The effect of different diets on the reproduction of two species of mosquitoes, *Aedes aegypti* and *Culex pipiens*

by

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This one's for you, Dad.

" Greater knowledge of nutritional requirements of mosquito larvae might lead to ways of making food less accessible ".

— WHO Technical Report No. 368

CONTENTS

INTRODUCTION

Mosquitoes are probably the least studied of all economically important arthropods. The three subfamilies Toxorhynchitinae, Anophelinae and Culicinae are comprised of 34 subgenera which includes more than 3 100 mosquito species (Service, 1986).

Mosquitoes have a world wide distribution, breeding in the high arctic tundra as well as dense tropical rain forests, but are absent from Antarctica (Service, 1986). They belong to the most primitive branch of the advanced order Diptera but in their feeding they are highly specialized. Other blood sucking members of the order have relatively short mouthparts, which make pit-like wounds into which blood seeps and is imbibed; this is described as pool feeding. Mosquitoes, on the other hand, have long, slender stylets which probe the flesh until they find a blood capillary from which they draw blood very rapidly (Busvine, 1975).

The rate of development of mosquitoes, like that of all arthropods, is dependent on climate. In hot countries, the life cycle is completed in a week or so, and breeding is continuous, though often augmented in rainy seasons and reduced in dry ones (Busvine, 1975).

All kinds of warm-blooded and some cold-blooded land animals may serve as sources of blood for the females but generally mosquitoes have host preferences (Busvine, 1975) and because of this they are pests in many areas, but their main importance is as vectors of disease. Medically, the most important genera are Aedes, Anopheles, Culex and Mansonia (Service, 1986).

In Africa, Aedes aegypti and Culex pipiens quinquefasciatus are more

important as pests but they are also able to transmit certain viral and nematode diseases (Christophers, 1960) and their potential as vectors in this field should not be disregarded or underestimated. Since the discovery of resistant strains of these mosquitoes to modern insecticides, it has been necessary to search for alternate means of control. These methods include control by parasites or predators, pathogenic micro-organisms, insect development inhibitors and the release of sterilized males. However, as there are many technical problems associated with the above mentioned means of control (Service, 1986), another possibility for consideration is through mosquito larval nutrition.

Nutrition has been shown to influence reproduction (Clements, 1963; Engelman, 1970; Moeur & Istock, 1980; Lillie & Nakasone, 1982). Different food types can affect adult development, the amount of eggs a female can lay (fecundity) and also the number of eggs that are able to hatch (fertility). As different foods influence the biology of mosquitoes, it is necessary to know what these effects may be as they will also have a bearing on bioassay studies carried out on laboratory bred mosquitoes.

The findings of this type of study would also enhance our knowledge of the nutritional requirements of A aegypti and C. p. quinquefasciatus. It was always believed that mosquito larvae were indiscriminate feeders but Aly (1985) and Dadd et al. (1982) have shown them to be selective in their choice of nutrients.

Some authors (Gahan & Smith , 1964; Muspratt, 1962) have raised mosquito larvae on a single diet and looked at the effects on larval and adult development. Diet types used can be classified as; mainly carbohydrate, mainly animal protein, artificial diets, yeasts and infusions. Only two

authors (Lillie & Nakasone, 1982) have compared the effects of different larval diets (cat, dog, rabbit and fish food) on mosquito adults reared simultaneously from the same breeding stock.

Temperature and humidity have been shown by Clements (1963) to be of extreme importance for the survival of mosquitoes under natural conditions. Very little attention has been paid to the effect of diet which has been shown to have a mojor role in controlling lifespan and fecundity (Nayar & Sauerman, 1971) and is probably the most important single factor in the majority of insect species. Much work has been done on elucidating the individual nutrients required for growth under sterile conditions (Dadd, 1980; Lang etal., 1972) but not on what influence each whole diet type has on the biology of mosquitoes.

The aim of the present study was to rear A. aegypti larvae under the same environmental conditions on five different diet types and to examine the effects on the following parameters;

(a) fecundity of resulting adult females
(b) fertility of the eggs they produce
(c) duration of the larval and pupal stages
(d) larval, pupal and adult mortality
(e) size of the ovaries

The experiment was repeated using C. p. quinquefasciatus larvae and the effects on the above mentioned parameters was studied.

The development of oogenesis in females reared on the different diets was also examined at the transmission electron microscope level as the effect of diet on oogenesis has not been studied before.

The diets used were; Pronutro and Tastee wheat, Epol rat cubes, Breeders Dogmor puppy chunks, Brewer's yeast and dessicated liver. Dessicated liver served as the control diet since it is the most commonly

used diet for rearing mosquitoes in insectaries.

It is hoped that the results of this study will have practical mosquito control applications from the point of view of discovering certain nutrients that are directly related to increased rates of reproduction. Even though a study of reproduction under controlled laboratory conditions is only a guide to what is happening under field conditions, it can help explain the biology in the wild, and as mosquitoes seem capable of invading the whole environment in town or country as a result of changes caused by the habits of the population and various aspects of modern life, all aspects of the study of mosquitoes are of primordial importance in order to better understand how to control them most effectively (Subra, 1980).

MATERIALS AND METHODS

1. THE DIETS

The following diets were used in the present study (Appendix 1);

1. Diet A - Pronutro and Tastee wheat (in equal quantities)
2. Diet B - Epol rat cubes
3. Diet C - Breeder's Dogmor puppy chunks
4. Diet D - Vital brewer's yeast powder
5. Diet E - Vital dessicated liver tablets

Diets A to D were the experimental diets while diet E was the control.

2. THE MOSQUITOES

2.1 **Culex** pipiens quinquefasciatus (from now onwards to be referred to as Culex quinquefasciatus as is common in the literature)

2.1.1 Stock mosquitoes

Larvae were field caught in Durban North and brought into the insectary and identified according to the WHO key. They were placed in plastic mosquito rearing dishes measuring 30cm in diameter and 11cm in depth (Fig. 1d) and containing 10ml tap water per larva. The larvae were maintained on a diet of dessicated liver. The water in the dishes was changed daily from the third day onwards and fresh food was added. Upon pupation, and using a 5ml plastic dropper, the pupae were transferred to wooden frame cages measuring 38cm square (Fig. 2) and left to emerge.

Figure 1. Bowls and rearing dishes used in the present study :

a = pill vial for hatching of egg rafts

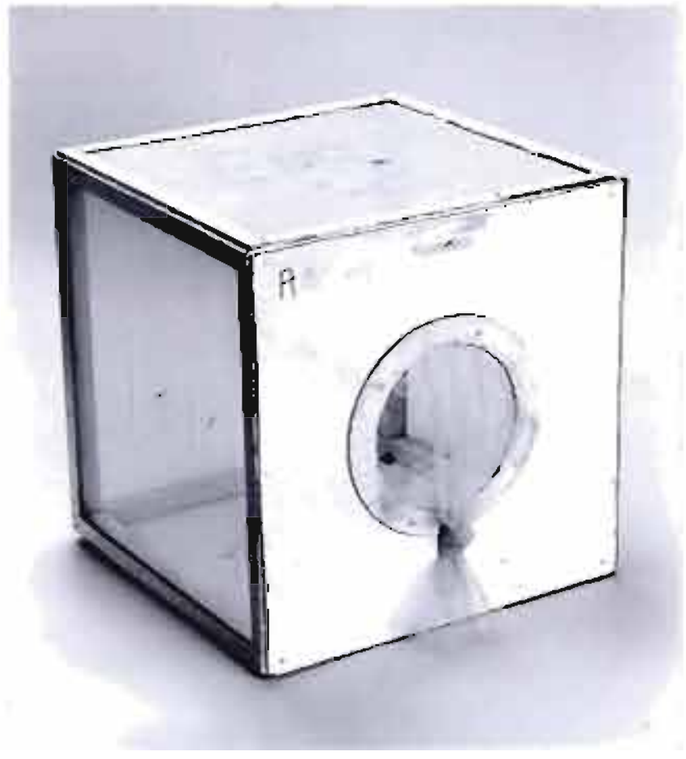
b = pupal emerging bowls

c = bowl for egg laying

d = larval rearing dish

(Scale : ruler = 30cm)

Figure 2. Mosquito rearing cage.



The adults were maintained on a diet of sugar cubes and water. Five days post emergence, the females were blood fed overnight on a pigeon after being starved for at least 6 hours.

Egg rafts were laid in a plastic bowl measuring 26cm x 6cm (Fig. 1c) containing tap water from 72 hours onwards post blood meal (PBM). These rafts were collected by gently lifting them off the water surface with a fine paint brush.

They were then placed individually in 20ml pill vials (Fig. 1a) containing approximately 16ml tap water and left for 48 hours to hatch.

The larvae were divided into five groups and transferred to plastic rearing dishes labelled A, B, C, D and E. They were treated as previously described except that the respective diets were added to each dish. Emerging adults were used as the experimental mosquitoes.

2.1.2 Experimental Mosquitoes

These were maintained on a diet of sugar cubes and water for five days post emergence. They were then starved for six hours and blood fed overnight on a pigeon. The abdomens of the female mosquitoes were examined before the bird was removed from the cage. This was to ensure that sufficient mosquitoes had taken a blood meal so that the maximum number of egg rafts would be obtained.

Egg rafts were oviposited from 72 hours PBM onwards in a plastic bowl and were removed and left to hatch as described previously.

The total number of eggs laid by each female was noted (fecundity) as was the number of larvae that hatched (fertility).

Approximately 150 larvae were transferred to the plastic rearing dishes labelled A, B, C, D and E containing 10ml tap water and 0,004g of the respective diet per larva. Each diet was set up in triplicate. From day three onwards the water was changed daily and fresh food added as scum formation on the surface started post 72 hours. Larval and pupal mortality as well as duration of the larval stage was recorded for each dish.

The pupae were sexed and transferred to small bowls (Fig. 1b) containing clean tap water. The bowls were then placed into their respective cages (i.e. A, B, C, D and E) where the pupae were left to emerge.

Adults were maintained as described previously and daily mortalities were recorded. Five females were removed prior to blood feeding. They were anaesthetized and their ovaries were removed for measurements.

The above experimental cycle was repeated twice (for a total of 3 runs).

2.2 Aedes aegypti

2.2.1 Stock mosquitoes

Larvae were field collected in Durban North and brought into the insectary and identified according to the WHO key. They were then maintained as described below.

2.2.2 Experimental mosquitoes

Both the stock and experimental mosquitoes were maintained in the same manner as the Culex spp. mosquitoes described above with the following minor differences;

(a) the adult female mosquitoes were blood fed for approximately one hour on a human forearm but most of the mosquitoes had fed within 20 minutes

(b) eggs were laid individually on moist paper towel lining the ovipositing bowls

(c) egg papers were dried for 48 hours, moistened, redried overnight and flooded with tap water so as to achieve maximum egg hatching (tap water was used for convenience as it resulted in as much hatching as deoxygenated water during pre-trials)

(d) fecundity values for each female were calculated as the mean number of eggs per female.

The above experimental cycle was repeated twice (for a total of 3 runs).

3. TRANSMISSION ELECTRON MICROSCOPY

A. aegypti and C. quinquefasciatus ovaries were treated as described below;

Five days post emergence females were given a blood meal and anaesthetized 12, 36 and 60 hours post blood meal (PBM). Four ovaries from each group were removed and processed as follows;

(1) the ovaries were fixed in 2,5% glutaraldehyde (in 0,1M sodium cacodylate buffer) for 30 minutes followed by one wash in buffer for 5 minutes

(2) they were then fixed in 1% osmium tetroxide for 60 minutes followed by three washes in distilled water for 2 minutes each

(3) the tissue was then placed in 0,5% uranyl acetate (in 80% acetone) for 30 minutes

(4) the ovaries were then dehydrated in 95% acetone for 10 minutes followed by three changes of 100% acetone for 10 minutes each

(5) thereafter, equal amounts of 100% acetone and Spurr resin were added and allowed to stand at room temperature for 90 minutes

(6) the tissue was then placed in two changes of pure Spurr resin for 60

minutes each

(7) finally, the ovaries were embeded in Spurr resin and left overnight at 70°C to polymerize.

Sections were obtained using a Reichert ultramicrotome and glass knives. For light microscopy 1µ sections were stained with toluidine blue. For electron microscopy 70nm sections (gold interference colour) were collected on 200 mesh copper grids, dried and stained with 2% aqueous uranyl acetate for 5 minutes, washed in distilled water and stained with Reynold's lead citrate for 2 minutes.

The sections were then viewed and photographed on a Philips 301 TEM.

4. HUMIDITY AND TEMPERATURE

Daily recordings were made on a New Sigma hygrothermograph. Mean values plus 95% confidence limits for each experimental run were calculated.

5. LIGHT / DARK RATIO

The ratio was controlled by an electrical timer switch and was set at 14 hours light and 10 hours dark.

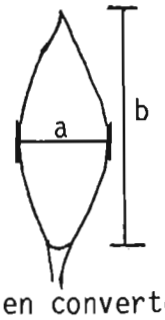
6. pH OF LARVAL WATER

A sample of water was collected from each bowl on a daily basis and the pH was measured on a pH meter.

7. MEASUREMENT OF OVARIES

Ovaries were dissected in 0,45% sodium citrate under a Bausch and Lomb

dissecting microscope. The length and width of the ovary was then measured on a Zeiss light microscope under the 5x objective using a Wild eye - piece micrometer. Measurements were made as follows;



a = width

b = length

Readings were then converted to micrometers as follows;

(1) before readings could be made it was necessary to calibrate the eye piece using a calibration slide

(2) the reference line in the eye - piece was moved across the scale of the calibration slide by turning the drum on the eye - piece

(3) the number of divisions on the slide that were covered was noted and also the number of revolutions on the drum

(4) one revolution of the drum represented 100 divisions

(5) the micrometer value was the number of divisions on the slide divided by the number of divisions on the drum

(6) the reference line on the drum was then moved across the width and length of the ovaries and the reading on the drum was then multiplied by the micrometer value

(7) the value obtained for the length was multiplied by the value obtained for the width to give the size of the ovaries.

8. STATISTICS

A difference of means test for the experimental diet groups A, B, C and D against the control diet group E was carried out.

T values were calculated for significance at the 95% level.

RESULTS

1. STATISTICS

All statistical analyses of the various parameters was done at the 95% confidence limits.

2. FECUNDITY

There was a wide range of values obtained in Culex quinquefasciatus for each diet type. Diet A varied from 79 eggs per raft to 251; diet B - 64 to 363 eggs; diet C - 74 to 275 eggs; diet D - 84 to 307 eggs and the control diet E - 96 to 301 eggs. For this reason there were no significant differences between the experimental diets A to D and the control diet E (Tables 1 and 2)

The fecundity for Aedes aegypti was calculated as the mean number of eggs per female. Once again, there was a wide range of values for each diet with diet A ranging from 28,1 to 39 eggs per female; diet B - 38,4 to 59,6 eggs; diet C - 28,9 to 39,3 eggs; diet D - 29,1 to 54 eggs and the control diet E had the widest range - 26,7 to 96,1 eggs. Because of the wide range of values there were no significant differences between the experimental diets A to D and the control diet E (Table 3).

3. FERTILITY

The fertility of the eggs laid by C. quinquefasciatus was relatively high with the lowest values obtained in diet A. This diet produced a mean fertility of 85,3% with a range of 60,5 to 98,2% Diet B had a mean of 94,7% with a range of 73 to 100% fertility. Diet C mean value was 97,1% - the highest - with a range of 86,4 to 100%. The mean fertility for diet D was 93,1% and the range was 57,3 to 100%. The control diet E

Table 1. Fecundity of Culex quinquefasciatus. Values expressed as the number of eggs per raft plus the mean and 95% confidence limits for each diet type.

Diet	Number of eggs per raft	$\bar{x} \pm$ 95% confidence limits
A	156, 170, 250, 251, 152, 105, 79, 81, 119.	151,4 ± 49,6
B	257, 300, 131, 66, 68, 64, 172, 140, 363.	173,4 ± 84,4
C	202, 160, 236, 256, 275, 161, 112, 87, 74.	173,6 ± 56,5
D	231, 174, 200, 230, 307, 225, 125, 84, 89.	185,0 ± 56,9
E	157, 179, 243, 255, 114, 129, 128, 96, 301.	178,0 ± 55,3

Table 2. The mean number of eggs laid per female Culex quinquefasciatus for each diet type.

Diet	Number of eggs per raft	Number of females laying	$\bar{x}$ number of eggs/female	$\bar{x}$ ± 95% confidence limits
A	156, 170, 79, 250, 251, 81, 152, 105, 119.	3 3 3	135,0 194,0 125,3	151,3 ± 92,3
B	257, 300, 131 66, 68, 64, 172, 140, 363.	3 3 3	229,3 66,0 225,0	173,4 ± 231,2
C	236, 256, 275, 202, 160, 74, 161, 112, 87.	3 3 3	255,6 145,3 120,0	173,6 ± 179,1
D	231, 174, 200, 230, 307, 225, 125, 84, 89.	3 3 3	201,6 254,0 99,3	184,9 ± 195,4
E	157, 179, 96, 243, 255, 301, 114, 128, 129.	3 3 3	144,0 266,3 123,6	177,9 ± 191,7

Table 3. Fecundity of Aedes aegypti. The values are expressed as the total number of eggs laid divided by the number of females that took a blood meal to give the mean number of eggs per female plus 95% confi - dence limits for each diet group.

Diet	Total number of eggs laid	Number of females fed	$\bar{x}$ number of eggs/female	$\bar{x} \pm$ 95% confidence limits
A	663	17	39,0	32,2 ± 14,5
	627	21	29,8	
	169	6	28,1	
B	298	5	59,6	51,2 ± 27,9
	192	5	38,4	
	558	10	55,8	
C	217	7	28,9	35,4 ± 14,0
	315	8	39,3	
	266	7	38,0	
D	150	4	37,5	40,2 ± 31,4
	162	3	54,0	
	291	10	29,1	
E	187	7	26,7	68,1 ± 90,9
	1 962	24	81,7	
	673	7	96,1	

produced a mean value of 94,9% with a range of 69 to 100% (Table 4).

The percentage fertility of the eggs laid by A. aegypti, on the other hand, is consdierably lower than that obtained above. Once again diet A produced the lowest values with a mean of 50,2% and a range of 44,3 to 55,6%. Diet B mean value was 73,1% and the range was 61 to 90%. As was the case with C. quinquefasciatus, diet C produced the highest fertility with a mean value of 74,3% and a range of 65,4 to 84,2%. Diet D mean was 65% with a range of 48 to 85,5% and the mean for the control, Diet E, was 67% with a range of 46,3 to 78,6% (Table 5).

When the 95% confidence limits were calculated, a wide range of values resulted for both mosquito species in all five diets. Because of this, there were no ensuing significant fertility differences between the control diet E and the experimental diets A to D.

4. DURATION OF LARVAL STAGE

In C. quinquefasciatus, the longest duration of 19 to 20 days was found in diet groups A and B respectively. Diet group C had the shortest larval duration of 15 days followed by diet group D with 16 days. The control group Diet E, had a larval period which lasted for 18 days (Table 6 and Fig. 3).

When comparing the mean values for each diet group, it was found that diet groups C and D produced larval periods that were significantly shorter than the control diet E. Diet groups A and B, on the other hand, were not significantly different from the control (Fig. 4).

In A. aegypti the shortest duration was found in diet group B. This was the opposite of C. quinquefasciatus where diet B produced the longest

Table 4. Percentage fertility of the eggs laid by Culex quinquefasciatus for each diet group.

Diet	Number of eggs per raft	Number of eggs hatched	Percentage fertility	$\bar{x}$ ± 95% confidence limits
A	156	113	72,4	85,3 ± 10,7
	170	167	98,2	
	250	205	82,0	
	251	240	95,7	
	152	149	98,0	
	105	77	73,3	
	79	72	91,1	
	81	79	97,5	
	119	72	60,5	
B	257	188	73,0	94,7 ± 6,6
	300	298	99,9	
	131	119	91,0	
	66	65	98,4	
	68	68	100,0	
	64	61	95,3	
	172	172	100,0	
	140	138	98,6	
	363	353	97,2	
C	202	196	97,0	97,1 ± 3,2
	160	159	99,0	
	236	228	96,7	
	256	254	99,3	
	275	275	100,0	
	161	158	98,2	
	112	109	97,3	
	87	87	100,0	
	74	64	86,4	
D	231	221	95,7	93,1 ± 10,4
	174	153	87,9	
	200	199	99,5	
	230	217	94,4	
	307	304	99,0	
	225	225	100,0	
	125	124	99,2	
	84	80	95,3	
	89	51	57,3	
E	157	109	69,0	94,9 ± 7,7
	179	178	99,5	
	243	241	99,2	
	255	253	99,2	
	114	113	99,1	
	129	127	98,4	
	128	128	100,0	
	96	88	91,6	
	301	297	98,6	

Table 5. Percentage fertility of the eggs laid by Aedes aegypti for each diet group.

Diet	Total number of eggs laid	Total number of eggs hatched	Percentage fertility	$\bar{x} \pm$ 95% confidence limits
A	663	337	50,8	50,2 ± 14,0
	627	278	44,3	
	169	94	55,6	
B	298	204	68,4	73,1 ± 37,4
	192	117	61,0	
	558	502	90,0	
C	217	142	65,4	74,3 ± 23,4
	315	265	84,2	
	266	195	73,4	
D	150	72	48,0	65,0 ± 47,1
	162	100	61,7	
	291	249	85,5	
E	187	147	78,6	67,0 ± 44,4
	1962	910	46,3	
	673	512	76,1	

Table 6. The duration of the larval stage in each diet type for Culex quinquefasciatus expressed in days.

Diet	Duration of larval stage in days	$\bar{x}$ ± 95% confidence limits
A	12, 12, 10, 19, 19, 18, 14, 12, 16.	14,6 ± 2,6
B	17, 15, 16, 15, 15, 13, 18, 19, 20.	16,4 ± 1,7
C	11, 10, 10, 12, 11, 12, 15, 12, 14.	11,8 ± 1,2
D	14, 12, 9, 10, 12, 10, 16, 12, 10.	11,6 ± 1,7
E	14, 11, 11, 18, 18, 18, 17, 16, 14.	15,2 ± 2,2

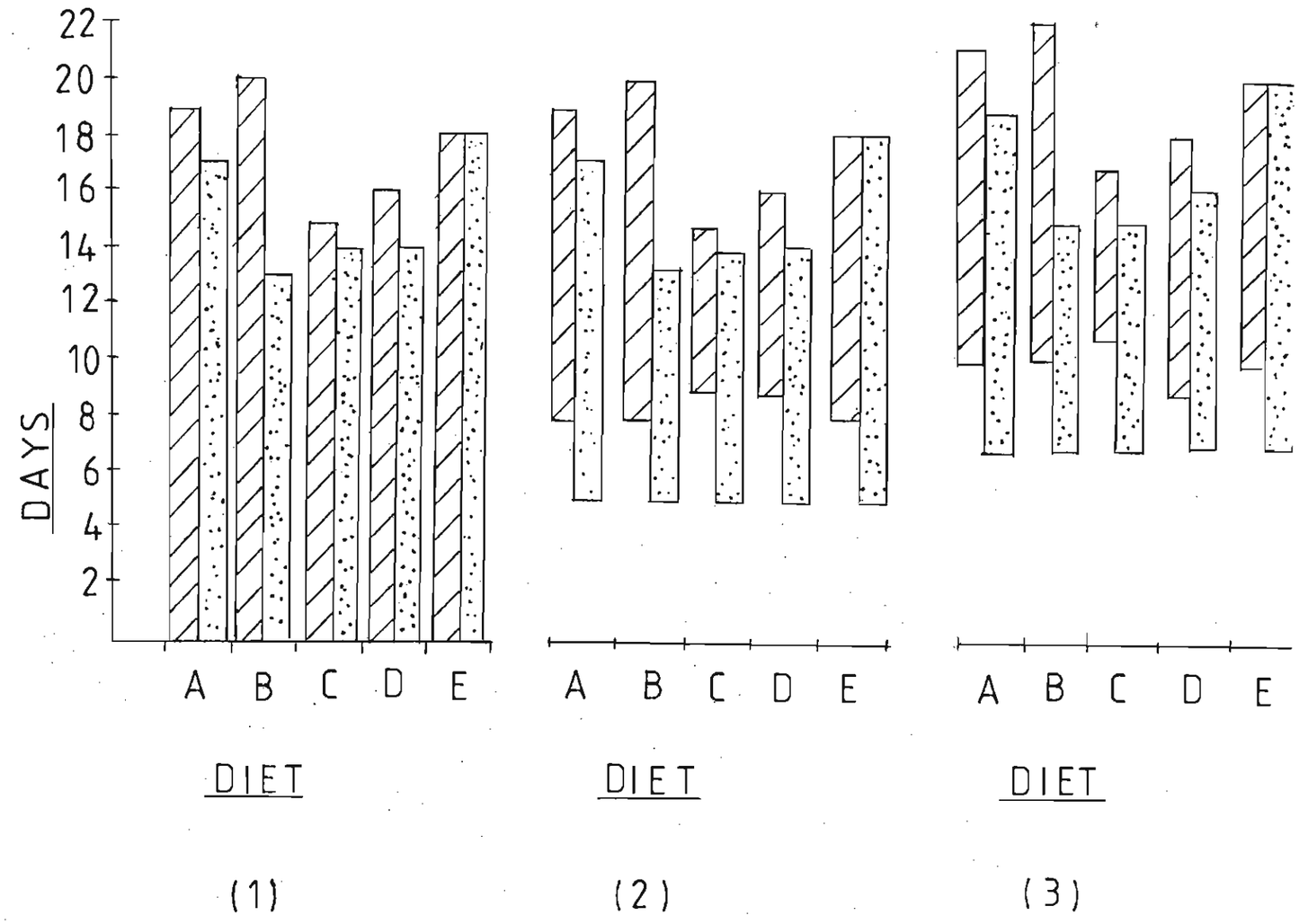


Figure 3. The duration of the larval stage (1), pupal appearance (2) and adult emergence (3) in days for Culex quinquefasciatus and Aedes aegypti for each diet type.

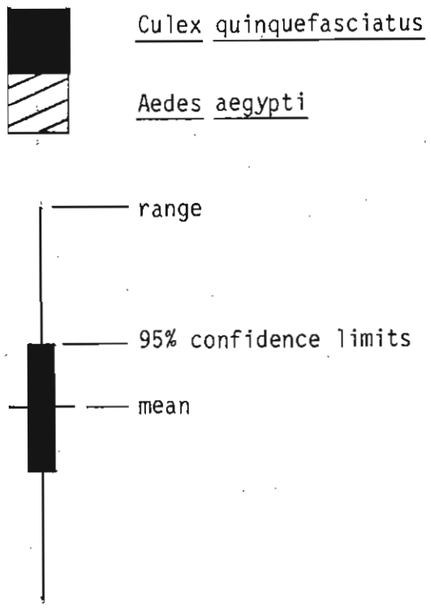
Key :

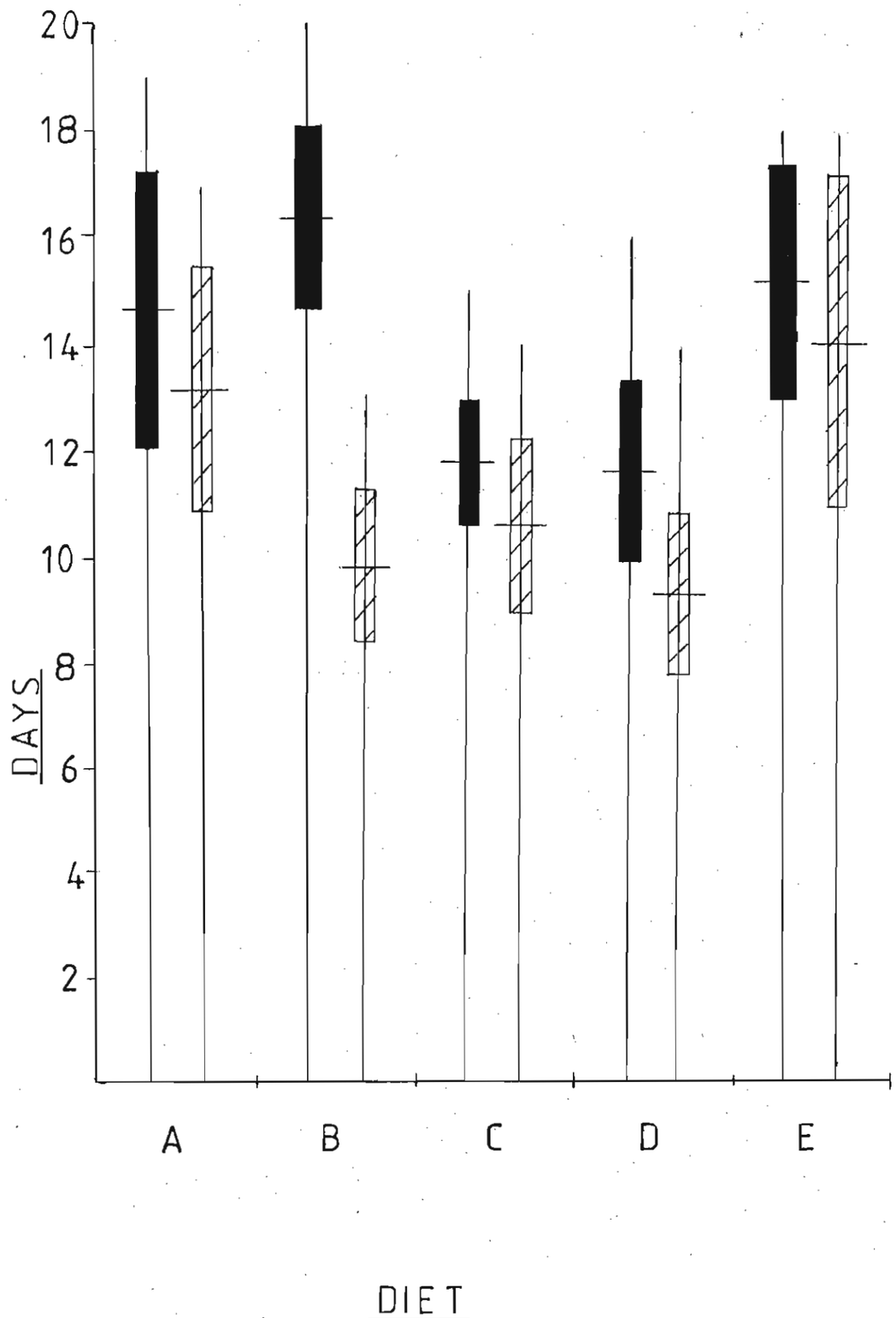
Culex quinquefasciatus

Aedes aegypti

Figure 4. A comparison between the duration of the larval stage in Culex quinquefasciatus and Aedes aegypti in days for each diet type.

Key :





larval period. Diet groups C and D had larval durations of 14 days each. The larval stage in diet group A lasted for 17 days and the longest was found in the control, diet group E, which lasted for 18 days (Table 7 and Fig 3).

Diet groups B and D had significantly shorter larval periods than the control as can be seen from figure 4 which summarises all the above information.

5. DURATION OF PUPAL STAGE

This period was calculated as the number of days over which pupae appeared in the rearing dish.

In C. quinquefasciatus the range of values for diet groups A, B and E were similar. The mean value for diet A was 6,1 days and the range was 2 to 11 days. Diet B produced a mean of 7,6 days with a range of 5 to 12 days. The control diet E had a mean value of 7,7 days and a range of 3 to 11 days. Diet C, on the other hand, produced a mean value of 4,3 days with a range of 2 to 8 days and diet D had a mean of 4,6 days with a range of 1 to 7 days (Table 8).

The duration of the pupal stage in diet groups C and D was significantly shorter than that of the control diet E. There were no significant differences between the control and diet groups A and B (Fig. 5).

In A. aegypti the shortest duration was found in diet group B with a mean of 4,3 days and a range of 3 to 8 days. This was followed by diet group D which produced a mean of 4,6 days and a range of 3 to 7 days. Diet C had a mean value of 5,6 days with a range of 3 to 8 days. The control diet E had a mean value of 7,2 days and a range of 4 to 10 days. The

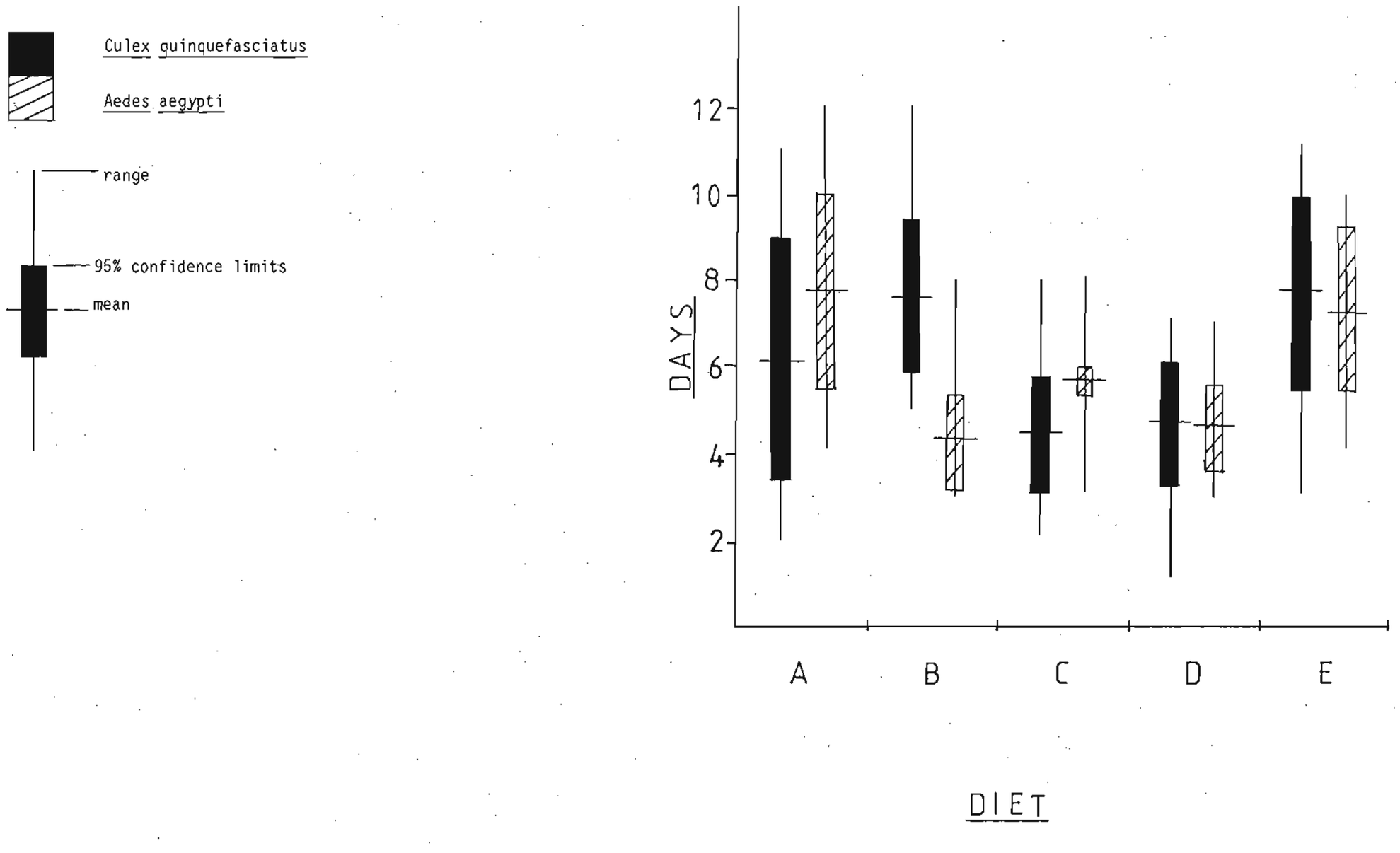
Table 7. The duration of the larval stage in each diet type for Aedes aegypti expressed in days.

Diet	Duration of larval stage in days	$\bar{x} \pm$ 95% confidence limits
A	17, 17, 16, 13, 12, 14, 10, 10, 10.	13,2 ± 2,3
B	13, 9, 8, 7, 11, 11, 11, 10, 9.	9,8 ± 1,4
C	9, 11, 7, 11, 10, 9, 14, 13, 12.	10,6 ± 1,6
D	7, 14, 9, 10, 9, 9, 8, 8, 10.	9,3 ± 1,5
E	18, 17, 17, 14, 17, 17, 8, 9, 9.	14,0 ± 3,1

Table 8. The duration of the pupal stage in Culex quinquefasciatus expressed in days for each diet type. The data represent the period of time over which pupae were appearing in the rearing dishes.

Diet	Duration of pupal stage in days	$\bar{x} \pm$ 95% confidence limits
A	2, 3, 2, 11, 11, 10, 6, 4, 6.	6,1 ± 2,8
B	5, 6, 7, 6, 8, 6, 9, 10, 12.	7,6 ± 1,7
C	3, 3, 2, 5, 4, 5, 8, 4, 5.	4,3 ± 1,3
D	7, 5, 1, 4, 6, 4, 7, 5, 3.	4,6 ± 1,4
E	7, 3, 4, 10, 11, 11, 9, 9, 6.	7,7 ± 2,2

Figure 5. A comparison of the duration of the pupal stage in days for each diet type in Culex quinquefasciatus and Aedes aegypti.



longest pupal stage was found in diet A with a mean of 7,7 days and a range of 4 to 12 days (Table 9)

Diet groups B and D produced pupal stages that were significantly shorter than the control diet E. The duration of the pupal stage in diets A and C were not significantly different from the control (Fig. 5).

6. LARVAL MORTALITY

The highest larval mortality in C. quinquefasciatus occurred in diet group A. The mean value for this diet was 42,1% with a range of 22,2 to 85,8%. Diet D had a mean mortality value of 26,5% and a range of 0 to 90,6%. The control diet E produced a mean value of 17,7% with a range of 1,1 to 30,6%. Diet B had a mean mortality of 15,8% and a range of 5,9 to 42,6%. The lowest larval mortality was experienced with diet group C. The mean value here was 12,6% and the range was 0 to 36,2% (Table 10).

Diet group A produced a larval mortality which was significantly higher than the control diet E (Fig. 6).

Larval mortality in A. aegypti was lower than that obtained for C. quinquefasciatus. The lowest value in A. aegypti with a mean of 8,9% and a range of 0 to 29,6% was found in diet group A. This was the opposite in C. quinquefasciatus where diet A produced the highest larval mortality. The second lowest value was obtained in the control diet E with a mean of 12,8% and a range of 0 to 30%. Diet B produced a mean larval mortality of 13,4% with a range of 0 to 44%. Diet group C had a mean value of 17,6% with a range of 2,7 to 28,2%. The highest larval mortality was experienced in diet group D with a mean of 30,1% and a range of 0 to 58,3%. This could be compared with C. quinquefasciatus where diet group D produced the second highest larval mortality (Table 11).

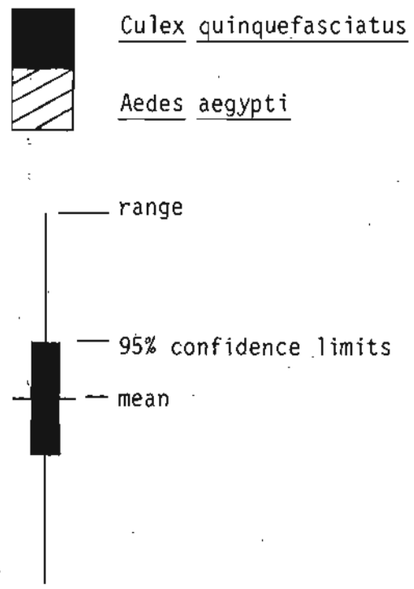
Table 9. The duration of the pupal stage in Aedes aegypti expressed in days for each diet type. The data represent the period of time over which pupae were appearing in the rearing dishes.

Diet	Duration of pupal stage in days	$\bar{x} \pm$ 95% confidence limits
A	12, 12, 11, 7, 6, 8, 6, 5, 4.	7,7 ± 2,2
B	8, 5, 3, 3, 4, 4, 5, 4, 3.	4,3 ± 1,15
C	5, 6, 3, 6, 5, 4, 7, 7, 8.	5,6 ± 1,2
D	3, 7, 3, 5, 5, 5, 5, 3, 6.	4,6 ± 1,08
E	10, 5, 9, 7, 10, 10, 4, 5, 5.	7,2 ± 1,9

Table 10. The percentage larval mortality for each diet type in Culex quinquefasciatus.

Diet	Number of larvae at begining	Number of larvae alive at end	% mortality	$\bar{x} \pm$ 95% confidence limits
A	113	16	85,8	42,1 ± 17,4
	150	65	56,6	
	17	12	29,5	
	152	109	28,2	
	144	112	22,2	
	151	96	36,4	
	149	116	22,1	
	149	48	68,2	
	150	105	30,0	
B	150	115	35,0	15,8 ± 10,0
	151	142	5,9	
	150	86	42,6	
	154	144	6,4	
	156	110	29,4	
	159	138	8,1	
	154	144	6,4	
	156	110	29,4	
	159	138	13,2	
C	155	147	5,0	12,6 ± 9,4
	150	145	3,0	
	50	49	2,0	
	136	114	16,0	
	151	151	0,0	
	150	127	15,3	
	158	115	27,2	
	147	133	9,5	
	127	81	36,2	
D	150	118	21,4	26,5 ± 24,9
	150	14	90,6	
	151	136	9,9	
	150	147	2,0	
	152	151	0,6	
	150	150	0,0	
	124	37	70,1	
	80	57	28,7	
	125	106	15,2	
E	150	104	30,6	17,7 ± 8,5
	150	117	22,0	
	150	137	9,0	
	100	63	37,0	
	100	84	16,0	
	53	43	18,8	
	113	94	16,8	
	127	116	8,6	
	169	167	1,1	

Figure 6. A comparison of the percentage larval mortality for each diet type in Culex quinquefasciatus and Aedes aegypti.



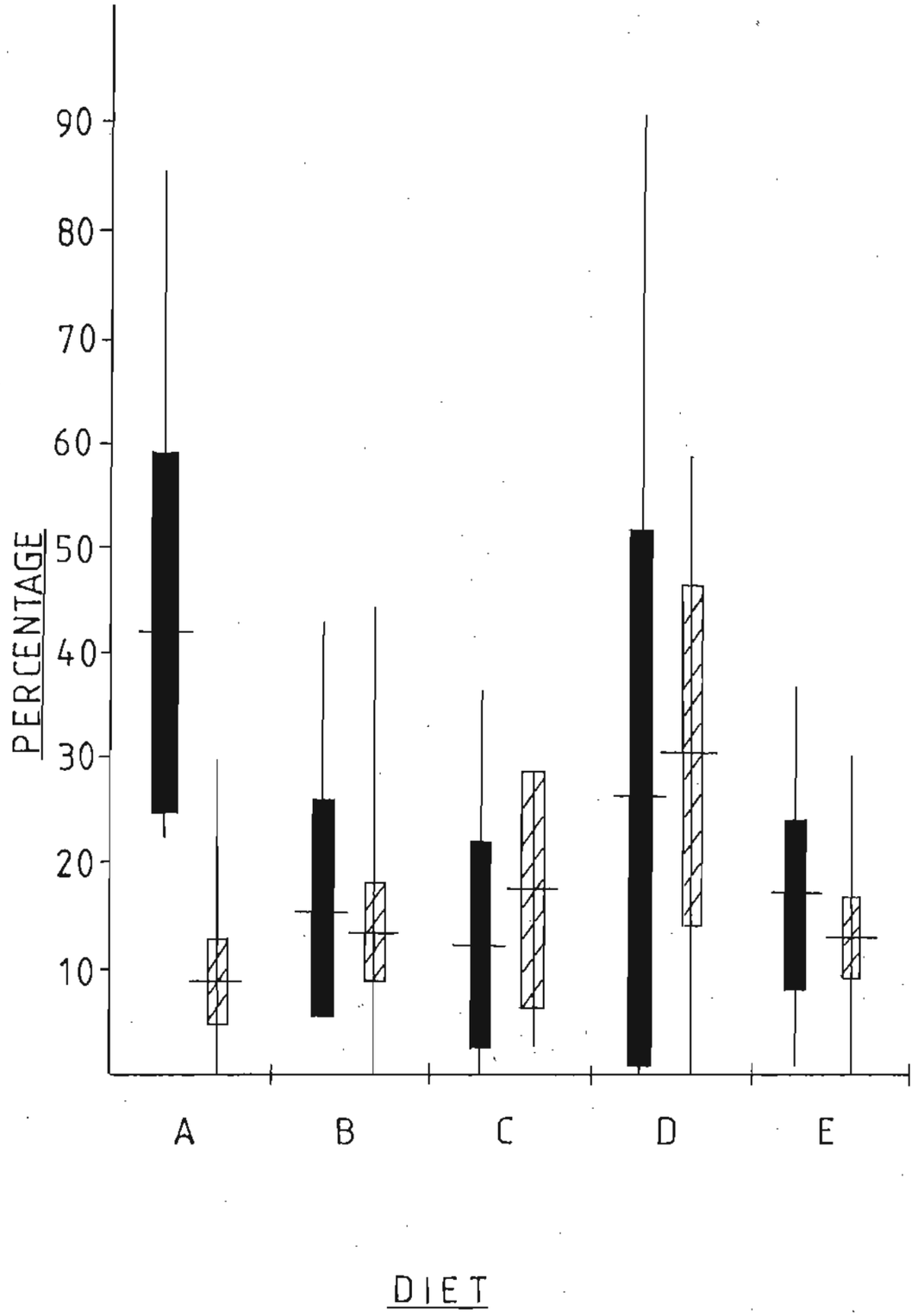


Table 11. The percentage larval mortality for each diet type in Aedes aegypti.

Diet	Number of larvae at begining	Number of larvae alive at end	% mortality	$\bar{x} \pm$ 95% confidence limits
A	150	138	8,0	8,9 ± 7,8
	98	87	11,2	
	76	76	0,0	
	50	50	0,0	
	54	38	29,6	
	68	68	0,0	
	82	80	2,4	
	74	67	9,4	
	15	12	20,0	
B	81	74	8,6	13,4 ± 9,7
	25	22	12,0	
	67	53	20,0	
	30	27	10,0	
	100	96	4,0	
	100	100	0,0	
	100	90	10,0	
	100	88	12,0	
	59	33	44,0	
C	37	36	2,7	17,6 ± 10,5
	78	65	16,6	
	27	14	48,0	
	70	66	5,7	
	61	52	14,7	
	72	64	8,3	
	39	28	28,2	
	80	65	18,7	
	56	47	16,0	
D	72	67	6,9	30,1 ± 16,8
	39	19	51,0	
	76	76	0,0	
	45	29	55,5	
	100	79	21,0	
	80	66	17,5	
	33	19	42,2	
	45	15	58,3	
	99	80	19,1	
E	120	84	30,0	12,8 ± 7,8
	79	56	29,1	
	100	86	14,0	
	100	91	9,0	
	100	96	4,0	
	103	103	0,0	
	151	132	12,5	
	150	137	8,6	
	150	138	8,0	

There were no significant differences between any of the experimental diets, A to D, and the control diet E (Fig. 6).

7. PUPAL MORTALITY

The highest pupal mortality in C. quinquefasciatus occurred in diet A as was the case with the larval mortality. The mean value was 5,5% with a range of 0 to 15,2%. Diet B produced a mean pupal mortality of 2,5% with a range of 0 to 7,7%. The mean for diet C was 1,2% with a range of 0 to 4,8%. Diet D had a mean value of 1,4% and a range of 0 to 4,4%. The mean value for the control diet E was the lowest at 0,6% and the range was 0 to 1,7% (Table 12).

The highest pupal mortality in A. aegypti was found in diet D. This diet also produced the highest larval mortality in this mosquito species. The mean value for diet D was 3,1% with a range of 0 to 10,5%. Diet A had a mean value of 2,1% and a range of 0 to 17,9%. Diet B produced a mean pupal mortality of 2,4% with a range of 0 to 8,8%. Diet C resulted in a mean value of 1,2% with a range of 0 to 3,5%. Once again, the lowest pupal mortality was found in the control diet group E with a mean value of 1,0% and a range of 0 to 3,8% (Table 13).

There were no significant differences between any of the experimental diets A to D and the control diet E in both mosquito species (Fig.7).

8. ADULT MORTALITY

In C. quinquefasciatus the highest value was found in diet B. The mean value was 28,5% and the range was 6,9 to 68,5%. Diet A had a mean value of 21,1% with a range of 17,7 to 27,1%. The control diet E had a mean value of 17,4% and a range of 9 to 30%. Diet C produced a mean of 16,4% with a range of 10 to 20,1%. The lowest adult mortality occurred in

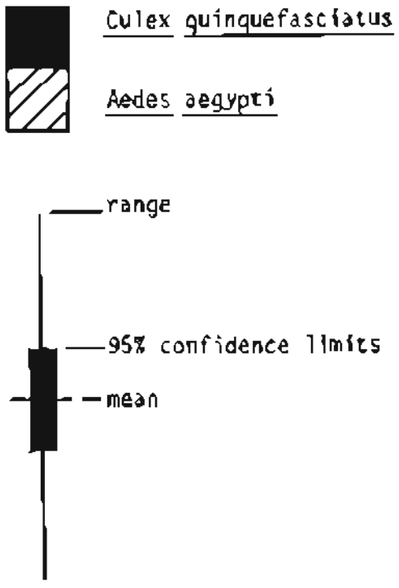
Table 12. The percentage pupal mortality for each diet type in Culex quinquefasciatus.

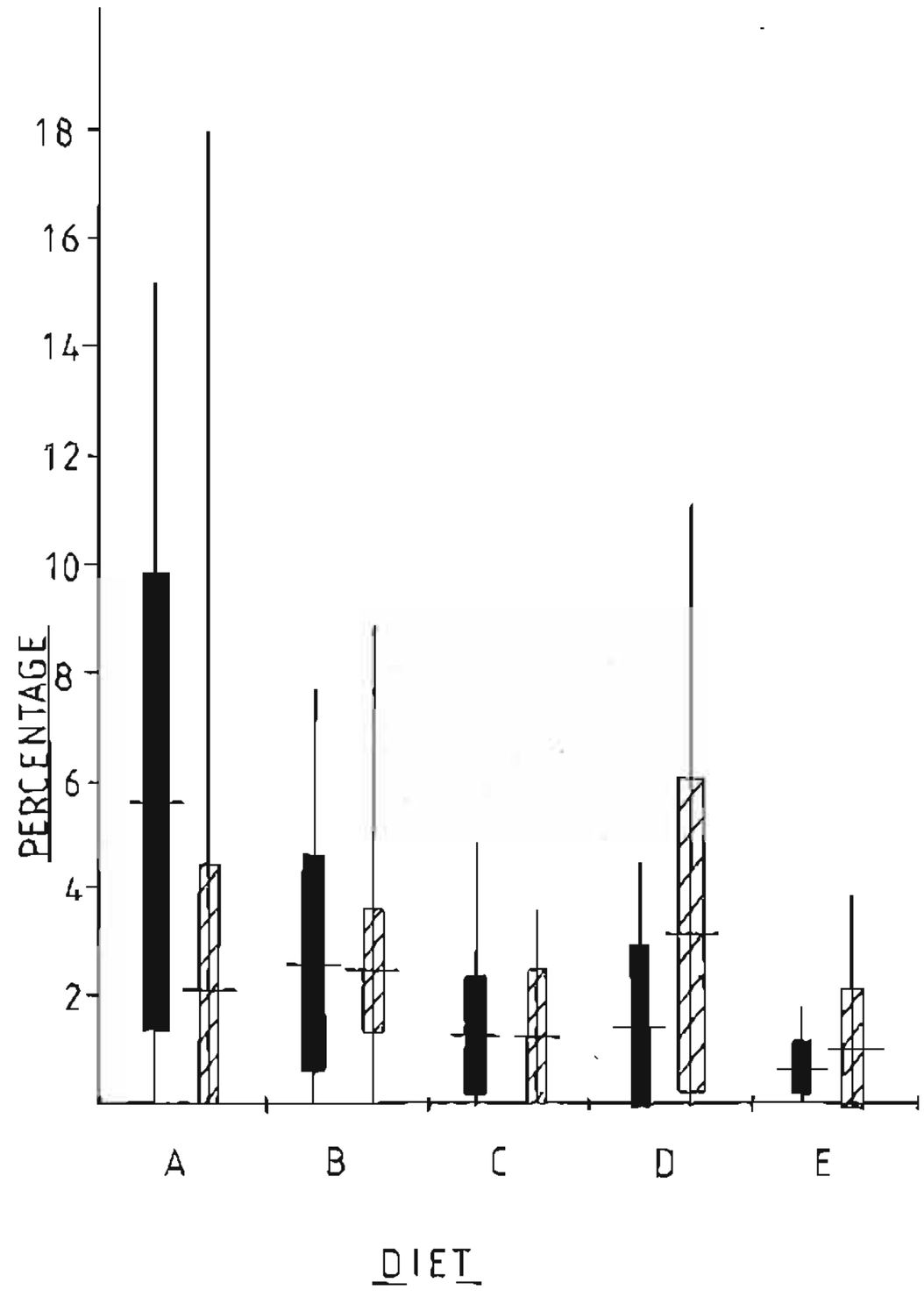
Diet	Total number of pupae	Number dying	% mortality	$\bar{x}$ ± 95% confidence limits
A	65	0	0,0	5,5 ± 4,3
	12	0	0,0	
	109	8	8,2	
	112	12	10,7	
	96	3	3,1	
	116	3	2,5	
	48	1	2,0	
	105	16	15,2	
B	115	2	1,7	2,5 ± 2,0
	142	11	7,7	
	86	1	1,0	
	60	2	3,3	
	64	0	0,0	
	56	3	5,3	
	110	0	0,0	
	144	0	0,0	
	138	5	3,6	
C	147	1	0,6	1,2 ± 1,1
	145	1	0,6	
	49	0	0,0	
	114	0	0,0	
	154	1	0,7	
	127	6	4,8	
	115	2	1,7	
	133	1	0,7	
	81	2	2,4	
D	118	5	4,2	1,4 ± 1,5
	14	0	0,0	
	136	6	4,4	
	147	1	0,6	
	151	0	0,0	
	150	1	0,1	
	37	0	0,0	
	57	2	3,5	
	106	0	0,0	
E	104	2	0,9	0,6 ± 0,5
	117	0	0,0	
	137	1	0,7	
	63	1	1,5	
	84	0	0,0	
	43	0	0,0	
	94	1	1,0	
	116	0	0,0	
	167	3	1,7	

Table 13. The percentage pupal mortality for each diet type in Aedes aegypti.

Diet	Total number of pupae	Number dying	% mortality	$\bar{x}$ ± 95% confidence limits
A	138	0	0,0	2,1 ± 4,5
	87	0	0,0	
	76	0	0,0	
	50	0	0,0	
	38	0	0,0	
	68	0	0,0	
	80	1	1,2	
	67	12	17,9	
	12	0	0,0	
B	74	1	1,3	2,4 ± 2,3
	22	1	4,5	
	53	0	0,0	
	27	0	0,0	
	96	0	0,0	
	100	0	0,0	
	90	8	8,8	
	88	4	4,5	
	33	1	3,0	
C	36	0	0,0	1,2 ± 1,2
	65	2	3,0	
	14	0	0,0	
	66	0	0,0	
	52	0	0,0	
	64	0	0,0	
	28	1	3,5	
	65	2	3,0	
	47	1	2,1	
D	67	2	2,9	3,1 ± 2,9
	19	0	0,0	
	76	8	10,5	
	29	0	0,0	
	79	2	2,5	
	66	3	4,5	
	19	0	0,0	
	15	0	0,0	
	80	6	7,5	
E	84	3	3,5	1,0 ± 1,1
	56	0	0,0	
	86	0	0,0	
	91	0	0,0	
	96	1	1,0	
	103	4	3,8	
	132	0	0,0	
	137	1	0,7	
	138	0	0,0	

Figure 7. A comparison of the pupal mortality for each diet type in Culex quinquefasciatus and Aedes aegypti.





diet group D where the mean was 10,9% and the range was 5 to 19,1% (Table 14).

Adult mortality was found to be higher in A. aegypti. The highest value within the species was found in diet A. The mean was 30,5% and the range was 20,1 to 42%. The next highest value occurred in diet group D where the mean was 30,1% and the range was 14,1 to 45,6%. Diet C had a mean of 25,5% and a range of 18,7 to 36,4%. Diet B produced a mean value of 21,1% with a range of 11,8 to 27,0%. The lowest adult mortality was found in the control diet E with a mean of 15,1% and a range of 6,0 to 27,0% (Table 15).

As there was a wide range of values in all the diet groups for both mosquito species, there were no significant differences between the experimental groups A to D and the control group E.

9. SIZE OF OVARY

The smallest ovaries in C. quinquefasciatus were found in diet group B where the mean value was 7,72 x $10^5\mu$, and the range was 3,74 to 14,46 x $10^5\mu$. Diet group A produced ovaries with a mean value of 14,39 x $10^5\mu$ (range = 7,19 - 22,88 x $10^5\mu$). The control diet E produced ovaries of a mean value of 17,48 x $10^5\mu$ (range = 11,13 - 28,36 x $10^5\mu$). The ovaries of diet C had a mean value of 17,6 x $10^5\mu$ (range = 7,76 - 30,28 x $10^5\mu$) and those of diet D were the largest with a mean size of 18,9 x $10^5\mu$ (range = 10,39 - 31,65 x $10^5\mu$) (Table 16).

In A. aegypti the smallest ovaries were found in diet group A where the mean size was 6,38 x $10^5\mu$ (range = 3,52 - 11,37 x $10^5\mu$). Diet group B produced ovaries with a mean value of 6,74 x $10^5\mu$ (range = 4,41 - 10,7 x $10^5\mu$). The ovaries of diet group C had a mean value of 6,9 x $10^5\mu$

Table 14. The percentage adult mortality for each diet group in Culex quinquefasciatus.

Diet	Total number of adults	Number dying	% mortality	$\bar{x}$ ± 95% confidence limits
A	80	15	18,7	21,1 ± 12,8
	299	81	27,1	
	253	45	17,7	
B	329	226	68,5	28,5 ± 86,1
	168	17	10,1	
	359	25	6,9	
C	348	66	19,1	16,4 ± 13,8
	327	66	20,1	
	312	31	10,0	
D	251	48	19,1	10,9 ± 18,1
	440	22	5,0	
	195	22	8,7	
E	353	106	30,0	17,4 ± 27,5
	178	24	13,4	
	363	33	9,0	

Table 15. The percentage adult mortality for each diet group in Aedes aegypti.

Diet	Total number of adults	Number dying	% mortality	$\bar{x} \pm$ 95% confidence limits
A	301	89	29,5	30,5 ± 27,2
	159	32	20,1	
	142	60	42,0	
B	144	17	11,8	21,1 ± 20,1
	214	53	24,7	
	195	53	27,0	
C	112	21	18,7	25,5 ± 23,6
	181	39	21,5	
	131	35	36,4	
D	149	68	45,6	30,1 ± 39,1
	177	22	14,1	
	101	31	30,6	
E	222	60	27,0	15,1 ± 26,5
	281	17	6,0	
	403	50	12,4	

Table 16. The size of the ovaries in Culex quinquefasciatus. Results shown as values x 10^5 micrometers plus the mean and 95% confidence limits .

Diet Group				
A	B	C	D	E
13,86	9,45	18,93	29,94	23,64
20,24	7,89	20,31	17,08	15,15
12,42	8,92	19,24	22,19	18,02
8,97	5,54	16,48	17,18	11,25
16,00	8,52	17,35	26,79	28,36
12,39	7,26	19,44	18,03	21,83
10,94	5,61	18,91	31,65	21,44
14,74	7,04	13,98	13,34	14,38
21,43	10,08	30,28	23,99	22,08
11,97	8,05	24,30	14,31	17,21
11,64	6,67	16,64	20,62	18,16
14,44	7,23	19,47	16,94	13,23
20,20	10,42	25,23	23,64	23,64
11,64	5,99	20,86	25,19	15,12
11,99	8,04	12,61	19,77	17,98
13,54	6,35	19,67	11,34	12,01
16,40	10,42	23,62	24,61	23,63
11,28	3,74	13,72	12,86	11,74
14,44	7,84	16,87	17,33	24,63
10,77	6,32	14,28	13,87	14,62
20,69	9,14	16,33	14,50	15,39
9,00	4,85	11,42	18,92	11,13
12,31	7,26	15,84	10,39	18,24
14,03	5,98	18,77	13,79	11,36
16,64	11,45	18,60	22,27	16,06
7,19	5,37	7,96	20,58	15,32
17,53	6,76	15,56	13,46	14,20
22,88	10,01	16,71	18,96	17,64
17,01	14,46	7,76	18,52	19,00
15,12	4,97	19,23	17,43	18,16
14,39	7,72	$\bar{x}$ 17,60	18,90	17,48
	± 95% confidence limits			
1,44	0,83	1,73	1,96	1,65

(range = 3,19 - 11,15 x $10^5\mu$). The control group diet E produced ovaries with a mean value of 7,13 x $10^5\mu$ (range = 2,67 - 11,22 x $10^5\mu$). The largest ovaries in A. aegypti were also found in diet group D as was the case with C. quinquefasciatus. Here the mean value was 8,42 x $10^5\mu$ (range = 3,58 - 12,76 x $10^5\mu$) (Table 17).

Only diet group B in C. quinquefasciatus produced ovaries that were significantly smaller than the control diet E. No other diet group in either mosquito species produced ovaries which were significantly different from the control (Fig.8).

10. SEX RATIO

In C. quinquefasciatus there was a significantly higher proportion of males than females produced in diets D and E where the p value was found to be greater than 95% in the former and 99% in the latter (Table 18).

In A. aegypti diets C and E produced significantly more males than females with the p value in both diet groups being greater than 95% (Table 18).

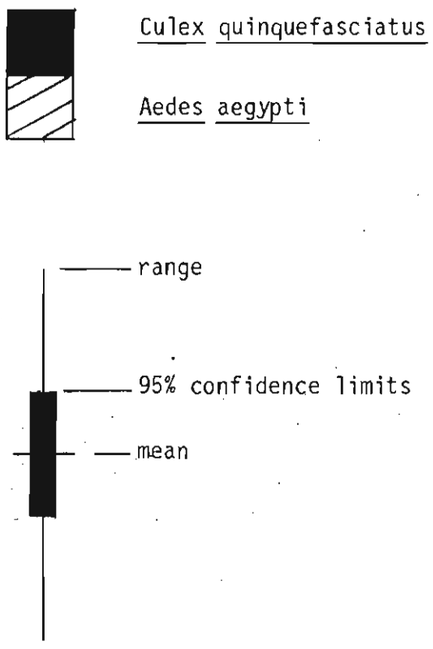
11. pH OF THE LARVAL WATER

The pH of the larval water ranged from 6,4 in diets B and C to 6,7 in diet D for both mosquito species (Table 19).

Table 17. The size of the ovaries in Aedes aegypti. Results shown as values x 10^5 micrometers plus the mean and 95% confidence limits.

Diet Group				
A	B	C	D	E
6,29	6,24	3,19	12,32	10,61
6,91	5,93	6,27	7,85	11,22
9,04	4,41	5,01	10,47	9,35
5,33	4,56	9,36	11,56	11,60
6,30	6,82	8,32	9,29	10,61
9,09	6,83	10,14	11,70	9,22
5,33	7,58	10,73	9,19	8,22
9,65	6,28	7,43	8,76	6,60
9,44	6,81	9,27	9,06	6,52
5,98	10,70	11,15	12,76	6,12
6,70	10,51	7,32	11,59	9,10
8,09	8,87	7,96	12,21	7,54
7,56	5,68	7,28	10,99	6,52
11,37	7,47	6,96	6,86	7,79
7,96	6,09	8,91	7,98	10,99
3,52	4,77	6,04	9,16	10,81
9,61	9,32	7,18	6,46	9,73
4,90	6,30	7,43	5,99	9,66
6,07	7,37	6,87	8,25	8,09
6,03	6,01	4,90	9,60	5,90
7,12	4,96	4,66	8,37	3,59
3,76	6,37	4,11	7,59	3,85
6,23	6,93	3,80	5,64	2,67
3,85	7,75	5,41	5,97	4,23
4,26	7,40	5,29	4,39	3,46
3,54	5,97	7,32	5,83	3,08
4,37	4,49	5,85	8,27	3,96
3,63	4,41	5,89	7,12	4,04
5,32	8,56	5,73	3,58	4,19
4,19	7,04	8,71	4,05	4,73
		$\bar{x}$		
6,38	6,74	6,90	8,42	7,13
	± 95% confidence limits			
0,70	0,60	0,70	0,90	1,04

Figure 8. A comparison of the ovary size for each diet group in Culex quinquefasciatus and Aedes aegypti.



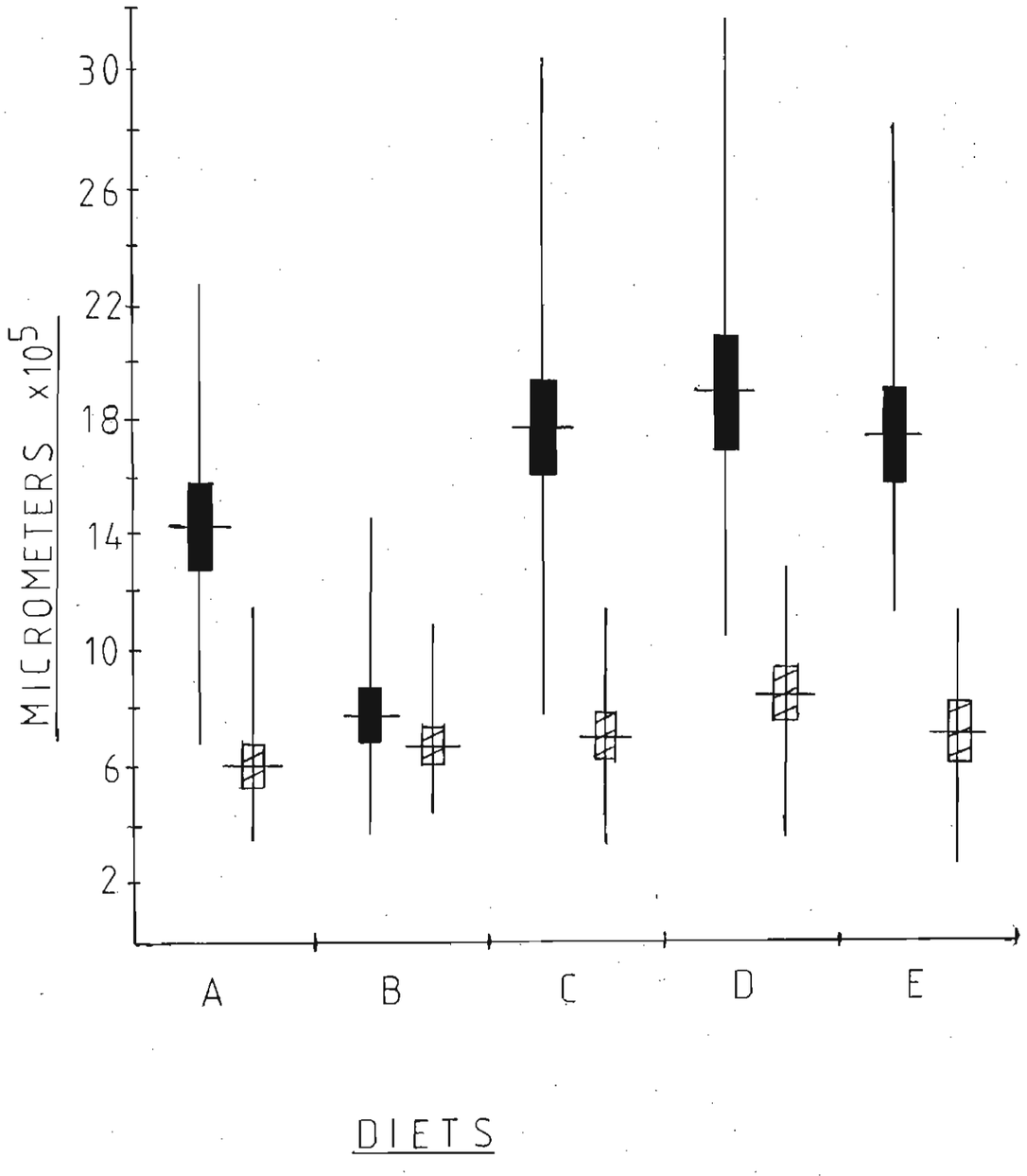


Table 18. A comparison of the male : female sex ratio in Culex quinquefasciatus and Aedes aegypti. Results expressed as a percentage plus p values for each diet type.

Diet	Culex quinquefasciatus			Aedes aegypti		
	% females	% males	p value	% females	% males	p value
A	45,2	54,8		43,5	40,2	
	31,8	68,2	p<95%	49,4	56,4	p<95%
	47,6	52,4		59,8	50,6	
B	47,3	52,7		46,7	63,3	
	50,0	50,0	p<95%	50,4	49,6	p<95%
	50,4	49,6		54,1	45,9	
C	45,8	54,2		45,3	54,7	
	53,4	46,6	p<95%	41,3	58,7	p>95%*
	50,4	49,6		49,3	50,7	
D	47,7	52,3		52,5	44,7	
	45,7	54,3	p>95%*	49,5	47,5	p<95%
	45,5	54,5		55,3	50,5	
E	47,1	52,9		46,5	53,5	
	47,8	52,2	p>99%*	38,3	61,7	p>95%*
	46,5	53,4		45,7	54,3	

* = significant

Table 19. The pH of the larval water for each diet group for both mosquito species.

Diet group	pH
A	6,5 ± 0,04
B	6,4 ± 0,04
C	6,4 ± 0,04
D	6,7 ± 0,09
E	6,6 ± 0,18

12. TRANSMISSION ELECTRON MICROSCOPY OF OVARIES

12 hours post blood meal (PBM)

Figures 9 to 18 show the follicular epithelium development at 12 hours PBM.

When examining the results for Culex quinquefasciatus (Figs. 9, 11, 13, 15 and 17) there are no visible structural differences between the experimental diet groups A to D and the control diet E. The follicle cells had formed a continuous layer around the oocyte. The cytoplasm was filled with ribosomes, rough endoplasmic reticulum and mitochondria were present. There was no obvious evidence of vitellogenesis. Chromatin in a condensed form was also present.

There was also no visible structural differences between the various diet groups in Aedes aegypti (Figs. 10, 12, 14, 16 and 18). The follicle cells had formed a continuous layer around the oocyte. Intercellular spaces filled with flocculent material were present and are characteristic of this stage (Pollard et al., 1986). The cytoplasm was filled with ribosomes and rough endoplasmic reticulum and mitochondria were present. Vitelline droplets were being deposited along the follicular epithelium - oocyte interface. Yolk deposition proceeded concurrently with vitelline membrane production and yolk droplets were seen in conjunction with lipid droplets. Chromatin in a condensed form was also evident.

If one compares the two mosquito species for each diet group, there are prominent differences seen between C. quinquefasciatus and A. aegypti. Vitellogenesis had begun in A. aegypti as had protein and lipid yolk deposition. On the other hand, these processes had not as yet begun in C. quinquefasciatus. There were also no intercellular spaces present

Figure 9. Follicular epithelium of Culex quinquefasciatus 12 hours post blood meal for diet A.

Key : fc follicle cell
n nucleus
r ribosomes
oo oocyte
Bar represents 1μ

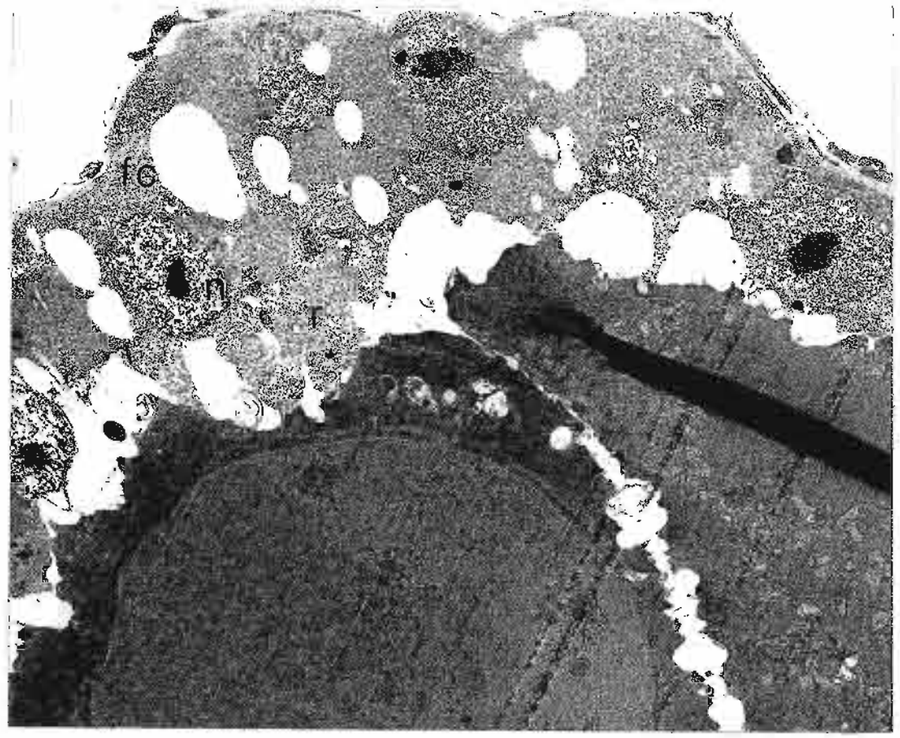
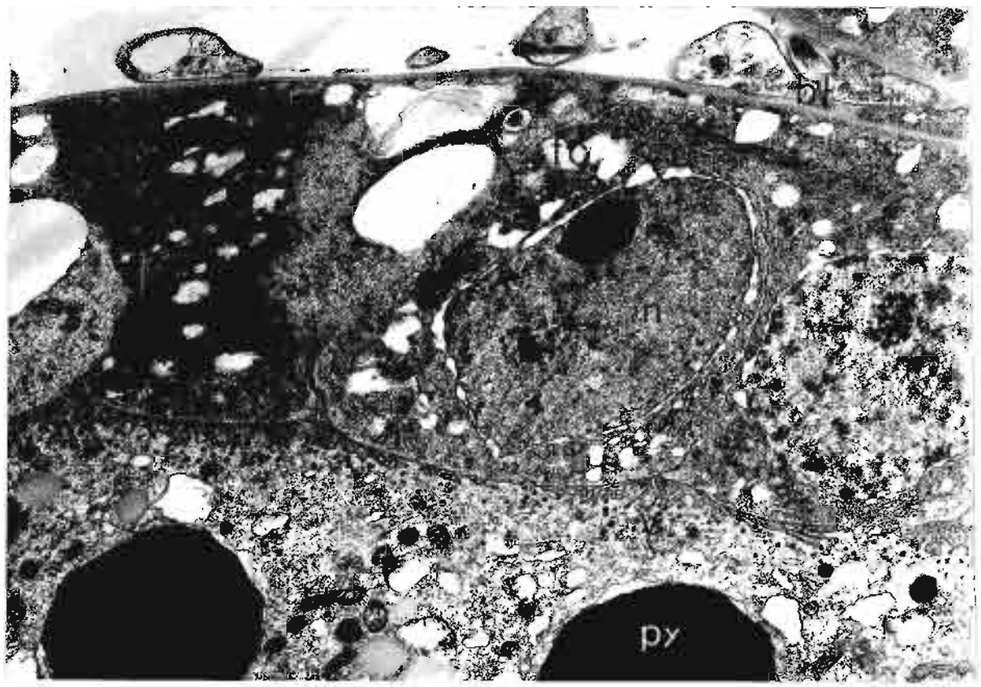


Figure 10. Follicular epithelium of Aedes aegypti 12 hours post blood meal for diet A.

Key : bl basement lamina
n nucleus
r ribosomes
v vitelline droplets
py protein yolk

Bar represents 1μ



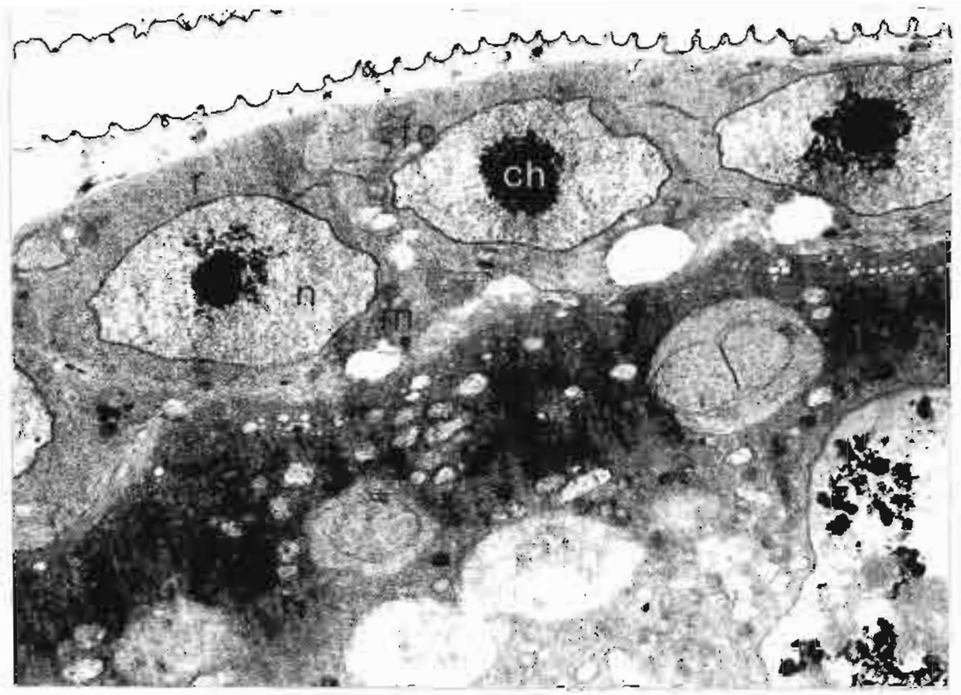


Figure 11. Follicular epithelium of Culex quinquefasciatus 12 hours post blood meal for diet B.

Key : fc follicle cell
r ribosomes
n nucleus
m mitochondria
ch chromatin
oo oocyte

Bar represents 1µ

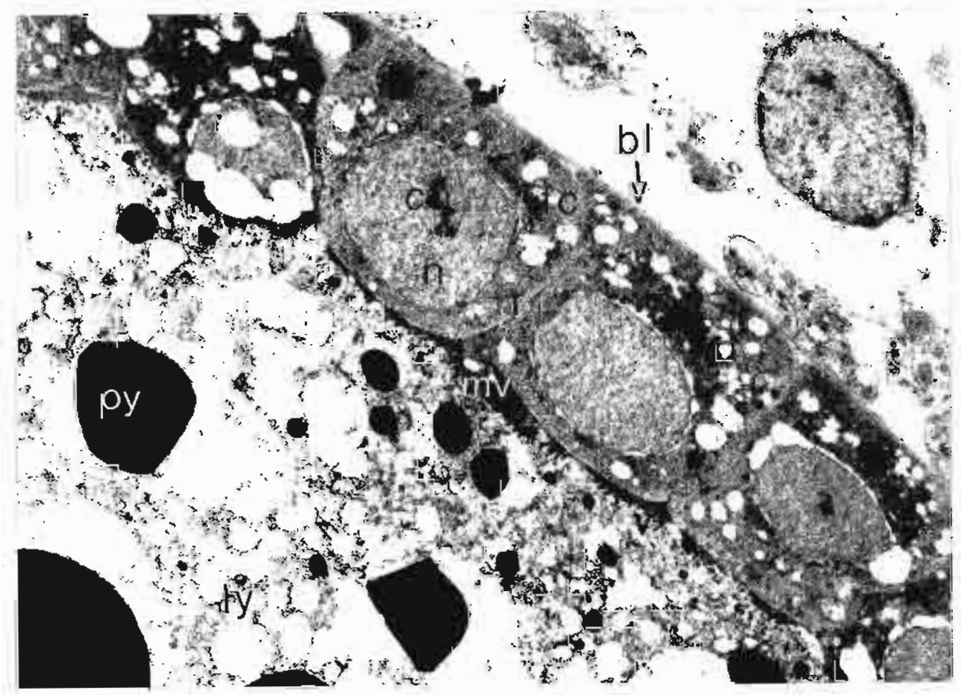


Figure 12. Follicular epithelium of Aedes aegypti 12 hours post blood meal for diet B.

Key : bl basement lamina
fc follicle cell
n nucleus
r ribosomes
mv microvilli
v vitelline droplets
py protein yolk
ly lipid yolk

Bar represents 1µ

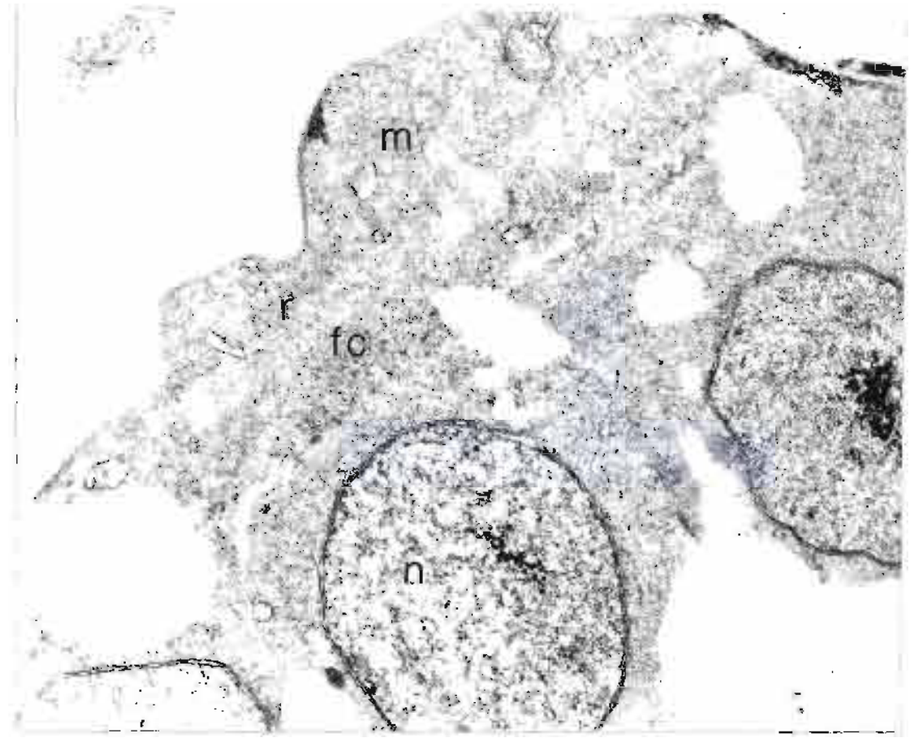


Figure 13. Follicular epithelium of Culex quinquefasciatus 12 hours post blood meal for diet C.

Key : m mitochondria
r ribosomes
fc follicle cell
n nucleus

Bar represents 1μ

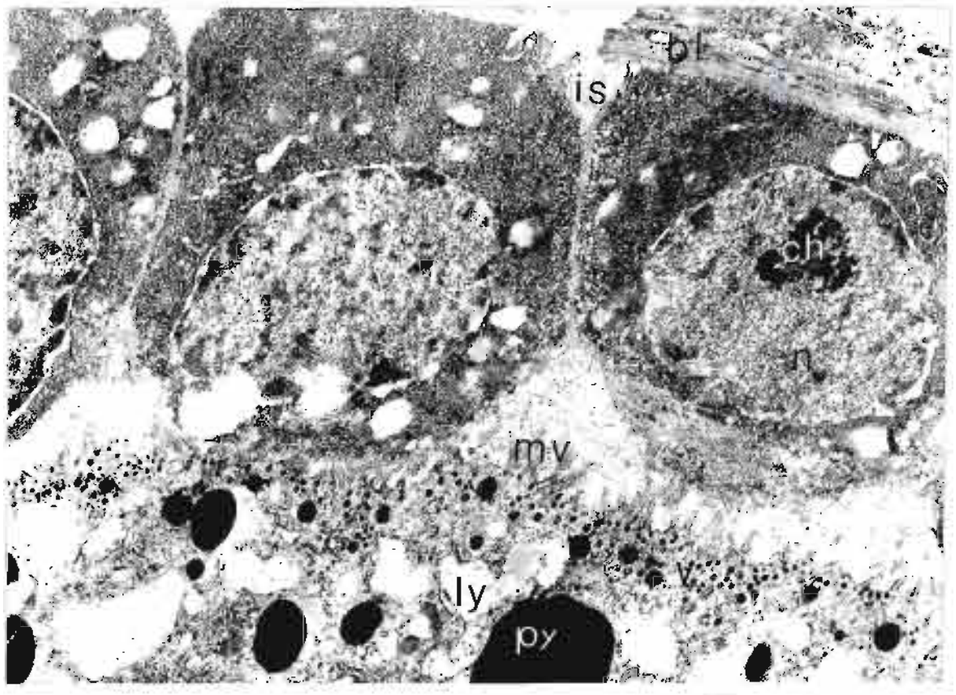


Figure 14. Follicular epithelium of Aedes aegypti 12 hours post blood meal for diet C.

Key : bl basement lamina
is intercellular space
fc follicle cell
r ribosomes
n nucleus
mv microvilli
ly lipid yolk
py protein yolk
v vitelline droplets

Bar represents 1μ

Figure 15. Follicular epithelium of Culex quinquefasciatus 12 hours post blood meal for diet D.

Key : fc follicle cell
m mitochondria
ch chromatin
n nucleus

Bar represents 1µ

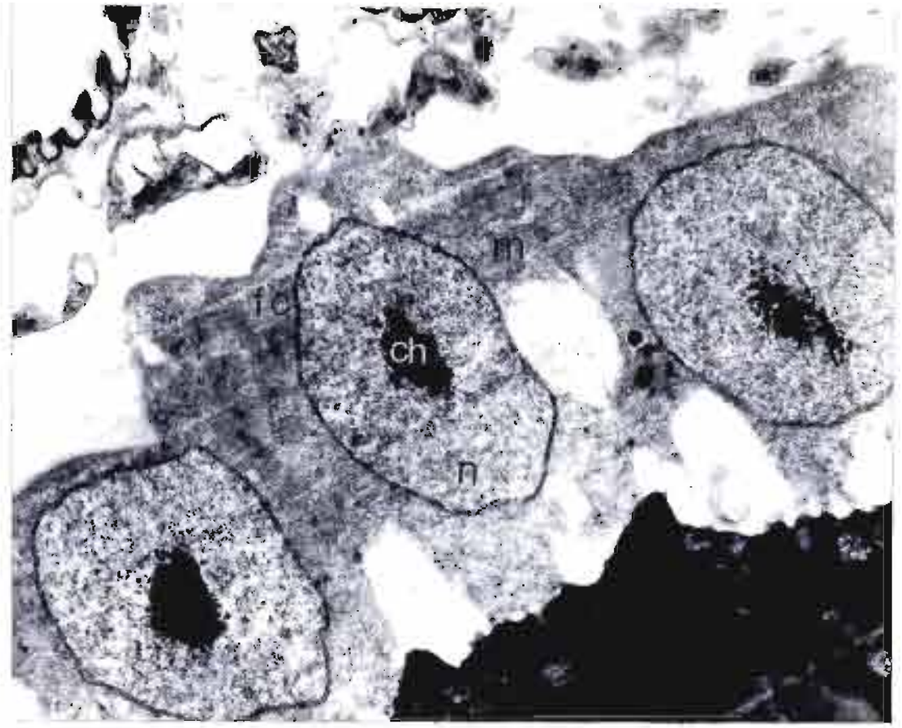
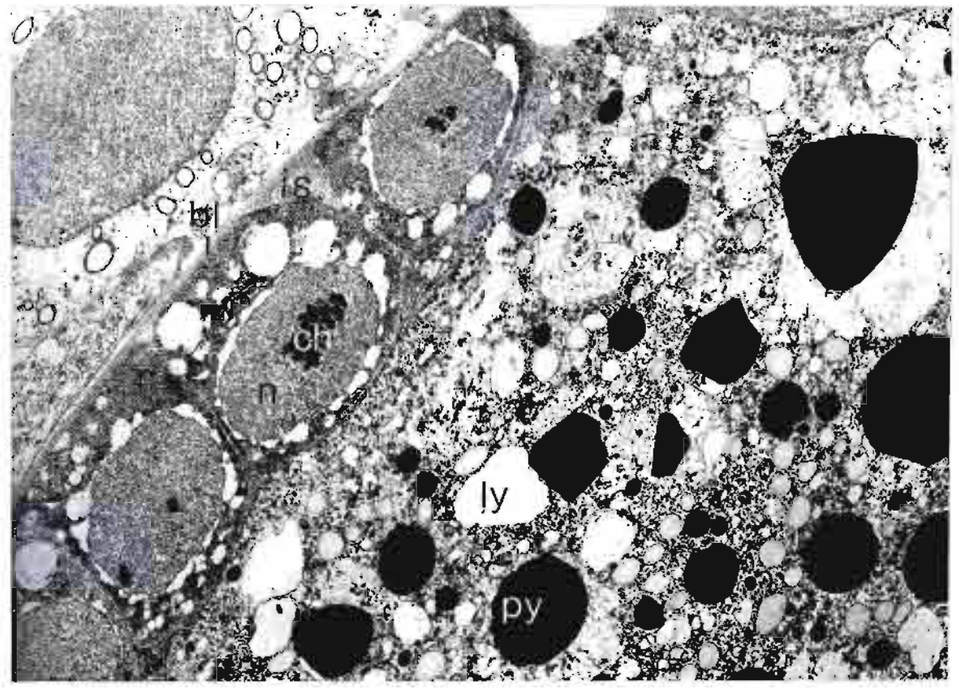


Figure 16. Follicular epithelium of Aedes aegypti 12 hours post blood meal for diet D.

Key : bl basement lamina
is intercellular space
fc follicle cell
r ribosomes
ch chromatin
n nucleus
ly lipid yolk
py protein yolk

Bar represents 1µ



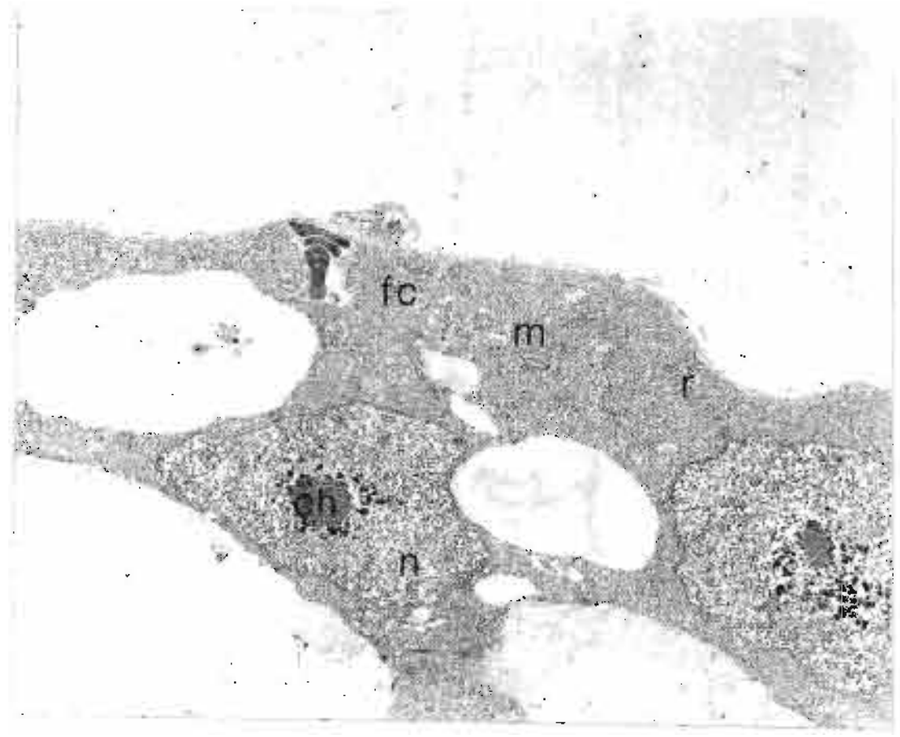


Figure 17. Follicular epithelium of Culex quinquefasciatus 12 hours post blood meal for diet E (the control).

Key : fc follicle cell
m mitochondria
r ribosomes
ch chromatin
n nucleus

Bar represents 1μ

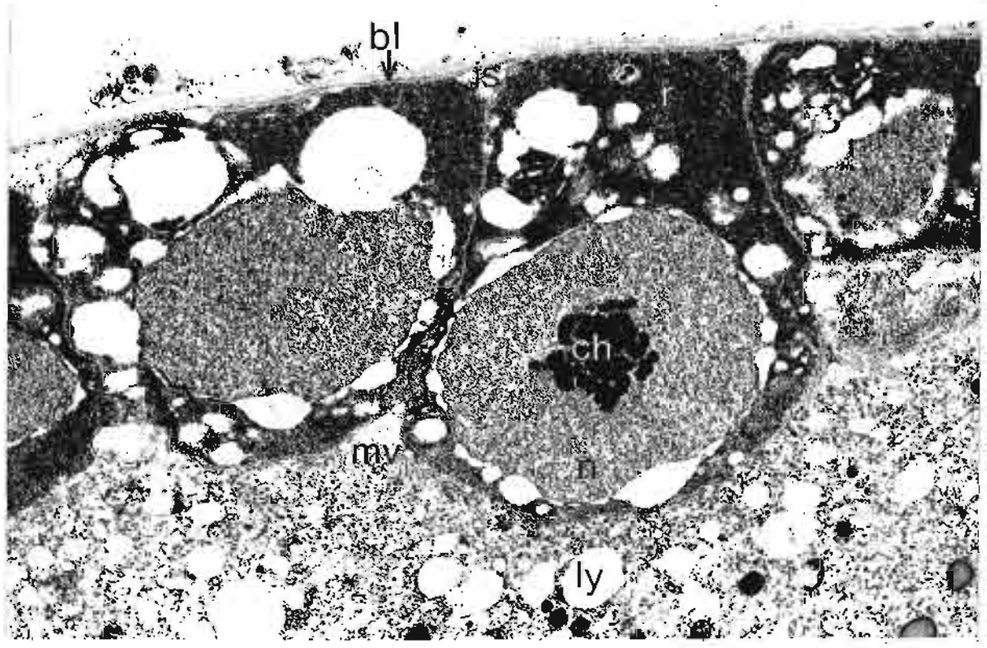


Figure 18. Follicular epithelium of Aedes aegypti 12 hours post blood meal for diet E (the control).

Key : bl basement lamina
is intercellular space
ch chromatin
n nucleus
r ribosomes
mv microvilli
ly lipid yolk

in C. quinquefasciatus.

The cellular structure was similar and the follicular epithelium had formed a continuous layer around the oocyte in the two mosquito species.

36 hours post blood meal

Figures 19 to 28 represent the follicular epithelium at 36 hours PBM.

Intercellular spaces were evident in C. quinquefasciatus and vitellogenesis had begun in diets B, C, D and E but not in diet A. The cytoplasm was filled with mitochondria, rough endoplasmic reticulum and secretory droplets were seen in Golgi complexes. Apart from there being no vitellogenesis in diet A, there were no other structural differences in the follicle cells between the various experimental diet groups and the control diet E (Figs. 19, 21, 23, 25 and 27).

There were no visible differences in A. aegypti between the experimental groups A to D and the control diet E. Oogenesis at this stage in this species was characterized by the coalescing of the vitelline plaques to form the vitelline membrane. The cytoplasm was still seen to be filled with ribosomes, rough endoplasmic reticulum, mitochondria and Golgi complexes (Figs. 20, 22, 24, 26 and 28).

The main difference at this stage between the two mosquito species is that vitellogenesis is only starting in C. quinquefasciatus but is almost complete in A. aegypti.

60 hours post blood meal

The follicular epithelium development at this stage can be seen in figures 29 to 39.

Figure 19. Follicular epithelium of Culex quinquefasciatus 36 hours post blood meal for diet A.

Key : is intercellular space
fc follicle cell
r ribosomes
m mitochondria
n nucleus
ch chromatin

Bar represents 1μ

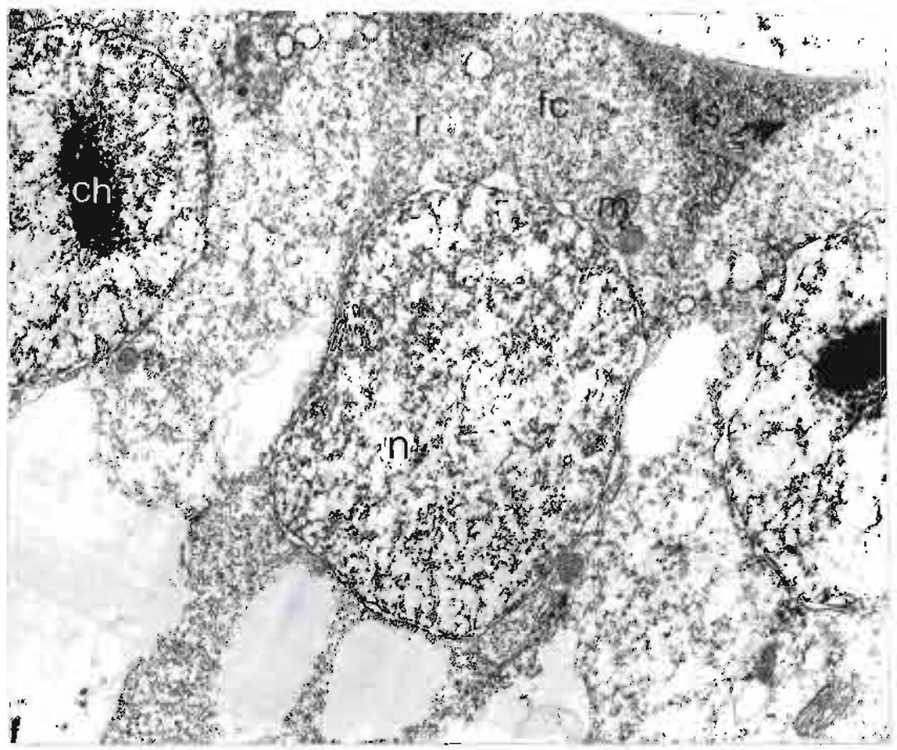
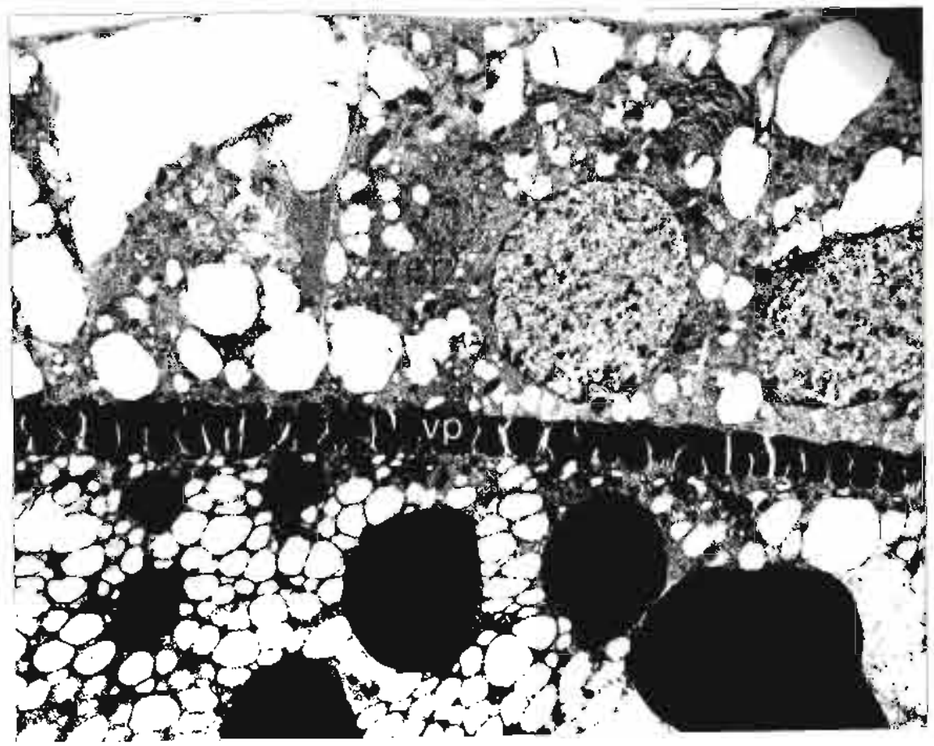


Figure 20. Follicular epithelium of Aedes aegypti 36 hours post blood meal for diet A.

Key : rer rough endoplasmic reticulum
vp vitelline plaques

Bar represents 1μ



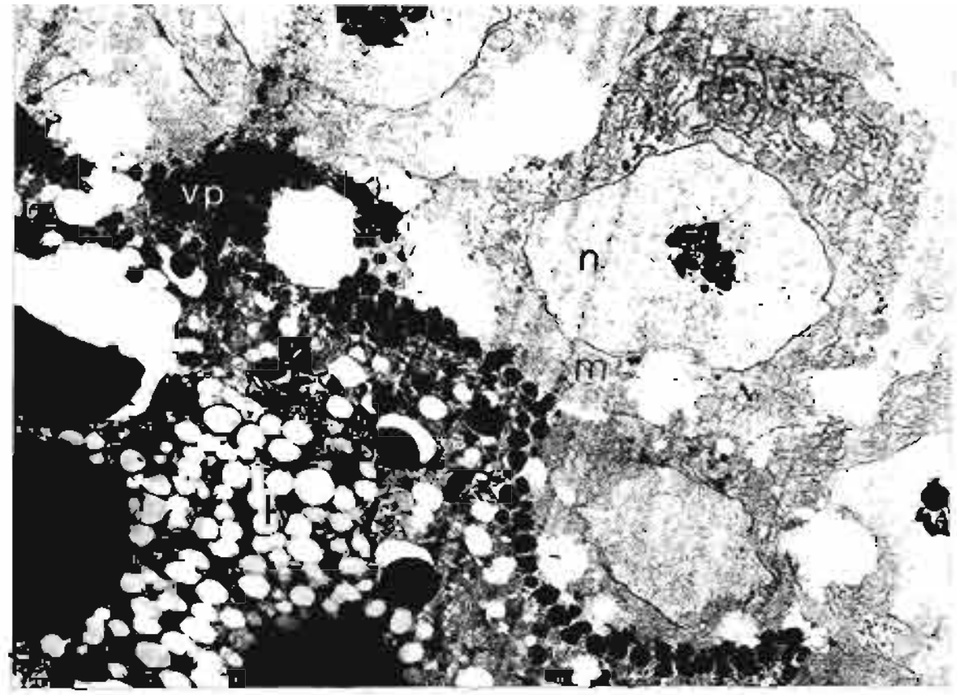


Figure 21. Follicular epithelium of Culex quinquefasciatus 36 hours post blood meal for diet B.

Key : rer rough endoplasmic reticulum
n nucleus
m mitochondria
vp vitelline plaque
ly lipid yolk

Bar represents 1µ

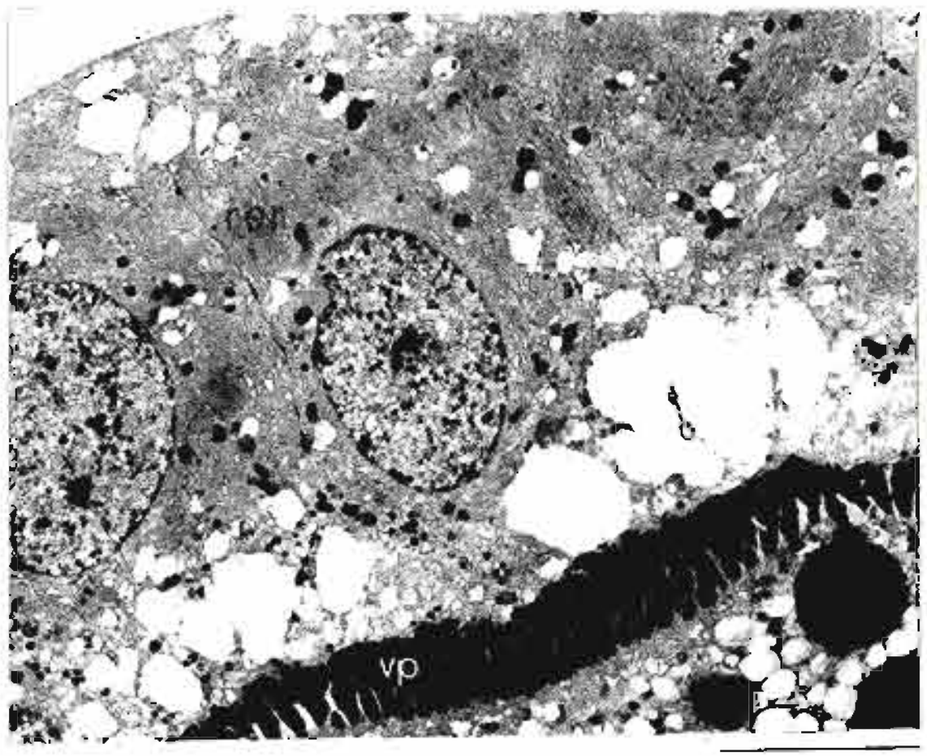


Figure 22. Follicular epithelium of Aedes aegypti 36 hours post blood meal for diet B.

Key : rer rough endoplasmic reticulum
vp vitelline plaque

Bar represents 1µ

Figure 23. Follicular epithelium of Culex quinquefasciatus 36 hours post blood meal for diet C.

Key : m mitochondria
r ribosomes
ch chromatin
n nucleus

Bar represents 1μ

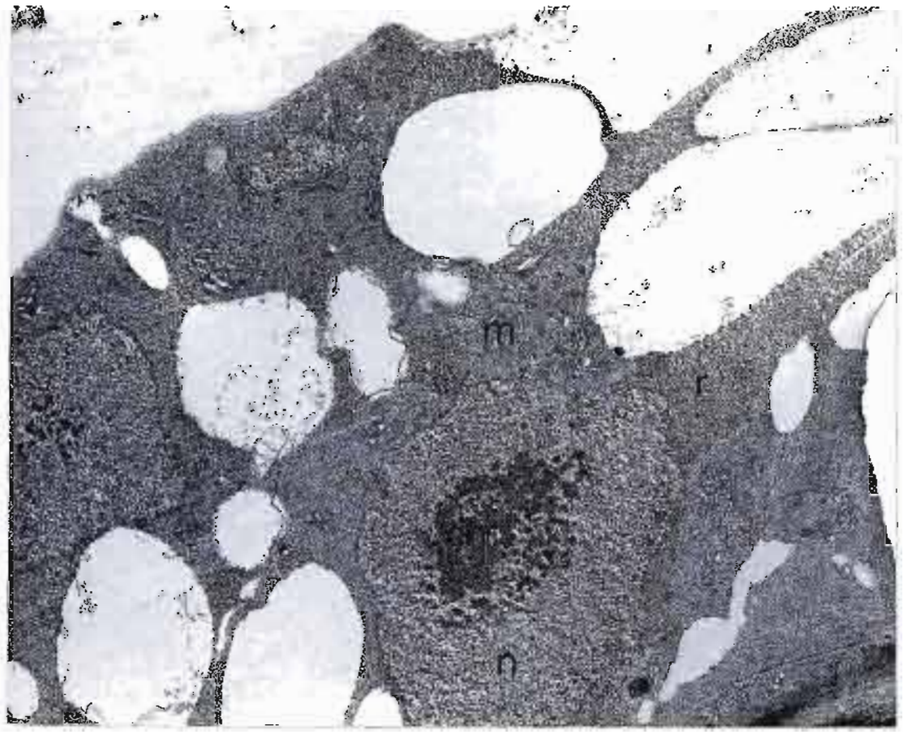


Figure 24. Follicular epithelium of Aedes aegypti 36 hours post blood meal for diet C.

Key : rer rough endoplasmic reticulum
vp vitelline plaque

Bar represents 1μ

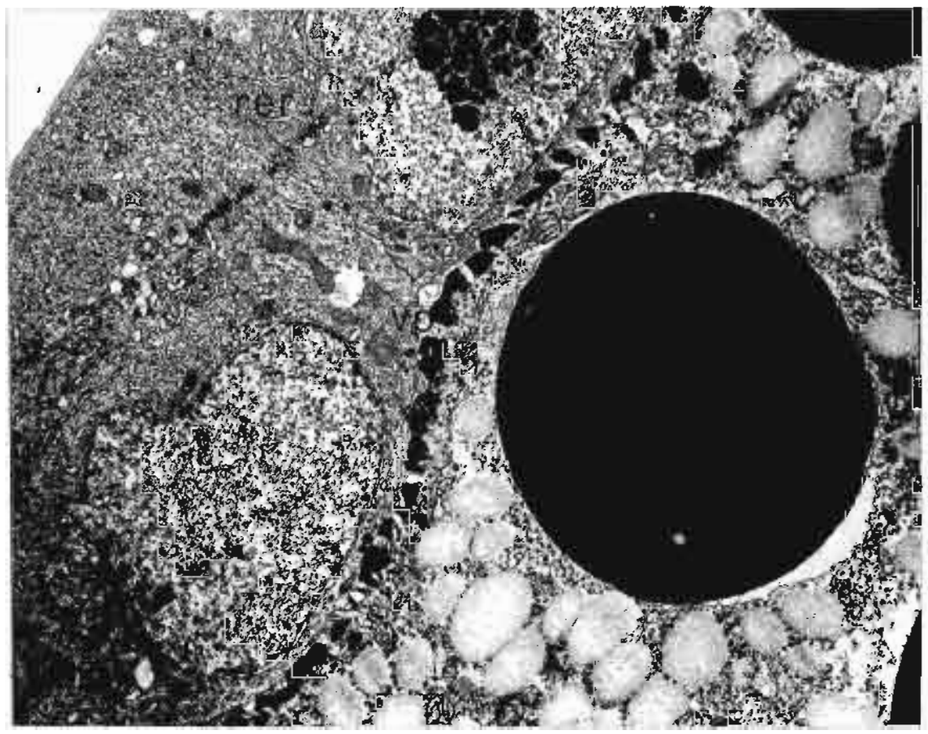


Figure 25. Follicular epithelium of Culex quinquefasciatus 36 hours post blood meal for diet D.

Key : rer rough endoplasmic reticulum
r ribosomes
m mitochondria
n nucleus
vp vitelline plaque
ly lipid yolk
py protein yolk

Bar represents 1µ

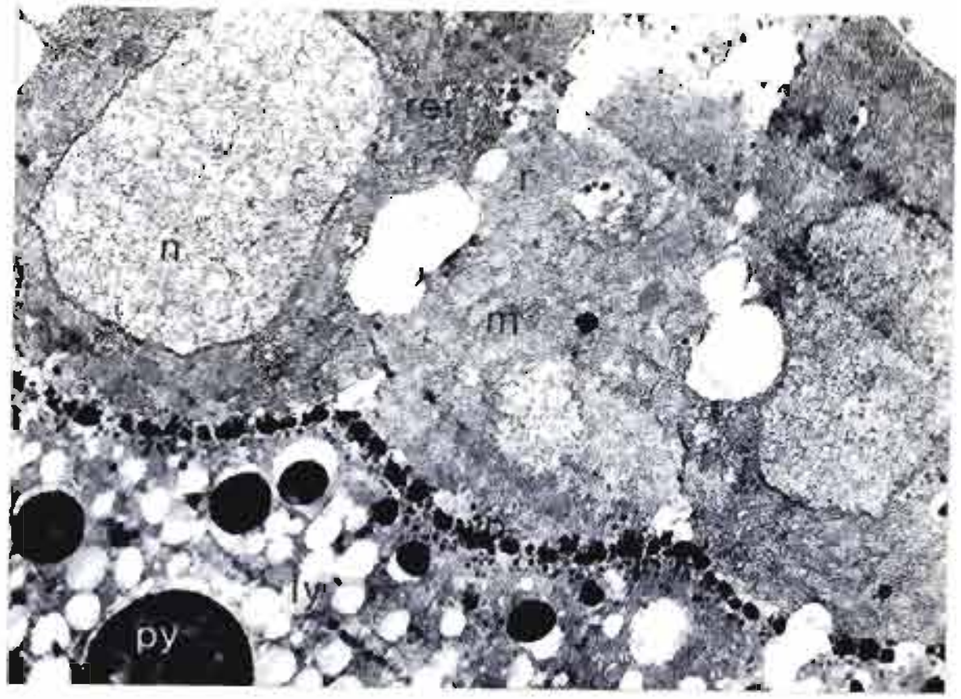
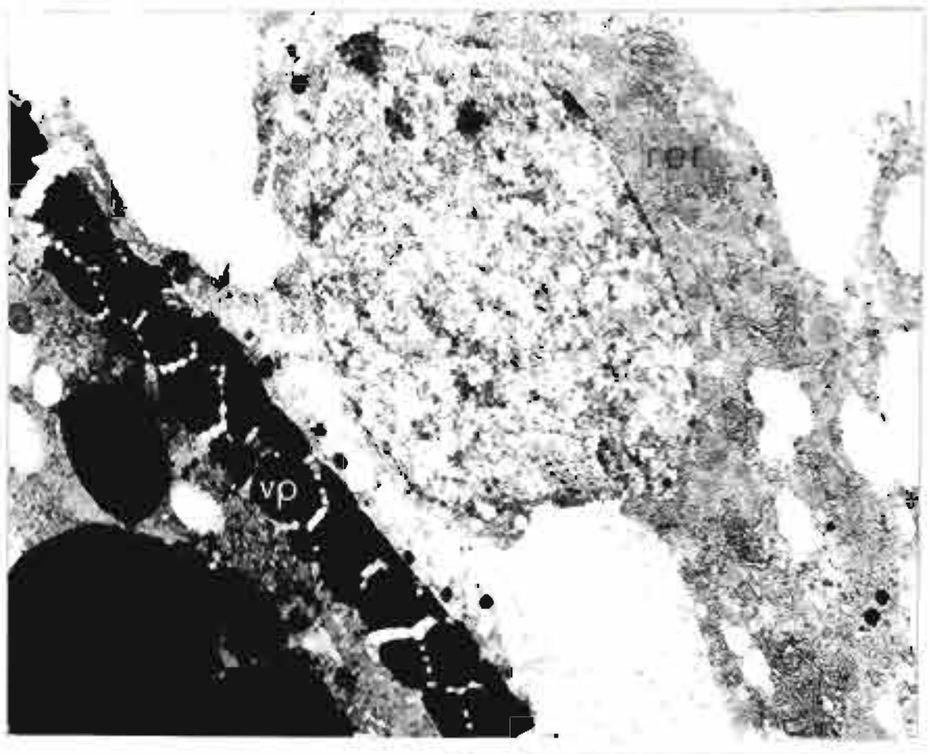


Figure 26. Follicular epithelium of Aedes aegypti 36 hours post blood meal for diet D.

Key : rer rough endoplasmic reticulum
vp vitelline plaque

Bar represents 1µ



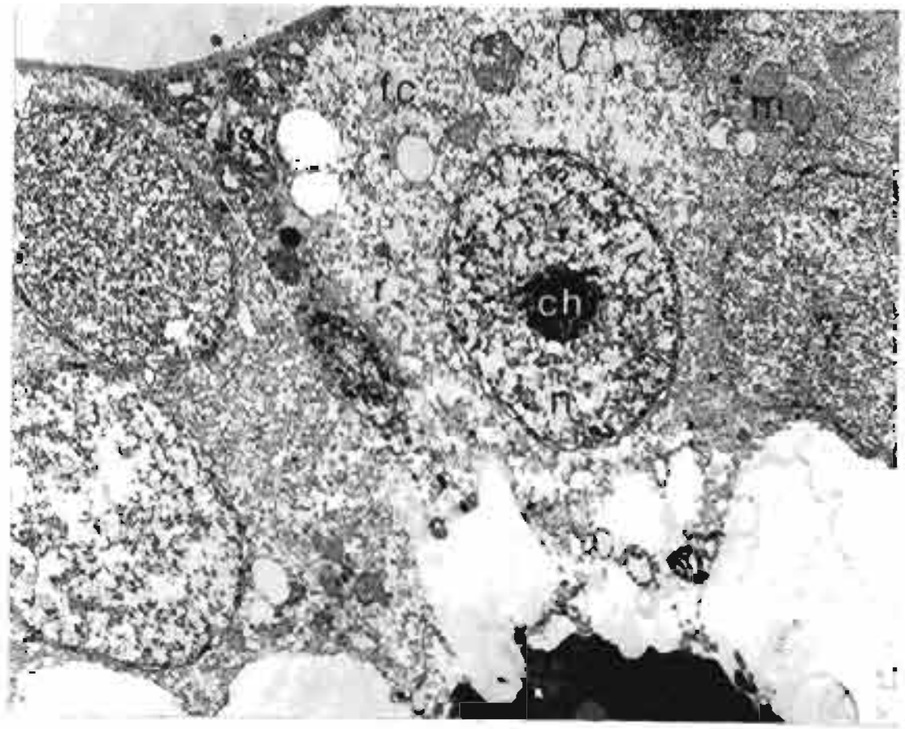


Figure 27. Follicular epithelium of Culex quinquefasciatus 36 hours post blood meal for diet E (the control).

Key : fc follicle cell
is intercellular space
m mitochondria
r ribosomes
ch chromatin
n nucleus

Bar represents 1μ

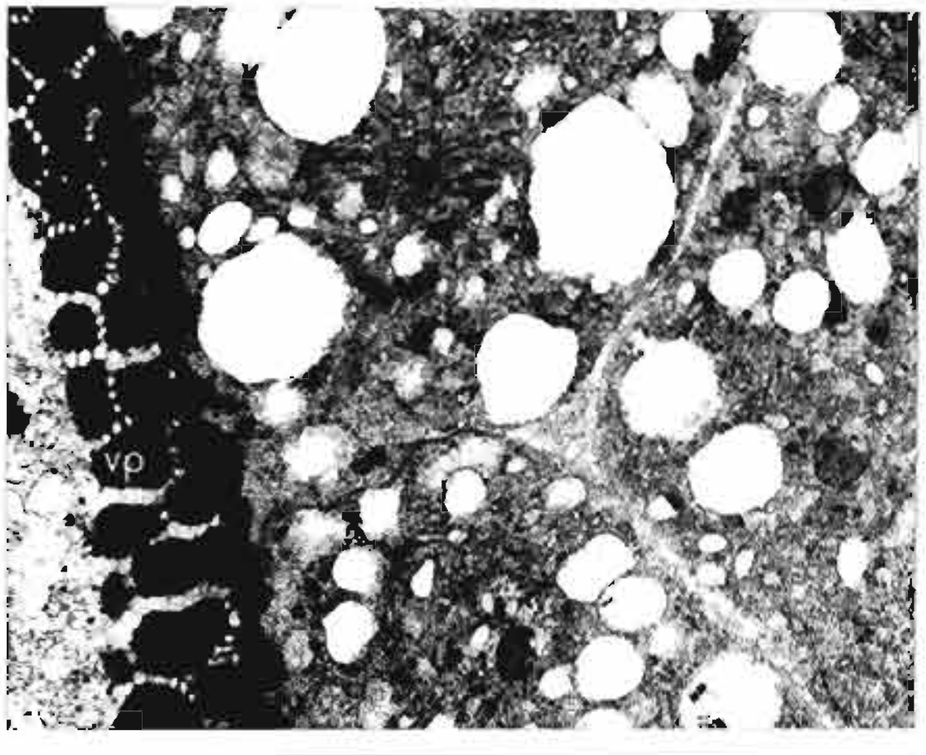


Figure 28. Follicular epithelium of Aedes aegypti 36 hours post blood meal for diet E (the control).

Key : rer rough endoplasmic reticulum
vp vitelline plaque

Bar represents 1μ

In C. quinquefasciatus vitellogenesis had begun in diet A. In all the other diet groups the nucleus was seen to be intact and the cytoplasm was filled with ribosomes, rough endoplasmic reticulum and mitochondria. There was no visible evidence of chorion formation yet. There were no structural differences in the follicle cells between the experimental diet groups A to D and the control diet E (Figs. 29, 32, 34, 36 and 38).

The results of A. aegypti can be seen in figures 30, 31, 33, 35, 37 and 39. Figure 31 represents chorion formation as seen in diet group A.

Structural differences between the different diet groups were observed for the first time at this stage.

In diet group A the follicular epithelial cell nucleus was still intact (Fig. 30) while rough endoplasmic reticulum, mitochondria and Golgi complexes were still visible.

In diet group B rough endoplasmic reticulum, mitochondria and Golgi complexes were still present in the follicular epithelial cells but only the begining of exochorion formation was visible. The nucleus was still clearly defined but had appeared to have taken on an elongate shape (Fig. 33).

In diet groups C, D and E the follicular epithelium had an abundance of ribosomes, mitochondria, very little rough endoplasmic reticulum and almost no Golgi complexes. The nuclear membrane had begun to degenerate and the cytoplasm was filled with fibrous tissue. The overall appearance of the follicular epithelium was one of degeneration (Figs. 35, 37 and 39).

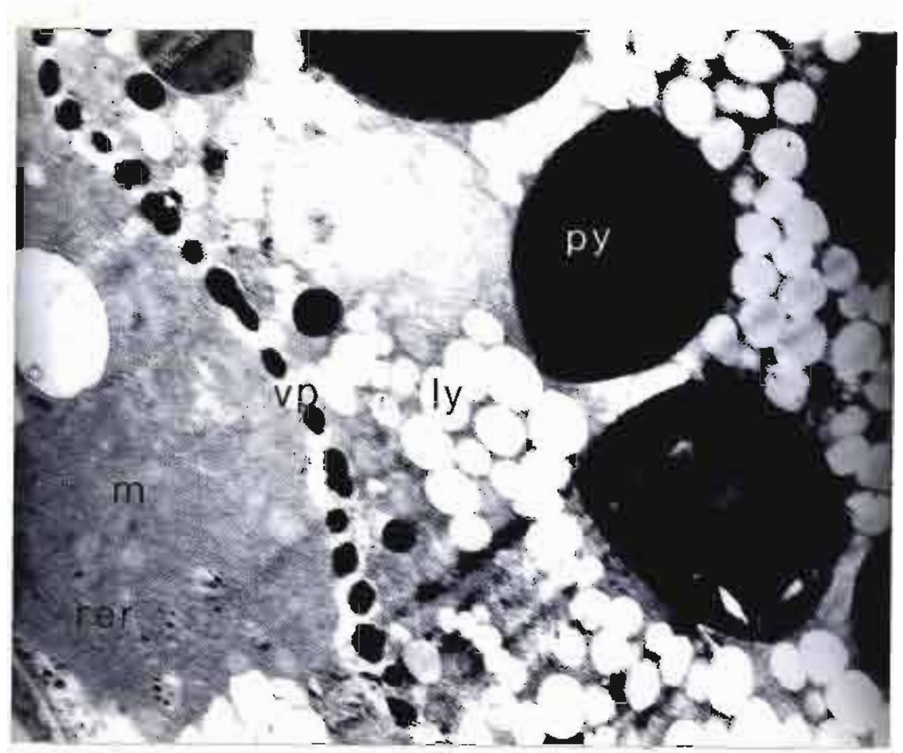


Figure 29. Follicular epithelium of Culex quinquefasciatus 60 hours post blood meal for diet A.

Key : rer rough endoplasmic reticulum
m mitochondria
vp vitelline plaque
ly lipid yolk
py protein yolk

Bar represents 1μ

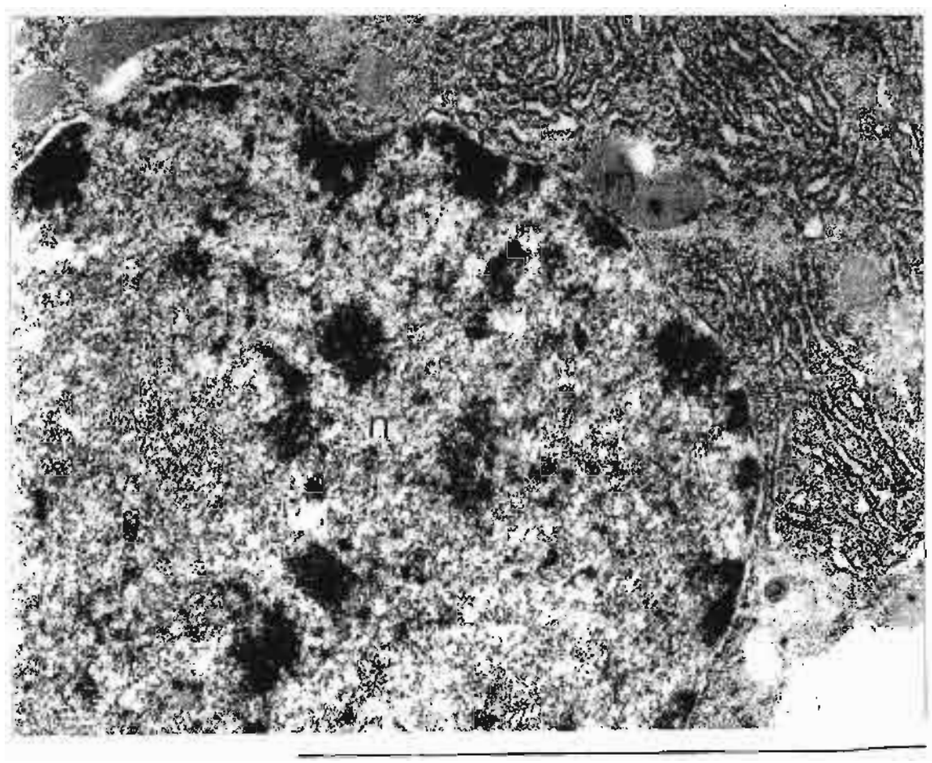


Figure 30. Follicular epithelium of Aedes aegypti 60 hours post blood meal for diet A.

Key : rer rough endoplasmic reticulum
m mitochondria
n nucleus

Bar represents 1μ

Figure 31. Chorion formation in Aedes aegypti in diet A 60 hours post blood meal.

Key : cp chorionic pillar

fm fibrous material

Bar represents 10μ

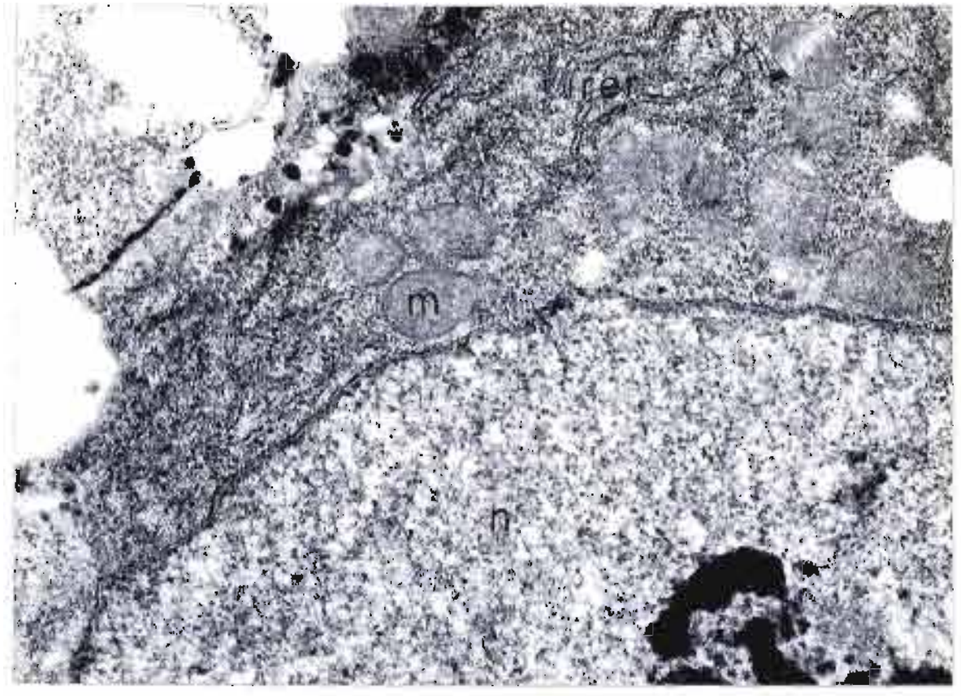


Figure 32. Follicular epithelium of Culex quinquefasciatus 60 hours post blood meal for diet B.

Key : rer rough endoplasmic reticulum
m mitochondria
n nucleus

Bar represents 1μ

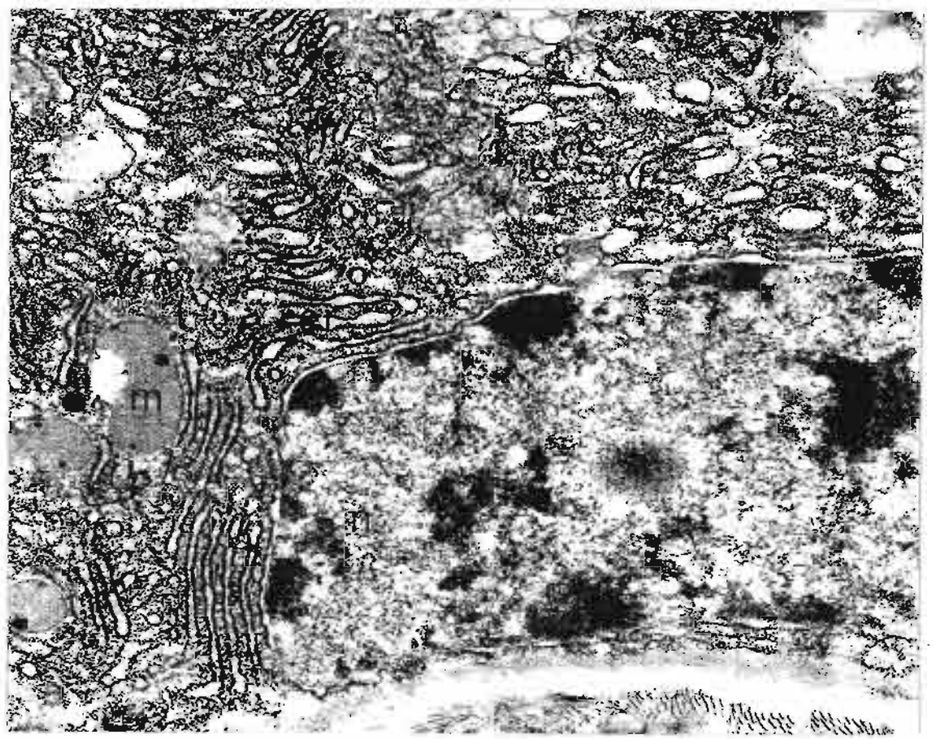


Figure 33. Follicular epithelium of Aedes aegypti 60 hours post blood meal for diet B.

Key : rer rough endoplasmic reticulum
m mitochondria
n nucleus

Bar represents 1μ

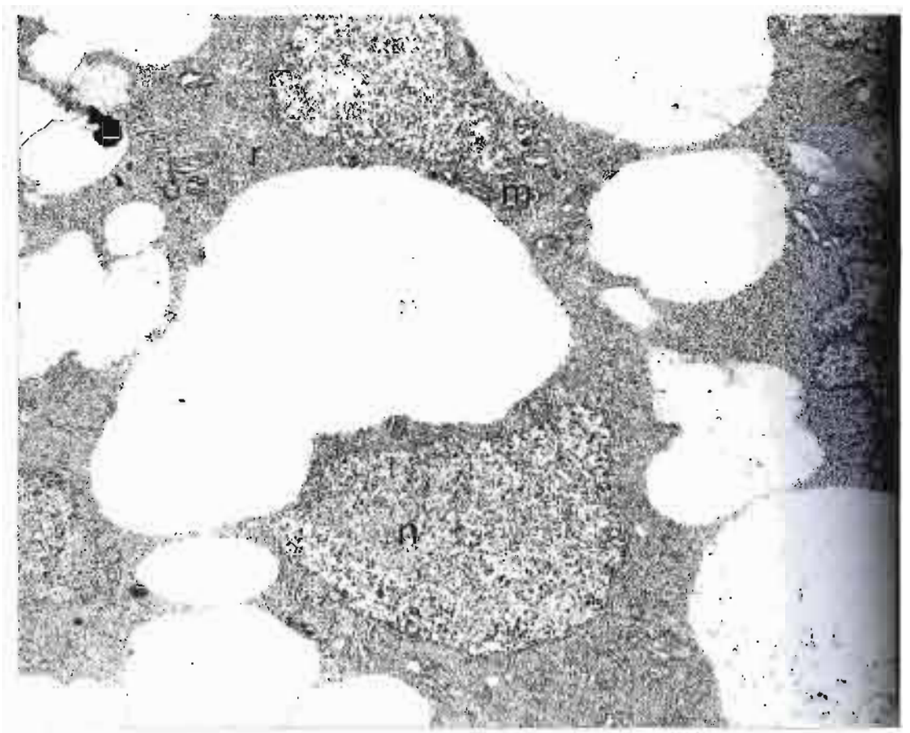


Figure 34. Follicular epithelium of Culex quinquefasciatus 60 hours post blood meal for diet C.

Key : r ribosomes
m mitochondria
n nucleus

Bar represents 1μ

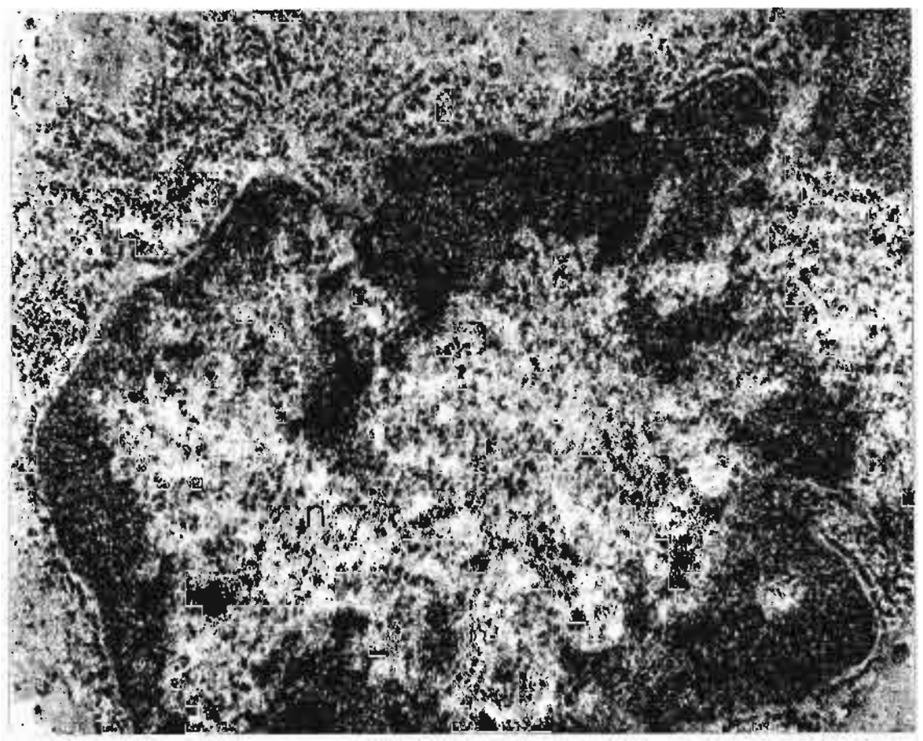


Figure 35. Follicular epithelium of Aedes aegypti 60 hours post blood meal for diet C.

Key : n nucleus

Bar represents 1μ

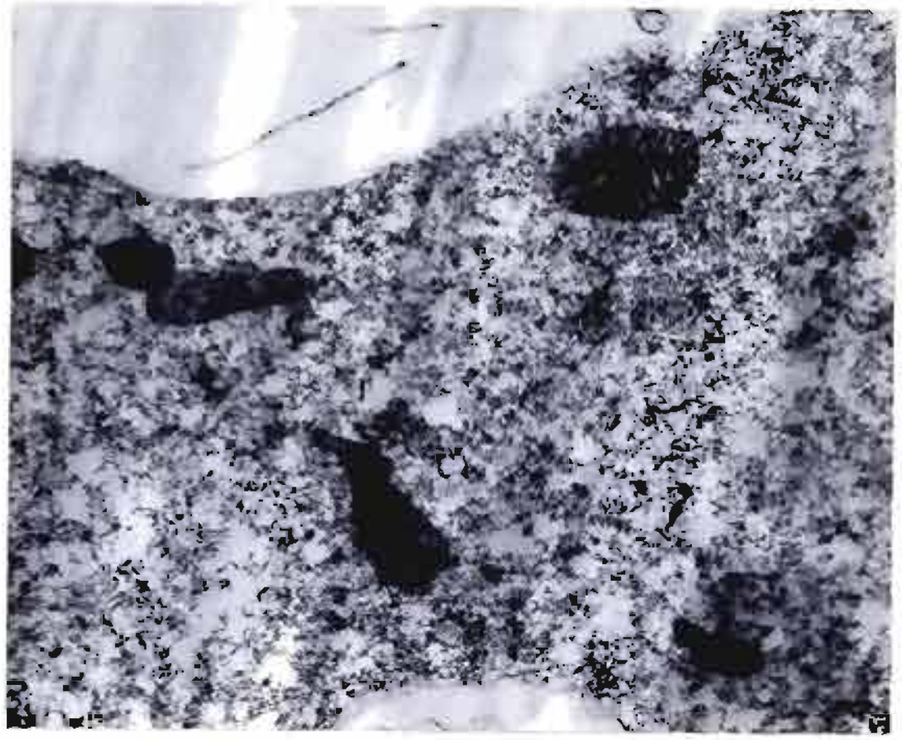


Figure 36. Follicular epithelium of Culex quinquefasciatus 60 hours post blood meal for diet D.

Key : m mitochondria

r ribosomes

Bar represents 1μ

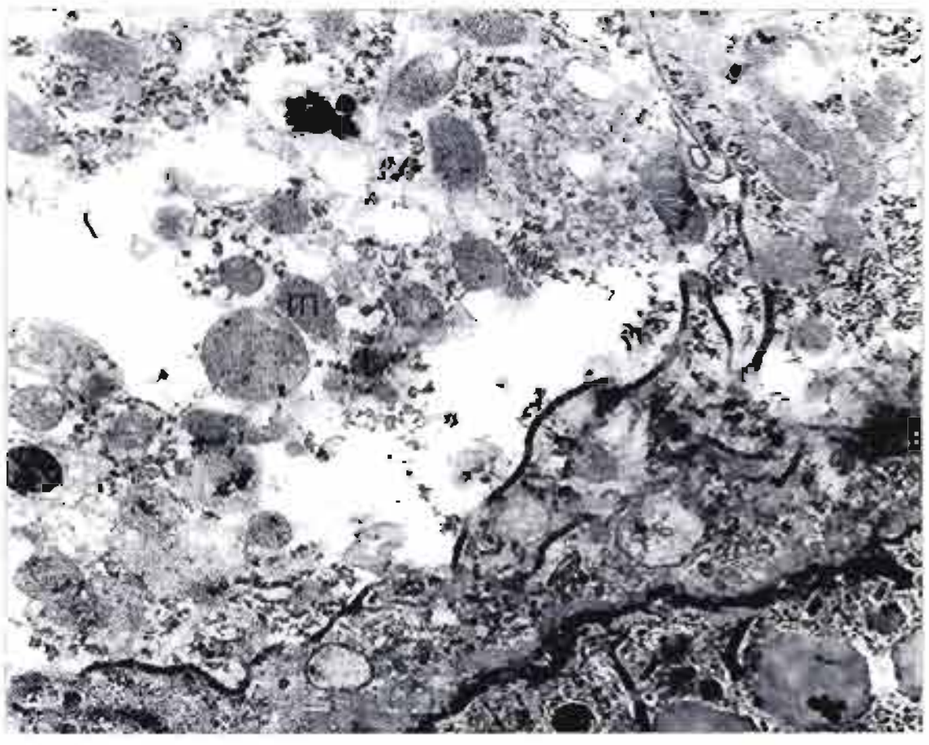


Figure 37. Follicular epithelium of Aedes aegypti 60 hours post blood meal for diet D.

Key : m mitochondria

Bar represents 5μ

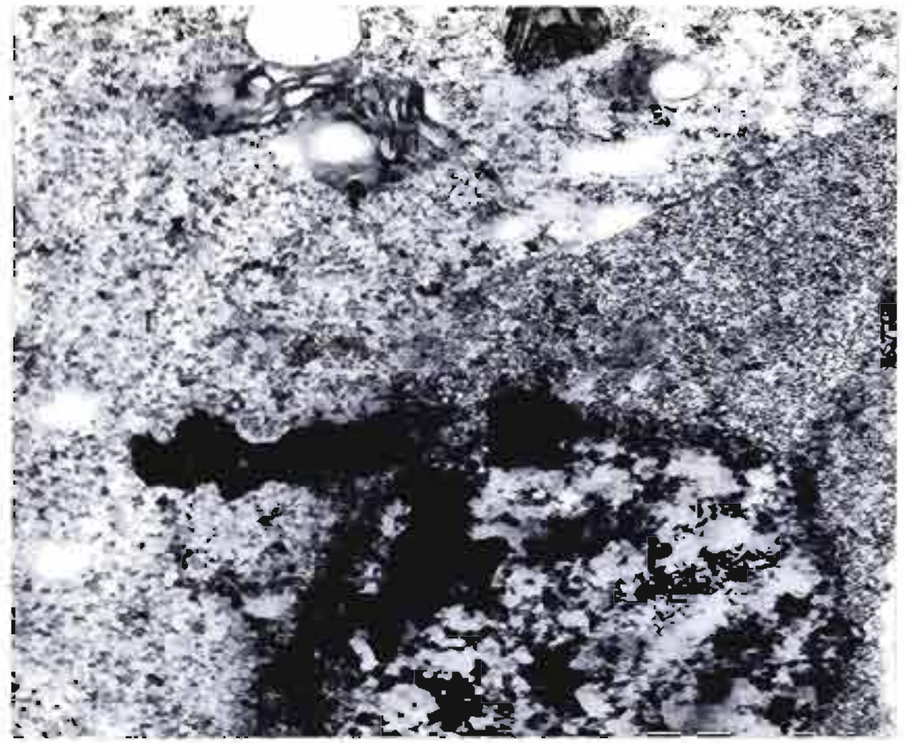


Figure 38. Follicular epithelium of Culex quinquefasciatus 60 hours post blood meal for diet E (the control).

Key : m mitochondria

r ribosomes

n nucleus

Bar represents 1μ

Figure 39. Follicular epithelium of Aedes aegypti 60 hours post blood meal for diet E (the control).

Key : n nucleus

m mitochondria

Bar represents 1μ

The main difference between the two species was in the development of the oocyte. In A. aegypti the oocyte had surpassed that of Culex quinquefasciatus. Maturity of an oocyte is characterized by the breakdown of the follicular epithelium and this had not yet begun in C. quinquefasciatus.

D I S C U S S I O N

1. FECUNDITY

There is much information scattered throughout the literature on the production of insect eggs as this is a major parameter in population dynamics (Gordon, 1968). This information, unfortunately, is seldom accompanied by data on food intake. However it is thought that mosquitoes are able to evaluate the nutrition potential of the blood meal before its complete digestion and thereby start the maturation of the appropriate number of oocytes (Bellamy & Bracken, 1971). In other words, some mechanism adjusts the number of maturing oocytes to fit the amount of nutrient potentially available.

Engelman (1970) supports this theory but also feels that the total egg production is dependent on the number of ovarioles present in the ovaries as well as the quantity of nutrients taken in by the adults and the larvae.

Nayar and Sauerman (1975) showed that the number of matured oocytes and the number of eggs laid varied with the species (for example, Culex mosquitoes can lay up to 300 and Aedes mosquitoes lay less than 100) depending on their nutritional status.

All the adult female mosquitoes in this study were given equal access to a blood meal and so it is possible to rule out the effect of the amount of nutrient available.

One must therefore consider the effect of larval diet on fecundity, but there was a very wide range of values obtained for both mosquito species (Culex = 74 - 363 eggs per female and Aedes = 28 - 96 eggs per female)

resulting in no significant differences between the test groups and the control group. Therefore, in the present study, the influence of larval diet can also be ruled out. However, nutrition in the larval stage is able to significantly influence fecundity in the resulting adults. Engelman (1970) showed in studies done on Aedes aegypti and Drosophila melanogaster that females from well fed larvae laid more eggs than those from poorly fed larvae and the number of ovarioles was also reduced in the latter.

Another factor to consider is sugar feeding by the adult. Nayar and Sauerman (1975) showed that sugar feeding of the female prior to and after blood feeding led to increased rates of fecundity and in some cases to eggs with higher nutritional reserves, which may affect the survival and growth of the progeny. All the adults in the present study were given equal access to sugar prior to and after blood feeding but there is a possibility that not all the females availed themselves thereof.

This is in direct contradiction to the results of de Meillon et al. (1967b) who found that cane sugar feeding in Culex pipiens led to erratic ovi - position which was delayed. This has been linked to survival in the wild; if no suitable oviposition sites are found, for example during droughts, gravid females will feed on sugar and thus prolong their lives and delay oviposition until a suitable environment is found. This is supported by Shroyer and Sanders (1977) who showed that sugar feeding delayed oviposition in Aedes vexans and led to increased rates of survival of the adults.

The effect of sugar could possibly be ruled out in this study as a suitable oviposition site was provided for the females but could account for a small percentage of the variation seen in the results.

One must therefore consider other factors such as the feeding behaviour of the mosquitoes as this has been shown by Friend and Smith (1977) to be affected by many factors such as diurnal rhythms, state of food and water deprivation, the host, etc. One must also consider the effects of hormones which have been shown by Bellamy and Bracken (1971) to affect ovarian responses.

Mating, light, temperature and humidity are all able to affect feeding and mating activities which in turn will affect egg production (Eldridge, 1986).

2. FERTILITY

Once again there were wide ranges of values (Culex = 57 - 100% fertility and Aedes = 44 - 90% fertility) precluding any significant differences between the test diet groups and the control diet group. This would rule out dietary influences and hence other factors such as the following must be brought into consideration;

(a) an increase in the percentages of males present in the cage was shown by Sebastian and de Meillon (1967) to lead to increased rates of insemination, but it must be remembered that uninseminated blood fed females can lay a few eggs (Clements 1963) and this will affect the results slightly. This could be overcome by floating the eggs on a saturated salt solution. Viable eggs will float and can be easily removed for culture.

(b) the effect of staggered hatching in Aedes aegypti was shown by Hotchkin (1985) who obtained fertility values of 94% within 72 hours. She kept egg papers for up to 10 weeks before flooding them. In the present study egg papers were dried out for approximately 48 hours before flooding.

Possibly one achieves higher hatch rates if the egg papers are left to dry for longer periods of time as Hotchkin (1985) obtained mean fertility values of 94% while those in the present study were much lower at 50 to 74% only. There is an advantage in staggered hatching as environmental conditions may be transient and as a flood water mosquito A. aegypti needs to ensure the maximum availability of water so that the larvae can complete their life cycle before the water dries up. This is also aided by second hatch larvae developing faster than first hatch larvae.

Fertility values obtained for C. quinquefasciatus were comparable with those obtained by other workers. Dziem and Cupp (1983) obtained a mean fertility value of 80,4% when raising Culex larvae on a diet of yeast and rabbit pellets.

3. DURATION OF LARVAL STAGE

Clements (1963) found that the duration of the larval stage of A. aegypti is 5 to 6 days. In the present study the larval period lasted from 5 to 18 days. These results are comparable with those obtained by Martinez-Palascios (1964) who experienced life cycles of 10 to 12 days. However, van Handel (1986) also obtained results of 5 to 6 days on either liver or yeast diets at a temperature of 28°C. One must therefore consider the effect of temperature here as the mean value for A. aegypti in the present study was 25,2°C (the mean humidity was 71%). This could possibly have led to the increased larval durations.

The mean results obtained for C. quinquefasciatus of 11 to 16 days, however, are comparable with those obtai ed by other workers. Dziem

and Cupp (1983) obtained a mean value of 12,7 days while using yeast and rabbit food at a temperature of 27°C and 80% humidity. Okazawa et al. (1985) obtained a mean value of 12 days while using powdered mouse pellets and yeast at a temperature of 28°C and 75 - 85% relative humidity. Van Handel (1986) and de Meillon et al. (1967a), on the other hand obtained mean values of 6,5 and 5 days respectively under similar conditions. The mean temperature for C. quinquefasciatus in the present study was 24,3°C and the mean relative humidity was 56,5%. Inspite of these factors being lower than those of the above mentioned workers, the duration of the larval stage was still similar implying that C. quinquefasciatus is able to easily accommodate a 4°C temperature difference. This would probably be a built-in mechanism related to coping with changing environmental conditions. This is unlike Aedes aegypti where lower temperatures tended to produce longer larval periods.

Within each species, the duration of larval life in the present study appears to be greatly affected by diet. When examining the experimental diets compared with the control diet E, it can be seen that the duration of larval life was significantly shortened in diet groups C (dog food) and D (yeast) in C. quinquefasciatus and in diet groups B (rat cubes), C and D in A. aegypti.

Dziem and Cupp (1983) claim inadequate nutrition is responsible for prolonged larval life. This is supported by Okazawa et al. (1985) and de Meillon et al. (1967a) who claim that the duration of larval life is greatly affected by feeding. Lichtenstein (1948) found that development is greatly increased by the addition of nicotenic acid and slightly by the addition of ascorbic acid. Without folic acid larvae are able to reach the 4th instar but do not pupate. Folic acid is found in yeast and liver (diets D and E respectively). This is supported by Goldberg et al. (1945) who also showed that folic acid and vitamin B were essential for larval growth and that these were contained in liver and yeast. Kettle (1984) showed that insects do not require ascorbic acid but they do require a source of sterols and some species need cholesterol. All

insects also require certain fractions of the B group of vitamins. This is further supported by Asahina (1964) who showed that certain food types contain growth promoters such as cholesterol and vitamin B - complex and these promoters are found in yeast and liver.

As the larvae were all grown under the same conditions the variations in the duration of the larval stage must be due to diet and it can therefore be said that a diet of dog biscuits or yeast (in the case of C. quinquefasciatus and A. aegypti) or rat cubes (in the case of A. aegypti) can shorten the larval period significantly when compared with a diet of dessicated liver. Clements (1963) has shown that growth is stimulated by the addition of bacteria or yeast. Christophers (1960) has shown that sterile food leads to no growth. Therefore one can conclude that living bacteria are needed for growth. One must therefore question what role bacteria played in the present study. Pronutro plus Tastee wheat appeared to have no effect on the duration of larval life when compared with the control diet E.

Dadd (1982) has shown that yeast has a strong phogostimulatory effect on mosquito larvae and this could explain some of the differences observed as the more the larvae eat the faster they will grow. Van Handel (1986) however showed that the accumulation of protein was faster in larvae fed on liver than those fed on yeast. The ultimate role played by these two diets would require further work as the duration of larval life was lengthened in both the mosquito species fed on liver (diet E) when compared with diets B, C and D. Dadd et al. (1982) also showed that larval water pH was important in the rate of ingestion. He found that pH values below 6 led to a marked inhibition of feeding. The pH values of the water in the various rearing dishes was above 6 in all diets in the present study.

Genetic factors have also been implicated by Hotchkin (1985) as she found that A. aegypti larvae from different hatches developed at different rates. These factors could possibly play a role within each diet group as the duration of the larval stage was lengthy but the strong influence of diet would appear to be the only factor to consider when making comparisons between the experimental and control diet groups.

4. DURATION OF PUPAL STAGE

As the pupal stage follows on from the larval stage one would assume that conditions affecting the larvae would therefore also affect the pupae. This is confirmed in C. quinquefasciatus in diet group C (dog food) and D (yeast) which differed significantly from the control diet E (dessicated liver). As was expected as the former two groups had shorter larval life spans they pupated sooner than the other diet groups.

However, the same is not true for A. aegypti as only diet group B (rat cubes) produced a pupal period which was significantly shorter than that of the control diet E.

Males were the first to pupate as was expected and this could be related to females needing more nutrition for subsequent reproduction, and therefore needing to spend longer in the larval stage. Resultant females were also larger than the males as can be expected.

The actual time spent as a pupa was approximately 2 days in all diet groups. This compares with the 2½ days obtained by Dziem and Cupp (1983) and the 1½ days obtained by de Meillon et al. (1967a).

5. MORTALITY

5.1 LARVAL MORTALITY

There were no significant differences between the experimental diet groups and the control group in A. aegypti. In the present study, mean mortality rates ranged from 8,9 to 30,1% which were higher than those obtained for C. quinquefasciatus (12 to 26% excluding diet group A) and considerably higher than those obtained by Okazawa et al. (1985) of 3.3%.

Only diet group A had a mortality value significantly higher than the control in C. quinquefasciatus. Diet groups B, C and E had considerably lower mortality values but diet group D had a relatively high mortality in both mosquito species (26% in C. quinquefasciatus and 30% in A.aegypti).

The higher incidence of larval mortality in diet groups A (Pronutro + Tastee wheat) and D (Brewer's yeast) can be related to the nature of the diet. Pronutro plus Tastee wheat swole rapidly when added to the water and formed a sticky mass. The larvae appeared to get caught up in this mass and drown. Brewer's yeast, on the other hand, rapidly spread across the surface of the water forming a scum layer which also caused the larvae to drown as they were prevented from breathing. This problem could be overcome by gently aerating the water (P. Hewitt, pers comm).

5.2 PUPAL MORTALITY

There were no significant differences between any of the experimental diet groups and the control diet in both mosquito species studied.

The mean values obtained for C. quinquefasciatus (0,6 - 5,5%) compared favourably with those obtained by other workers. Dziem and Cupp (1983) experienced mortalities of 7,6% while Okazawa et al. (1985) showed mortalities of 1,7%.

Pupal mortalities for A. aegypti were relatively low at 1,0 to 3,1% These results compared favourably with those of Okazawa **et al.** (1985)

The highest pupal mortality ranges were experienced in diet groups A (Pronutro + Tastee wheat) and D (Brewer's yeast). Muspratt (1962) claims that pupal mortality is due to bacterial infection. This could explain the reason for the high pupal mortality in diet A as Pronutro is a substrate which allows rapid bacterial growth. Fouling of the water could also lead to increased pupal deaths as the pupae would be prevented from breathing by the scum layer on the water surface. This could be the mechanism in operation in diet D. Moya and Botella (1985) working on Drosophila found that pupal mortality was a density dependent process. This could also be taken into consideration when examining mosquito pupal mortality but further work is required to ascertain the maximum density per millilitre of water for rearing mosquito pupae.

5.3 ADULT MORTALITY

Diet would appear not to have any effect on adult mosquito mortality as there was a wide range of results obtained in the present study. Consequently no one experimental diet group differed significantly from the control diet group.

Various theories have been proposed to explain mortality rates and much work has been done in determining the longevity of adult mosquitoes (Gahan and Smith, 1964; de Meillon et al., 1967a; Clements, 1963; Nayar and Sauerman, 1975). The main factors under consideration are temperature and humidity. The temperature in the present study was considered to be too high for C. quinquefasciatus (P. Jupp, pers comm) as some daily recordings of 28°C were made. It is felt that 24°C is preferable.

Relative humidity values of 90% were obtained on some days during mid - summer and this is also felt to be too high for both mosquito species. At these high humidity values biting and oviposition are halted (Clements, 1963).

Temperature and humidity could account for most of the deaths but during periods when daily temperatures were cooler other factors must be brought into consideration.

Dadd et al. (1979) have shown that a respiratory substrate must be included in the diet of adults if maximum longevity is to be achieved. In the case of female mosquitoes, triglycerides are the respiratory substrate and are synthesized from sucrose. They also showed that arachidonic acid was necessary for flight in Culex.

6. SIZE OF OVARY

In C. quinquefasciatus only diet group B (rat cubes) produced ovaries that were significantly smaller than the control diet E (dessicated liver). There were, however, no significant differences between the experimental diet groups A to D and the control diet E in A. aegypti.

Although diet B, and to a lesser extent diet A, gave rise to smaller ovaries in C. quinquefasciatus, there was no concomitant decrease in the number of eggs produced i.e. the size of the ovary was not related to fecundity. It was also noted that the adults were not smaller in size than those from the other diet groups.

Mori (1979), working on C. quinquefasciatus, claimed that larval density coupled with type of diet led to a decrease in ovary size. As the larval density was constant for all diet groups, and only the food type varied,

one can deduce that larval diet appeared to influence the size of the ovaries in the present study.

7. SEX RATIO

The sex ratio in A. aegypti usually approximates 1:1 (Hickey & Craig, 1966). However, certain males have very few females in their offspring. This predominance of females has been shown to be due to an inherited factor and not due to differential mortality (Craig et al., 1960).

McClelland (1962) demonstrated that sex is determined by a single pair of alleles designated M and m. The sex alleles are located on chromosome one. Females are homogametic mm, and males are heterogametic Mm. Thus, the male parent determines the sex ratio in the progeny.

As most workers in the past have obtained a 1:1 sex ratio (Qutubuddin, 1953; Dziem & Cupp, 1983; Clements, 1963; Hotchkin, 1985) the skewing of the sex ratio with a bias towards males in both mosquito species studied (C. quinquefasciatus in diets D and E and A.aegypti in diets C and E) was an unexpected finding.

Christophers (1960) however, has observed a slight excess of males in past literature and concluded that frequencies of 35 to 45% female was characteristic of A. aegypti.

Engelman (1970) found that exposure of larval A. stimulans to temperatures of 26°C caused males to develop into females. In the present study the temperatures were approximately the same but an increased number of males arose. Furthermore, all the larvae were exposed to the same temperature regimes with the only different factor being diet.

Diet therefore appears to play a role in sex determination but the mechanism would require further work for elucidation as a male producing phenomenon could be used to control or manipulate natural populations of these mosquitoes. As these species are involved in disease transmission, any measure which would modify the sex ratio in favour of males should be beneficial since blood feeding and hence, disease transmission, is limited to females.

8. TRANSMISSION ELECTRON MICROSCOPY OF OVARIES

The results of this study indicate that diet does play a role in oogenesis. In C. quinquefasciatus there were no differences between the follicular epithelial cells in the different diet groups at 12 hours PBM. However, from 36 hours PBM, development in diet group A appeared to lag behind that of the other diet groups. Vitellogenesis had commenced at 36hours PBM in diet groups B, C, D and E but did not start until 60 hours PBM in diet group A.

In A. aegypti there were no visible differences in the follicular epithelium until 60 hours PBM. At this stage the follicular epithelium of diets A and B had not developed as far as the control diet E. The development in diets C and D, however, had progressed as far as that of the control diet E.

In each diet the main common ingredient was protein. The amount of protein available per larva was as follows; A = 2,24mg, B = 1,92mg, C = 1,08mg, D = 0,75mg and E = 0,76mg. Since diet A in both species and diet B in C. quinquefasciatus lagged behind in oogenesis, one may conclude that increased amounts of protein in the larval diet prolongs the process of vitellogenesis in C. quinquefasciatus and A. aegypti. The mechanism or reason for this effect would require further work for elucidation as no

work has been done on the effect of diet on the follicular epithelium of developing oocytes.

There was also a marked contrast in the overall rate of development of the oocytes between the two mosquito species. Development in C. quinquefasciatus appeared to be much slower than that in A. aegypti. One would presume this to be a genetic effect because of differences in the nature of the eggs and the different life-styles of the two species. A. aegypti eggs have to undergo a period of drying before maximum hatching is achieved and, furthermore, the eggs are able to withstand months of dessication. C. quinquefasciatus eggs, on the other hand, cannot with-stand dessication and have to be laid on water. C. quinquefasciatus eggs hatch within 48 to 72 hours after being laid whereas A. aegypti eggs can hatch within hours of being re-hydrated.

This would tend to indicate a longer period of embryogenesis in Culex as opposed to Aedes.

The procedure used in the transmission electron microscopy study carried out in this thesis was a new one developed for this project and deserves mention. Previously the fixation, dehydration and embedding of mosquito ovaries was a time consuming exercise of up to four days (Pollard et al., 1986). The method described herein is time saving in that all pre - sectioning steps can be carried out in one day. The quality of micro-graphs obtained is also comparable with those of other workers (Mathew & Rai, 1975; Pollard et al., 1986).

SUMMARY

Larvae of Culex quinquefasciatus and Aedes aegypti were raised on five different diets and the effects thereof on various parameters was studied. The diets used were: A = Pronutro and Tastee wheat, B = Epol rat cubes, C = Breeder's Dogmor puppy chunks, D = Vital Brewer's yeast powder and E (the control) = Vital dessicated liver. The following results were obtained.

1. Fecundity appeared not to be affected by diet in both species.

2. Fertility did not appear to be affected by diet either.

3. Diets C and D significantly shortened the duration of the larval stage in C. quinquefasciatus when compared with the control Diet E. In A. aegypti diets B, C and D produced larval periods that were significantly shorter than the control.

4. The pupal stage in C. quinquefasciatus in diets C and D was also significantly shorter than that of the control diet E. In A. aegypti only diet B produced a pupal stage that was significantly shorter than the control.

5. Larval mortality was significantly higher than the control diet E in C. quinquefasciatus in diet group A. There was no significant differences between the experimental diet groups A to D and the control in A. aegypti.

6. Pupal mortality appeared to be unaffected by larval diet in both species.

7. Larval diet did not affect adult mortality either.

8. In C. quinquefasciatus the size of the ovaries was significantly smaller than the control in diet group B. Ovary size appeared to be unaffected by larval diet in A. aegypti.

9. Sex ratio appeared to be greatly affected by larval diet. There were significantly more males than females in diets D and E in Culex quinquefasciatus and in diets C and E in Aedes aegypti.

10. Oogenesis also appeared to be affected by larval diet. In C. quinquefasciatus oogenesis was found to be slower from 36 hours PBM in diet A. In A. aegypti oogenesis was slower in diets A and B from 60 hours PBM. The rate of oogenesis in C.quinquefasciatus was also found to be slower than that in A.aegypti.

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APPENDIX 1

Substance	Diet				
	A	B	C	D	E
protein	x	x	x	x	x
fat	x	x	x	x	x
linoleic acid	x		x		
fibre		x	x		
nicotinamide	x				
vitamin C	x				
calcium	x	x	x	x	
phosphorous	x	x	x	x	
potassium			x		
sodium chloride			x		
magnesium		x	x	x	
iron	x	x	x	x	
copper	x	x	x		
manganese			x		
zinc		x	x		
iodine	x	x	x		
sesium			x		
vitamin A	x	x	x		
vitamin D		x	x		
vitamin E	x	x	x		
vitamin B1	x	x	x	x	
vitamin B2	x	x	x	x	x
pantothenic acid	x	x	x	x	
niacin		x	x	x	x
vitamin B6	x	x	x	x	
folic acid	x	x	x	x	x
biotin			x	x	
vitamin B12	x	x	x		x
choline		x	x		
carbohydrates		x		x	x
cholesterol		x			

List of ingredients as supplied by manufacturers.

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