	...	...	...	33·3

In this experiment (VIII), fifty-eight resistant eggs were treated with lysol, whilst thirty-four were used as controls. Of the former, thirty-eight hatched within one to two hours following their re-immersion in water after the lysol treatment, and thirteen others followed during the succeeding twenty-four hours. In all 87·9 per cent. hatched. Of the thirty-four controls, none hatched during the first one to two hours following the commencement of the experiment, and two only during the first twenty-four hours.

The effect of 2·5 per cent. soap solution on resistant eggs

Bacot, experimenting with larvicides, found that petroleum soft-soap emulsion 1-8000, acted as a stimulus to the hatching of

resistant eggs in a similar manner to lysol. The present observations show that soap solutions without petroleum also have a similar action.

In my experiment (Experiment IX) eggs were taken from the stock-breeding cages and kept continuously in or floating upon water up to the time of their immersion in the soap solution. The overdue period was reckoned, as in the former experiments, from three days after laying to the time of immersion in soap solution. The eggs were placed in the solution without preliminary drying and, after about five minutes' immersion, were returned to tap water.

EXPERIMENT IX

Expt.	No. of eggs treated	No. of control eggs	Date of treatment	No. of days overdue in hatching	No. of eggs hatched inside 2 hours		No. of eggs hatched in 24 hours		Percentage
					Treated	Control	Treated	Control	
1	4	3	20.6.17	13	3	0	1	0	100·0
2	4	3	20.6.17	13	1	0	0	0	25·0
3	5	2	20.6.17	15	1	0	0	0	20·0
4	6	...	22.6.17	17	5	...	0	...	83·3
5	4	3	22.6.17	20	3	0	0	0	75·0
6	6	3	24.6.17	20	4	0	0	0	66·7
7	5	5	25.6.17	23	4	0	0	1	80·0
8	4	5	25.6.17	23	4	0	...	0	100·0
9	5	5	25.6.17	23	3	0	0	0	60·0
10	10	...	27.6.17	25-27	7	...	0	...	70·0

Thus fifty-three resistant eggs were treated with soap solution and twenty-nine used as controls, thirty-five of the former hatched within one to two hours of their re-immersion in tap water following five minutes' immersion in the solution, and one hatched during the twenty-four hours following the treatment. In all 60·2 per cent. hatched within twenty-four hours. Of the seventeen which failed to hatch, two (one in No. 2 and one in No. 3) were found to be infertile and fifteen to contain either living or dead larvæ. Only one of the control eggs hatched.

The influence of submergence of eggs upon hatching

Experiment X

In order to ascertain the difference, if any, in the hatching period of submerged eggs and those floating on the surface film, forty-five eggs laid during the night of 28th April were divided into two batches on the following morning, 'A' containing twenty-two eggs and 'B' twenty-three eggs. The former ('A') were submerged on filter paper in a dish containing 300 c.c. of tap water, whilst the latter ('B') were allowed to float on the surface film of a similarly prepared dish.

Batch 'A' (submerged) hatched as follows: eighteen on the third day and one on the twenty-third day. The three remaining eggs were taken from the water, dried and dissected on the twenty-eighth day, when they were found to be collapsed. In batch 'B' (floating), twenty-one eggs hatched on the third day and one on each of the fourth and fifth day.

From this one experiment it would appear that the position of the eggs in the containers, i.e., whether floating or submerged, has little influence upon the hatching, since in 'A' 81·8 per cent. of the total eggs, i.e., 94·7 per cent. of the total *fertile* eggs in the batch, hatched on the third day, and in 'B' 91·3 per cent. of the total. This view is supported by observations during the routine work of breeding *Stegomyia*.

The effect of sea water upon the hatching of eggs

On dry storage eggs

Experiment XI

Three hundred eggs, after having been dry stored for a period of three weeks, were divided into two equal batches, 'A' being placed in 300 c.c. of sea water and 'B' in 300 c.c. of tap water. The effects of this immersion are shown in Table 3.

Table 3.

"A" In sea water		"B" In tap water	
Total number of eggs hatched at the end of :—		Total number of eggs hatched at the end of :—	
15 minutes	20	15 minutes	nil
30 minutes	40	30 minutes	30
60 minutes	96	90 minutes	34
27 hours	110	2 hours	70
		5 hours	85
		27 hours	125
Percentage hatched	77·3	Percentage hatched	83·3

On freshly laid eggs

Experiment XII

Two hundred eggs which were laid during the previous night were transferred in the morning from the dish of fresh water to a jar containing sea water. After fifteen days' immersion in sea water they were divided into ten batches of twenty eggs each, and placed in bottles, (1) containing tap water, (2) containing 10 per cent. sea water, (3) containing 20 per cent. sea water, and so on up to 90 per cent. sea water. The subsequent history of these eggs is shown in Table 4.

Table 4.

No.	Contents of bottle	No. of eggs hatched	Length of larval life or subsequent stage of development reached
1	Tap water	17	16 adults reared
2	Tap water plus 10% sea water ...	5	5 adults reared
3	" " 20% " ...	4	4 adults reared
4	" " 30% " ...	5	4 adults and 1 pupa reared
5	" " 40% " ...	2	1 adult reared, 1 larva lived three days
6	" " 50% " ...		5 larvæ lived three days
7	" " 60% " ...		lived one day
8	" " 70% " ...		lived under 48 hours
9	" " 80% " ...		lived under 48 hours
10	" " 90% " ...		

The effects on hatching of submergence in small or large quantities of water

Bacot's experiments to elucidate the above question failed to bring forth any very conclusive evidence, but it appeared from them that slightly increased mortality and delayed hatching followed as a result of submergence in a small quantity of water. Nor do the present experiments afford convincing evidence, but such as has been adduced is in support of Bacot's finding regarding the increased mortality amongst eggs submerged in small quantities of water.

In the first of these experiments, thirty eggs laid during the night of 25th May were divided into two batches and submerged on filter paper, 'A' in 300 c.c. and 'B' in 1,300 c.c. of tap water.

In the second, sixty eggs laid on 30th May were similarly treated. The results are readily seen in Tables 5 and 6.

TABLE 5.

Batch	Day																	Mortality
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	28	
'A'	6	1	...	...	...	...	...	2	4	...	...	...	...	...	...	...	...	13·3%
'B'	8	2	1	...	...	2	...	...	1	1	...	...	...	...	...	...	...	nil

The two remaining eggs in 'A' failed to hatch by the twenty-eighth day.

TABLE 6.

Batch	Day																			Mortality
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
A	...	25	...	...	2	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	6·6%
B	...	20	...	3	4	...	...	2	...	1	...	...	...	...	...	...	...	...	...	...	nil

The two remaining eggs in 'A' which failed to hatch by the sixtieth day were dried and found to be collapsed (dead).

The influence of temperature and environment on the hatching of eggs and the development of the resulting larvae

In Experiment XIII, ninety eggs recently laid were divided into three batches each containing thirty eggs. Each batch was placed in 200 c.c. of tap water with 0·5 gram of polished rice.

'A' was kept in the laboratory at room temperature, i.e., about 78° F.

'B' was kept in an incubator at a temperature of about 95° F.

'C' was kept in an ice chest at a temperature varying from 56° to 59° F.

For details of the results see Experiment XIII.

EXPERIMENT XIII

Batch	Eggs	Day												
		3	4	5	6	7	8	9	10	11	12	13	14	15
'A'	30	1L.	5L.	3L.	1L.	2L.	12L.	...	...	1P.	2P.	1P.	...	3P. 3M.
'B'	30	1L.	...	...	...	2L.	9L.	...	2L.	...	...	3L.	...	1P. (c)
'C'	30	...	...	...	...	...	2L.	5L.	1L.	...	1L.	...	1L.	2L.

EXPERIMENT XIII (*continued*)

Batch	Eggs	Day												
		16	17	18	19	20	22	23	24	25	26	27	28	29
'A'	30	1P. 2M.	3P. 3M.	3M.	2M.	2M.	2P.	2P.	6P.	1P. 3M.	2M.	6M.		
'B'	30									1 (b)				
'C'	30												1P.	1 (d)

NOTE.—L. = larva.
P. = pupa.
M. = adult.

(a) pupated and died on 15th day.
(b) the last larva died on 25th day.
(c) this pupa died two days later.
(d) the last larva died on 29th day.

Thus of thirty eggs kept under the above conditions in the laboratory, twenty-four, or 80 per cent., produced larvae, all of which pupated and subsequently produced adults. Of the thirty kept in the incubator, seventeen, or 56·6 per cent., produced larvae, only one of which pupated; this one died on the same day. The last surviving larva died on the twenty-fifth day. Of the thirty eggs placed in the ice chest, fifteen, or 50 per cent., hatched, of which number only one pupated. This pupa died two days later. The last surviving larva died on the hundred and third day.

In Experiment XIV, one hundred and ten eggs were divided into three batches, 'A' and 'B' containing thirty-eight eggs and 'C' thirty-four eggs. The methods of storing and the temperature were the same as the corresponding batches in Experiment XIII. The results are shown in the following statement:—

EXPERIMENT XIV

Batch	Eggs	Day 5	6	7	8	9	10	11	12	13
'A'	38	...	...	4L.	8L.	2L.	1L.	...	9L. 1P.	...
'B'	38	4L.	...	...	6L.	...	...	8L.	...	1P. (a)
'C'	34	...	...	...	...	1L.	...	...	...	1L.

EXPERIMENT XIV (*continued*).

Batch	Eggs	Day 14	15	16	17	18	19	20	21	47
'A'	38	... 3P.	2P. 1M.	1P.	2P. 4M.	... 2M.	2P.	... 1M.	... 2M.	...
'B'	38	...	...	...	...	...	...	...	L. (b)	...
'C'	34	...	...	12L.	1L.	...	...	...	...	L. (c)

(a) This pupa lived only two days.
(b) The last surviving larva died on 21st day.
(c) The last surviving larva died on 47th day.

Thus of thirty-eight eggs kept in the laboratory twenty-four (63·1 per cent.) hatched. Of these twenty-four larvae eleven (45·8 per cent.) pupated, and ten of the eleven (90·9 per cent.) produced adults. The percentage of adults reared from the thirty-eight eggs was therefore 26·3. Of thirty-eight eggs placed in the incubator eighteen (47·3 per cent.) hatched, one of which reached the pupal stage but survived only two days. Fifteen of the thirty-four eggs placed in the ice chest produced larvae, none of which pupated, although one lived to the forty-seventh day. The low percentage of hatching in 'A,' Experiment XIV, as compared with 'A,' Experiment XIII, is not accounted for.

The influence of sunlight, diffused light, and darkness on the hatching of eggs and development of subsequent stages

EXPERIMENT XV

In this experiment, one hundred and five eggs were divided into three batches, which were treated in the following manner:—

Batch 'A,' containing forty-five eggs placed in 1,300 c.c. tap water with a small quantity of fowl faeces, was stored on the verandah, where it was exposed to direct sunlight during the day and to the lower temperature of night.

Batch 'B,' containing thirty eggs placed in 1,300 c.c. tap water with a small quantity of fowl faeces, was stored in a photographic dark room.

Batch 'C,' containing thirty eggs, was treated similarly to 'A' and 'B,' excepting that it was stored in the laboratory.

The results are shown in the tabulated statement XV.

EXPERIMENT XV

Batch	Eggs	Day 3	4	5	6	7	8	9	10	11	12	13
'A' exposed to direct sunlight	45		35L.								1P.	
'B' darkroom	30		24L.			5P.	5P.	4P.	3P. 3M.			
'C' exposed to diffused sunlight	30	16L.		2L.	1L.	8P.		8P.		3P. 3M.	2P. 1M.	

EXPERIMENT XV (*continued*).

Batch	Eggs	Day 14	15	16	17	18	19	21	29	32
'A' exposed to direct sunlight	45	2P. 5M.	6P. 2M.	... 15M.	1P. 1M.	... 1M.	...	... 1M.	1P.	... 1M.
'B' darkroom	30	... 1M.	...	...	...	1P.	... 1M.	...	...	...
'C' exposed to diffused sunlight	30	... 3M.	... 1M.	...	...	...	...	...	...	...

Thus in 'A,' thirty-five (77·7 per cent.) larvae hatched from forty-five eggs, and twenty-seven (77·1 per cent.) of these larvae ultimately produced adults.

In 'B,' twenty-four (80 per cent.) larvae hatched from thirty eggs, of which number fifteen (62·5 per cent.) produced adults.

In 'C,' twenty (66·6 per cent.) larvae hatched from thirty eggs, all of which larvae produced adults.

It will be seen that the lowest percentage of hatching occurred in the laboratory (Batch 'C'), where 100 per cent. of those which did hatch ultimately produced adults. It should be noted, however, that no observations were made on the unhatched eggs after the thirty-second day; it is quite possible, therefore, that all, or a large proportion of them, may have been naturally resistant.

2. LARVAE

The effect of light or absence of light on the development of larvae

EXPERIMENT XVI

Twenty larvae which hatched between 9.45 a.m. and 11.45 a.m. on 24th March were divided into two equal batches, and each batch placed in a bottle containing 5 or 6 grains of rice and 10 ozs. tap water. One bottle ('A') was stored in the photographic darkroom, the other ('B') on the laboratory windowsill. All the larvae, excepting two of those in 'A,' pupated between 5 p.m. on 27th March and 8.45 on 28th March. On the next day (29th) one of the pupae in 'A' died. On the 30th (about 10 a.m.) the remaining seven pupae in 'A' and the full number (ten) in 'B' produced adults.

In this experiment it would appear that the development of the larvae under artificial conditions and at room temperature is not influenced by the presence or absence of light.

EXPERIMENT XVII.

'A.' On verandah exposed to the sun
'B.' In dark-room
'C.' In workroom
'D.' In ice chest and room temperature alternately

Food given, 0·3 per cent. in 1300 c.c. of water.

Batch.	Larvae.	Day. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
'A'	25	...	...	...	...	...	...	...	...	2P.	5P. 1F.	2P. 2M. 3F.	6P. 2M. 2F.	1P. 5F.	2P. 1M.	1P. 1M.	1P. 2M.
'B'	24	...	...	...	5P.	...	5P.	3P.	1P. 6F. 3M.	4P. 2F.	1P. 3F. 1M.	... 4M.	...	...	...	1P.	
'C'	20	...	...	...	...	...	8P.	...	8P.	... 10F. 3M.	2P. 2F. 1M.	2P.	... 3F.	... 1F.	...	...	
'D'	32	..	...	...	...	...	...	...	...	...	1P.	1P.	2P.	3P.	2P. 1M.	2P. 3F.	2F. 4M.

EXPERIMENT XVII (*continued*).

Batch.	Larvae.	Day. 17	18	19	21	22	23	24	25	26	27	28	29	30	31	33	36
'A'	25	...	1M.							1P.		...	1M.				
'B'	24	1M.															
'C'	20																
'D'	32	1P. 2F.	5P. 2F. 1M.	1F. 1M.	1F. 3M.	1M.			3F. 2M.	1F. 1M.		1P.		1P.			

NOTE.—M. = Male. F. = Female. P. = Pupa.

Thus in 'A' 21 larvae completed their life cycle—84 per cent. reared in 28 days. In 'B' 20 larvae completed their life cycle—83·3 per cent. reared in 16 days. In 'C' all larvae completed their life cycle—100 per cent. reared in 12 days. In 'D' 31 larvae completed their life cycle—96 per cent. reared in 36 days.

The effect of temperature and darkness on the development of recently hatched larvae

The larvae in these two experiments (Experiments XVIII and XIX) hatched overnight. In Experiment XVIII, thirty larvae were divided into two equal batches, 'A' and 'B,' each of which was placed in 100 c.c. tap water containing 0·25 grams of fowl faeces. 'A' was kept in an incubator at 98·6° F. and 'B' in a second incubator which was not artificially heated, the temperature being about 78·8° F. The results are shown hereunder:—

EXPERIMENT XVIII

Batch	Larvae	Day.										
		1	5	7	8	9	11	12	13	14	16	18
'A'	15	...	1P. (*a*)	...	L. (*b*)	...	...	...	...	...	...	...
'B'	15	...	...	...	6P.	6P. 1M.	... 4M.	2P. 4M.	... 1M.	... 3M.	1P.	... 1M.

NOTE.—'L' = Larva. 'P' = Pupa. 'M' = Adult.

(*a*) The first larva pupated on the fifth day and died on the seventh.

(*b*) The remaining larvae were all dead on the eighth day.

Thus in 'A' (temperature 98·6° F.) only one larva pupated, and this lived only two days. The remaining larvae died by the eighth day.

In 'B' (temperature 78·8° F.) only 6·7 per cent. of the larvae failed to produce adults.

EXPERIMENT XIX

Batch	Larvae	Days					
		10	11	12	13	14	15
'A'	10	L. (*a*)	...	...	...	...	...
'B'	10	3P.	5P.	1P.	1P. 4M.	... 2M.	... 1M.

(*a*) All the larvae were dead on the tenth day.

Thus in 'A' (temperature 98·6° F.) none of the larvae survived, but in 'B' (temperature 78·8° F.) only 10 per cent. of the larvae failed to produce adults.

From these experiments it would appear that excessive heat and not darkness was responsible for the mortality. In both experiments (XVIII 'B' and XIX 'B') the larval period was longer than usual, i.e., from ten to thirteen days in the latter.

Subsequent records of experiments will show that the lengthened larval period was probably due to unsuitable feeding, since it has been shown in Experiment XVI that the absence of light does not retard larval development.

Quantity and nature of food, and its influence on larval development

In this experiment (XX) forty newly hatched larvae were placed in each of five vessels containing 300 c.c. tap water and 0·1 per cent. of food as follows:—

P.R. 0·3 grm. polished rice.
M.L. 0·3 grm. dry mango leaf.
F.F. 0·3 grm. dry fowl faeces.
B. 0·3 c.c. broth previously exposed to the air for two days, with luxuriant growth of bacteria.
F.M. 0·3 grm. fresh fly maggot (dead).

The average laboratory temperature during the experiment was about 71° F. The results are shown on the corresponding statement (XX).

Experiment XX

Batch	Larvae	Day 8	9	10	11	12	13	14	15	16
P.R.	40	1P.	11P.	4P.	8P. 3M.	3P.	1P.	...	3P. 6M.	... 1M.
M.L.	40	...	...	...	...	...	...	...	...	1P.
F.F.	40	1P.	3P.	4P.	1P.	1P. 4M.	3P. 5M.	... 2M.	1M.	1P.
B.	40*	...	...	...	...	...	...	...	...	...
F.M.	40	1P.	...	... 1M.	1P.	...	2P.	... 1M.	...	... 2M.

Experiment XX (*Continued*).

Batch	Larvae	Day 17	18	19	20	21	22	23	24
P.R.	40	... 1M.	... 2M.	...	2P.	...	...	... 1M.	...
M.L.	40	...	...	... 1M.	...	...	...	...	...
F.F.	40	1P.	... 1M.	1P.	...	1P. 1M.	... 2M.	1P.	... 1M.
B.	40*	...	...	...	...	...	...	...	...
F.M.	40	...	...	...	...	...	...	...	...

* All died in larval stage.

The mortality totals in the above 0·1 per cent. foods are therefore seen to be:—P.R. 65·0 per cent., M.L. 97·5 per cent., F.F. 57·5 per cent., B. 100 per cent., and F.M. 90 per cent.

Experiment XXI

The number of larvae used in this experiment varied between ten and thirteen to each batch. All were recently hatched. Each batch was liberated in a vessel containing 400 c.c. tap water and 0·25 per cent. of food as follows:—

M.L. 1·0 gram dry mango leaf.
D.C. 1·0 " dried cockroach.
H.D. 1·0 " dried horse faeces.
P.R. 1·0 " polished rice.
G.S. 2·0 c.c. goat serum.

The results are shown in the corresponding statement (XXI).

Experiment XXI

Batch	Larvae	Day 5	6	7	8	9	10	11	12	13
M.L.	13	1P.	1P.	1P.	... 3M.	2P.	1P. 2M.	1P. 1M.	1P. 1M.	1M.
D.C.	10	...	4P.	2P.	... 4M.	2P. 2M.	... 2M.	...	...	...
H.D.	10	...	...	...	...	1P.	...	2P.	...	1P.
P.R.	13	11P.	... 11M.	...	...	...	...	...	...	...
G.S.	13	...	1P.	2P.	1P. 2M.	3P. 2M.	...	... 3M.	...	...

Experiment XXI (*continued*).

Batch	Larvae	Day 14	15	16	17	18	19	20	21
M.L.	13	...	...	1P.	...	1P. 1M.	1P. 1M.	...	... 1M.
D.C.	10	...	1P.	...	... 1M.	...	...	...	...
H.D.	10	1P.	... 1M.	...	... 1M.	...	...	...	...
P.R.	13	...	...	...	...	1P.	...	... 1M.	...
G.S.	13	2P.	1P.	1P.	3M.	1M.	1M.	...	...

Thus the mortality totals in 0·25 per cent. of the foods shown above are as follows:—M.L. 15·4 per cent., D.C. 10 per cent., H.D. 50 per cent., P.R. 7·7 per cent., and G.S. 7·7 per cent.

Experiment XXII

From nine to seventeen recently hatched larvae were liberated in each of nine vessels containing 400 c.c. tap water and 0·3 per cent. of food, as follows:—

M.L. 1·2 gram. dry mango leaf.
D.C. ,, dry cockroach.
H.D. ,, dry horse faeces.
P.R. ,, polished rice.
P. ,, peptone.
P.S. 0·15 per cent. peptone solution, tweny-four hours old, filtered.
B.C. 1 ,, agar slant, B. coli.
B.C.2 $\frac{1}{2}$,, ,, ,, ,,
H.U. 4 c.c. human urine.

The results are shown in the corresponding statement (XXII).

Experiment XXII.

Batch	Larvae	Day 5	6	7	8	9	10	11	12	14	18
M.L.	17	3P.	6P.	... 4M.	5P. 4M.	...	1P. 6M.	...	1P.	... 2M.	...
D.C.	17	2P.	5P.	6P. 1M.	... 4M.	3P. 5M.	...	... 4M.	...	...	...
H.D.	9	2P.	2P.	... 2M.	...	...	... 2M.	...	...	...	...
P.R.	9	5P.	2P.	...	... 7M.	...	...	...	...	...	...
P.	15	...	1P.	...	...	...	...	...	...	...	...
P.S.	9	5P.	3P. 1M.	... 3M.	1P. 4M.	...	... 1M.	...	...	...	...
B.C.	11	7P.	2P. 1M.	... 5M.	... 2M.	...	...	1M.	...	...	...
B.C.2	10	5P.	1P.	... 5M.	...	...	... 1M.	1P.	...	1P. 1M.	... 1M.
H.U.	9	4P.	... 1M.	... 2M.	1P. 1M.	1P. 1M.	... 1M.	...	...	1P.	... 1M.

Thus the mortality totals in 0·3 per cent. of the foods shown are as follows:—M.L. 5·9 per cent., D.C. 5·9 per cent., H.D. 55·5 per cent., P.R. 22·2 per cent., P. 9·3 per cent., P.S. nil, B.C. 18·2 per cent., B.C.2 20 per cent., and H.U. 22·2 per cent.

The results of these three experiments are tabulated in Table 7.

Table 7.

Batch	Nature of food	Percentage of food to water	Percentage of mortality
P.R.	Polished rice	0·1 0·25 0·3	65·0 7·7 22·2
M.L.	Mango leaf	0·1 0·25 0·3	97·5 15·4 5·9
F.F.	Fowl faeces	0·1 0·25 0·3	57·5 ... ...
B.	Broth	0·1 0·25 0·3	100·0 ... ...
F.M.	Fly maggot	0·1 0·25 0·3	90·0 ... ...
D.C.	Cockroach	0·1 0·25 0·3	... 10·0 5·9
H.D.	Horse faeces	0·1 0·25 0·3	... 50·0 55·5
G.S.	Goat serum	0·1 0·25 0·3	... 7·7 ...
P.	Peptone	0·1 0·25 0·3	... ... 9·3
P.S.	Peptone Solution ...	0·15	nil
B.C.	Agar slant *B. coli* ...		18·2
B.C.2	Agar slant *B. coli* ...		20·0
H.U.	Human urine ...	1·0	22·2

Experiment XXIII

'A.' 200 c.c. of tap water containing 0·5 per cent. rice starch.
'B.' ,, ,, ,, with the addition of 5 per cent. sugar.
'C.' ,, ,, ,, with the addition of half the white of an egg.
'D.' ,, ,, ,, containing piece of banana (removed after the third day).

The results are shown in the corresponding tabulated statement (XXIII).

Batch	Larvae	Day 7	8	9	10	11	12	13	14	15
'A'	22	...	...	...	...	...	...	3P.	...	...
'B'	20	L. (a)	...	...	...	...	...	...	...	...
'C'	20	...	...	9P.	1P.	... 1M.	... 6M.	3P.* 2M.	...	1P. 3M.
'D'	30	1P.	8P.	5P.	6P.	... 1M.	4P. 4M.	... 2M.	1P. 9M.	2P. 5M.

Experiment XXIII (*continued*).

Batch	Larvae	Day 16	17	18	19	21	22	23	24	25
'A'	22	... 3M.	...	11P.	...	1P.	1P.	... 11M.	2P.	... 4M.
'B'	20	...	...	...	...	...	...	L. (b)	...	...
'C'	20	2P. 1M.	...	(c)	(d)	...	...	...	...	1P.
'D'	30	...	... 6M.	...	(e)	...	...	...	...	...

(a) Some of the larvae now in second skin.
(b) Larvae in second and third skin.
(c) Two pupae and one larva died.
(d) One pupa died.
(e) The remaining larvae died.

Mortality 'A' ... 18·2 per cent.
'B' ... 100 ,,
'C' ... 35 ,,
'D' ... 10 ,,

The effects of an increase of temperature on larvae and pupae of Stegomyia

A number of recently hatched larvae were placed in water in beakers heated over a water bath. When the temperature reached 114·8° F., 75 per cent. of the larvae were found to be dead, and the remainder failed to develop when the water was gradually reduced to room temperature.

Further experiments were then carried out with older larvae, i.e., larvae in their third skin, and with pupae. The procedure was the same as in the first experiment. The results of these experiments are seen in the tabulated statement (XXIV).

Experiment XXIV

	Temperature	Number used: Larvae	Number used: Pupae	Number subsequently reared	Number killed by heating	Mortality
1	90° F.	10	3	all	nil	nil
2	95° F.	10	3	all	nil	nil
3	100° F.	10	3	all	nil	nil
4	105° F.	10	3	10	3 larvae	23%
5	110° F.	10	3	4	2 pupae 7 larvae	69·2%
6	115° F.	10	3	nil	all	100%

From the foregoing statement it will be seen that the approximate maximum temperature that the larvae and pupae are capable of withstanding without great mortality is about 105° F.; beyond this temperature there is a very great increase in the mortality. Bacot showed that a temperature of 112° F. caused a mortality of 50 per cent. and that higher temperatures caused the death of all larvae and pupae experimented with.

The survival of larvae and pupae out of water

Experiment XXV

(1) On filter paper kept slightly moist.

Thirty-four larvae and five pupae were placed on wet filter paper and kept moist by adding a few drops of water each day. Two

adult mosquitoes emerged on the following day, two others on the second day and the remaining one on the fourth day. The larvae were left on the paper until the fifteenth day, when only two of them remained alive. These two were then transferred to a bottle of water, but failed to reach the pupal stage.

(2) On wet filter paper and allowed to dry in muslin covered dish

Larvae and pupae were placed on wet filter paper in a muslin covered dish and allowed to dry gradually. Two larvae and one pupa were removed to water at the end of thirty-two hours, when the paper was nearly dry, and all of them eventually developed into adults.

Of two larvae and one pupa removed at the end of forty-seven and a half hours, only the latter was alive, but this died soon after in a dish of water. Another pupa, however, was alive after fifty and a half hours on the paper, but was dead at seventy-two hours. None of the larvae appeared to have survived beyond thirty-two hours.

(3) On wet filter paper and allowed to dry in a glass covered dish

Thirty-one larvae were placed on wet filter paper and put on a glass covered petri dish. After sixty-five hours in this dish seven larvae were removed to water, where one of the seven eventually matured. The remaining twenty-four larvae were transferred to water after having been on the paper for eighty-nine hours. Of these five were alive, but one died during the following twenty-four hours. The remaining four lived for nine days, after which no further record of them was kept.

3. ADULTS

Length of life of adult mosquitoes in captivity when unfed and fed on various foods

The mosquitoes used in this experiment (XXVI) were recently emerged laboratory-bred insects, all of which (except A) were given an opportunity of feeding daily during the course of the experiment. The various batches were kept in similar cages and treated similarly

to each other, excepting with regard to the food given. The average daily temperature was between 71° F. and 80° F.

A. Not fed.
B. Fed on syrup.
C. Milk and sugar.
D. Banana.
E. Blood once (females); banana afterwards.
F. Blood for sixty-one days, then bananas (females); banana only for males.

The results are shown in the following tabulated statement:—

EXPERIMENT XXVI

Expt.	Number of adults used	Day 2	4	7	12	15	19	26	30	35	47	51	58	61	68
'A'	25F. 12M.	9F. 8M.	15F. 4M.	1F.	...	...	...	...	...	...	...	...	...	...	...
'B'	7F. 4M.	...	2M.	2F.	...	2F.	2F. 1M.	1F.	1M.	...	...	...	...	...	...
'C'	7F. 2M.	...	...	1F.	1F. 2M.	2F.	2F.	...	...	...	...	...	...	...	...
'D'	5F. 5M.	...	...	...	...	...	1F. 1M.	1M.	1F. 1M.	1F. 1M.	1F. 1M.	...	...	...	1F.
'E'	3F. 1M.	...	...	...	...	...	...	1F. (e)	1M.	1F.	...	...	...	1F.	
'F'	15F. 5M.	...	...	...	5F.	...	5F. 1M.	...	1F. 2M.	...	1F. 1M.	1F.	1F.	1M.	

(e) = Escaped from cage. F. = Females. M. = Males.

Retention of eggs by gravid females

Oviposition on dry surfaces

It is a generally accepted belief that either water or a damp surface is essential to oviposition, and that if such is not available the gravid female will retain her eggs for a long period—until her death or until favourable conditions are made available. My experience in the routine of breeding *Stegomyia*, as well as the results of a number of experiments undertaken especially to confirm former observations, convince me that this belief is well founded

Oviposition on oily surface

That similar results follow the provision in the breeding cages of only water having a surface film of oil is shown by the following experiments:—

EXPERIMENT XXVII

On the morning of 11th September, two gravid females and four males were placed in a breeding cage containing a dish of tap water covered with a thin film of kerosene. For several days previously the females had fed on human blood and the males on banana, which foods were offered daily during the progress of the experiment. The following observations were subsequently recorded:—

11th September (afternoon), one female fed. 13th, one female fed. 14th, one female fed; three males drowned. 17th, a female drowned. 19th, the remaining male drowned, three others liberated in cage. The remaining female fed on 20th September and on 4th October. No eggs laid up to this date. A dish of tap water without oil on surface was put into the cage, and the first dish (with oil) removed. 5th October, forty-six eggs laid over-night, the first oviposition during her twenty-four days of captivity. Female fed in afternoon. 15th October, seventy-nine eggs laid over-night. Dish of fresh water removed and replaced by one containing water with oily film. 16th October, female fed. 22nd October, female fed, and at intervals of about three days until her death. One male drowned. 24th October, one male drowned. 25th October, one male drowned. 8th November, female drowned.

Egg laying by unfertilised females

We have noticed on several occasions that certain batches of eggs produced females only, and that when segregated and fed on human blood these unfertilised insects were capable of laying eggs.

In this connection the following experiment is of interest as showing the increase which takes place in egg production after fertilisation.

The mosquitoes used in this experiment, seven in number, were reared from a batch of eggs which produced females only, and as soon as possible after hatching they were segregated in such manner as to preclude the possibility of fertilization. Opportunities for feeding

on human blood were given frequently from the date of emergence, i.e., 7th September. After thirty days of captivity in this manner two males were liberated in the cage with the five surviving females for seventeen hours, with the results shown in Experiment XXVIII.

EXPERIMENT XXVIII

Dates on which eggs were laid	Number of eggs laid	Approx. number proved fertile	Fertility	Deaths to date	Feeds given to date	Remarks
22.9.17	5	...	...	2	14	
24.9.17	6	...	...	...	16	
1.10.17	85	...	...	...	19	Total number of eggs laid by unfertilised females: 105
8.10.17	9	...	...	...	23	Total number of feeds given: 23
4 p.m. 8.10.17 to 9 a.m. 9.10.17	(17 hours) 2 males liberated in cage with 5 surviving females.					
10.10.17	60	30	+	...	28	
12-13.10.17	78	78	+	...	...	
15.10.17	12	3	+	...	31	
17.10.17	31	31	+	...	33	
18.10.17	60	60	+	...	35	
22.10.17	140	140	+	...	39	
25.10.17	52	38	+	1	41	
29.10.17	120	100	+	...	...	
2.11.17	72	60	+	1	...	
7.11.17	140	100	+	1	44	Total number of eggs laid by fertilised females: 762
						Total number of feeds given to fertilised females: 44

From the foregoing it will be seen that seven unfertilised females did not lay eggs during the first fifteen days, and that during this

period two died. During the following fifteen days the remaining five females laid one hundred and five infertile eggs and were then mated with two males. During the thirty days following the introduction of the males seven hundred and sixty-five eggs were laid, of which number approximately six hundred and forty proved fertile.

Experiments to ascertain whether Stegomyia fasciata *would lay eggs when fed on food other than blood*

From time to time various batches of mosquitoes were given food other than blood for periods ranging from twenty-five days to one hundred and forty days. The following foods were experimented with:—

Concentrated sugar solutions	... ...	4 experiments.
Concentrated peptone and sugar solution		3 ,,
Concentrated sugar and haemoglobin solution	... ...	3 ,,
Milk and sugar	... ...	2 ,,
Banana	... ...	1 ,,
Peptone solution	... ...	2 ,,
Syrup	... ...	1 ,,
Honey	... ...	1 ,,
Dates	... ...	1 ,,
Apple	... ...	1 ,,

In each of the three experiments in which peptone and sugar were given as food a number of eggs were laid, about 60 per cent. of which were fertile. In all other experiments there was no egg production.

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ANCYLOSTOMA CEYLANICUM IN THE CAT IN DURBAN

BY

B. BLACKLOCK

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Material from Durban was sent by Dr. F. G. Cawston on 10th June, 1919, with the following notes:—

'*Ankylostoma duodenale* from a cat in Durban. The gut also contained a tapeworm, and death seems to have been caused by the numerous Ankylostoma.'

The material consisted of ten female worms, of which nine were complete and in a good state of preservation.

It is not possible in the absence of specimens to describe the characters of the male bursa, but the general appearance of the females, the measurements and the arrangement of the chitinous buccal capsule and teeth have been studied.

Of the nine complete specimens the minimum length is 7·0 mm., the maximum 9·5 mm., the average 8·3 mm. The arrangement of the teeth is characteristic. There is a pair of large ventral teeth and also a very small pair situated at the base of these on a deeper plane. The measurements and mouth structure are, in fact, such that this parasite cannot be distinguished from *A. ceylanicum* (Looss). This parasite was recorded by Yorke and Blacklock (1915) in seven dogs in Sierra Leone, and was further referred to by them (1917) subsequent to Macfie's discovery of the same parasite in four of ten dogs at Accra (1916).

That this parasite should be found in Durban is of interest, more especially so if, as Dr. Cawston surmises, it should be the cause there of fatal disease in cats.

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