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Comparative larval development of *Culex pipiens* L. and *Aedes aegypti* L. : the influence of food, space and light.

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COMPARATIVE LARVAL DEVELOPMENT OF CULEX PIPIENS L.
AND AEDES AEGYPTI L.; THE INFLUENCE OF FOOD, SPACE
AND LIGHT

A Dissertation Presented

By

Victor A. Vazquez

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AND AEDES AEGYPTI L.; THE INFLUENCE OF FOOD, SPACE
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INTRODUCTION

The role of insects as both pests and benefactors of man and his interests is well known. Insect pests attack crops and other plants, along with foods and fibers, as well as man and other higher animals directly. The damage may be actual injury, irritation, or contamination, or the insect may serve an important role as a vector of a disease of either plants or animals. Yet only a relatively small percentage of known insect species are important pests. By far the majority are either beneficial or neutral from man's standpoint.

Mosquitoes occupy an important ecological niche so far as man is concerned. Out of a total of 110 known virus diseases of man and higher animals, 84 are transmitted by mosquitoes (Anon., 1961). Important diseases caused by other pathogens (i.e., malaria by protozoans and elephantiasis by nematodes) are also transmitted by mosquitoes. Along with this important role, mosquitoes are notorious pests of man and most higher animals because of the blood-sucking activities of the females.

Among the better known and more important of the disease vectors is Aedes aegypti Linn, the key vector of yellow fever in many parts of the world. Since 1945, leading government and foundation health agencies have

been engaged in a long-term project to eradicate this mosquito with the hope of thus eradicating yellow fever. Good progress in this direction has been made in many Central and South American countries.

The eradication program mentioned above, along with the usual aspects of mosquito control, have led to many excellent studies on mosquito taxonomy, physiology, ecology and chemical control. The present study was undertaken to extend our present knowledge of the ecology of mosquito larvae. The two mosquito species selected for study were Aedes aegypti L., which has already been mentioned, and Culex pipiens Linn. The latter is not known to be a disease vector, but it is commonly represented in Massachusetts by two strains which look alike but show quite different habits. One breeds in natural water and feeds mostly on birds in rural area, whereas the other breeds primarily in man-made containers and feeds mainly on humans. In the present work the former strain was used, and the culture was obtained from the Entomology Department at Rutgers University.

The ecological factors which were selected for special study were:

1. Food consumption during the larval stage (total and by instars), using dried brewer's yeast as the food source.

2. The minimum volume of water and the minimum surface area of water needed per larva to permit what appear to be normal growth and development.
3. In the case of Aedes aegypti L. to determine the effect of light on larval development by rearing under various light conditions.

REVIEW OF LITERATURE

The factors important for the completion of larval development in mosquitoes were presumed by Fay (1964) to be:

1. Volume of water
2. water temperature
3. Crowding
4. Quality and quantity of the food

Trager (1935), working with Aedes aegypti L., showed that normal larval development could be obtained by appropriate feeding with autoclaved yeast and liver extract. He also showed that autoclaved yeast by itself was inadequate but that the addition of the ammonium salt of folic acid to the yeast gave satisfactory growth. Similar results were also reported by Goldberg and Lavoipierre (1944). Goldberg and De Meillon (1948), also working with Aedes aegypti L. showed that deficiencies in proteins and amino acids prevented larvae from reaching the second instar.

Buddington (1941) studied the development of Culex pipiens L. in pond water. He found no satisfactory growth in larvae reared in filtered and autoclaved pond water. He also developed a synthetic medium which included 0.1 per cent oyster meal protein and 0.1 per cent Osborne-Mendel salt mixture in which the larvae grew rapidly at a temperature of 28°C. and a pH of 6.8 to 7.0.

Matheson and Hinman (1928) reported some development of Aedes aegypti L. in filtered pond water. They concluded that solutes and colloids are not by themselves completely adequate for normal larval growth. Casannges, McGovran and Chiles (1947) put about 2000 Aedes aegypti L. larvae in two liters of pond water, along with 50 mg. of powdered brewer's yeast and a piece of whole wheat bread as food. As the larvae grew, the level of feeding was increased. They concluded that 500 first instar larvae consumed 40 to 50 milligrams of food and that 500 fourth instar larvae consumed 1.1 to 1.2 grams. Kenneth (1950) in hybridization experiments used 0.1 gram of food for each egg raft. Morlan, Hayes and Shoof (1963) found that larval growth could be obtained in mass production by feeding 2, 3, 4 and 6 milligrams of laboratory chow per larva on 1, 2, 3, and 4 to 6 days at a suitable temperature. The best results were obtained with food that passed a 40-mesh screen. Marcovitch (1960) found that larvae of Aedes aegypti L. could pupate in five days under favorable conditions. He also concluded that their normal food consists of microorganisms, particularly bacteria, yeast and protozoa, along with any detritus which may be picked up by the mouth brushes in filtering the water.

It has long been known that a low level of feeding makes it possible for larvae to grow, but not to complete their development (Shanon and Putnam, 1934). Also, very

heavy larval populations are found in nature; 233 larvae in a pint of water were once found in Nigeria (Horsfall, 1928). Shanon (1931) reported that Aedes aegypti L. larvae were quite sensitive to both light and vibration, and that they were negatively phototropic. However, Jobling (1937) reported that light had little or no effect on the rate of development of these larvae.

Most of the work on mosquito larval development has been related to temperature. Headlee (1942) found the minimal effective temperature to be in the range of 11.5° to 12.5°C. He also reported that with temperatures of 15.9°, 17.6° and 19.8°C complete larval development required 29, 24 and 19 days respectively; also MacGregor (1915) had reported similar findings. Bar Zeev (1957) found that Aedes aegypti L. can survive only briefly at temperatures from 6.0 to 7.7°C. He also showed that any temperature above 31.9°C adversely affected the development of the larvae.

MATERIALS, METHODS AND RESULTS

Three different experiments were carried out to determine the influence of certain factors on the development of two species of mosquito larvae, Culex pipiens L. and Aedes aegypti L. The factors specifically investigated were food, space and light.

A preliminary study was made to determine the level of solids present in natural surface water in which mosquitoes breed. In August, 1965 four one-liter samples were taken from different parts of a pond in which mosquito larvae were living. (In these preliminary tests only larvae of Culex pipiens L. were used.) The larvae in it were counted at once and removed, after which subsamples were centrifuged for 15 minutes at a speed of 1500 r.p.m. to separate the solids from the water. The water was drained off and the residues dried for 48 hours at about 28°C., after which they were weighed. Table 1 gives the results obtained from this preliminary study.

Table 1. Amount of solids and number of mosquito larvae present in surface water collected from a mosquito breeding pond near Amherst, Massachusetts, August, 1965.

Replicate no.	No. larvae present in one liter	Weight of residues centrifuged out	Average amount of solids/larva
1	74	.11 gms.	1.4 mg.
2	67	.09 gms.	1.3 mg.
3	58	.10 gms.	1.7 mg.
4	60	.08 gms.	1.4 mg.
Average	64.7	.095 gms.	1.4 mg.

Nutrition Experiments

In the nutrition experiments we were concerned with:

1. The minimum amount of food needed for normal development of larvae of Culex pipiens L and Aedes aegypti L respectively.
2. The minimum amount of food consumed by a larva in each instar.

Yeast was added as food to distilled water, with the realization that this would lead to bacterial growth if other conditions were favorable and sufficient time was allowed. In a preliminary experiment on food requirements,

which was continued for 48 hours at room temperature (about 24°C.), the following comparison was made, using four replicates of each treatment:

1. 250 ml. distilled water plus 250 mgs. yeast, plus 120 third instar larvae.
2. Same as above, except without mosquito larvae.

At the end, these samples were centrifuged at a speed of 1500 r.p.m. for 15 minutes, after the larvae had been removed. The results of this preliminary experiment are shown in Table 2.

Table 2. Results from preliminary experiment on larval food requirements in which populated and non-populated environments were compared.

Treatments	Replications (weight in gms.)				
	1	2	3	4	Average
250 ml. distilled water plus 250 mg. yeast plus 120 third instar larvae	.0550	.0381	.0467	.0521	.0479
Same as above, except that no larvae were added	.247	.248	.250	.244	.247

A second preliminary experiment on food was carried out to give a better basis for further work. In this case, the following treatments were employed, again at room temperature for 48 hours. The samples were treated the same

as in the first preliminary experiment.

1. 250 ml. distilled water plus 250 mg. yeast plus 120 third instar larvae.
2. Same as above except that no larvae were added.
3. 250 ml. of natural pond water plus 120 third instar larvae.
4. Same as No. 3, except that no larvae were added.

The results from this second preliminary experiment on food requirements are presented in Table 3.

Table 3. Results from preliminary experiment on larval food requirements in which populated and non-populated environments were compared.

Treatments	Net weights of dried residues:
250 ml. distilled water plus 250 mg. yeast plus 120 third instar larvae	.0550 gm.
Same as above but no larvae added	.2455 gm.
250 ml. water from a mosquito breeding pond plus 120 third instar larvae	.0184 gm.
Same as above but no larvae added	.2182 gm.

Table 3 indicates that the average food consumption per larva during the third instar was approximately 1.6 mg.

On the basis of these preliminary findings, the main experiments on the food requirements of mosquito larvae were set up.

In the main nutrition experiment six different dosage levels were employed, using yeast in distilled water as the culture medium and petri dishes as containers. The dosage range was from .0078 to 150 gms. of yeast for 20 larvae. The precise dosages and the calculated milligrams per larva are shown below:

.150	grams	of	yeast	which	represents	7.5	milligrams	per	larva
.125	"	"	"	"	"	6.25	"	"	"
.062	"	"	"	"	"	3.1	"	"	"
.031	"	"	"	"	"	1.55	"	"	"
.015	"	"	"	"	"	0.75	"	"	"
.0078	"	"	"	"	"	0.39	"	"	"

Each treatment was replicated three times and two different volumes of water were used per larva (2 and 3 milliliters). Also extra larvae were kept at each food level so that any dead larvae appearing in the experimental containers could be replaced. Water and food were changed after all the larvae reached the next instar, and after each change, the dishes were thoroughly cleaned with soap and hot water before further use. The temperature averaged about 24°C. This experiment was conducted separately with larvae of Culex pipiens L. and Aedes aegypti L.

After each run the residues were centrifuged, dried at room temperature, and weighed. By subtraction from the original totals it was possible to determine the approximate average food consumption of each larva during each instar. When these values were available, the total food consumption for the entire larval stage could be determined by addition.

Records were also kept during the course of the nutrition experiments to determine the rates of development of the larvae on different food levels. Finally, the pupae were dried and weighed to show any weight differences that might result from the different feeding levels. Only the older pigmented pupae were preserved (in 75% alcohol) for subsequent weighing. They were dried at room temperature before weighing so that average pupal weights could be calculated for each feeding level. The results from the main nutrition experiments are shown in Tables 4a, 5a, 6a, for Culex pipiens L., and in Tables 4b, 5b and 6b for Aedes aegypti L.

Table 4a. Rate of development of Culex pipiens L. larvae (time required to pupate expressed in days) at different levels of dried brewer's yeast as food.

Level of food	Volume of liquid medium:							
	2 ml./larva:				3 ml./larva:			
	Replicates:				Replicates:			
	1	2	3	Average	1	2	3	Average
.150 gm./20 larvae	10	9	10	9.7	10	10	10	10
.125 gm./20 larvae	9	10	11	10	9	10	10	9.7
.062 gm./20 larvae	11	10	10	10.3	10	10	10	10
.031 gm./20 larvae	11	12	10	11	9	11	10	10
.015 gm./20 larvae	14	16	16	15.3	14	14	16	14.7
.0078 gm./20 larvae	18	16	18	17.3	16	17	18	17

Table 4b. Rate of development of Aedes aegypti L. larvae (time required to pupate expressed in days) at different levels of dried brewer's yeast as food.

Level of food	Volume of liquid medium:							
	2 ml./larva:				3 ml./larva:			
	Replicates:				Replicates:			
	1	2	3	Average	1	2	3	Average
.150 gm./20 larvae	8	9	9	8.7	9	9	8	8.7
.125 gm./20 larvae	9	8	9	8.7	8	8	9	8.3
.062 gm./20 larvae	8	9	8	8.3	11	10	10	10.3
.031 gm./20 larvae	9	10	11	10	11	11	11	11
.015 gm./20 larvae	13	14	13	13.7	13	14	14	13.7
.0078 gm./20 larvae	14	14	15	14.3	14	13	15	14

When the results presented in Tables 4a and 5a were tested for significance by the analysis of variance procedure, no significant differences were found between the four highest feeding levels (.150, .125, .062 or .031 gram for 20 larvae) for either species. However, there was a difference between these four highest levels and the two lowest feeding levels (.015 and .0078 gram for 20 larvae) which was significantly different for each species at the five per cent level of probability.

Pupal dry weights were also recorded as a second measure of the effects of the different feeding levels on larval growth in mosquitoes. These results are presented in Tables 5a and 5b.

Table 5a. Total weights of 30 Culex pipiens L. pupae reared on five different levels of yeast as food.

Level of food	Volumes of liquid medium:		
	2 ml./larva	3 ml./larva	Total
.150 gm./20 larvae	.0189 gm.	.0172 gm.	.0361 gm.**
.125 gm./20 larvae	.0191 gm.	.0167 gm.	.0358 gm.**
.062 gm./20 larvae	.0144 gm.	.0149 gm.	.0293 gm.**
.031 gm./20 larvae	.0126 gm.	.0126 gm.	.0252 gm.**
.015 gm./20 larvae	.0092 gm.	.0110 gm.	.0202 gm.
.0078 gm./20 larvae	.0088 gm.	.0077 gm.	.0165 gm.
Total	.0830 gm.	.0801 gm.	.1631 gm.

** significant difference at .01 level
 LDS .05 = .0050064 LDS .01 = .0069280 gm.

The results for Culex pipiens L. in Table 5a show significant differences in pupal weights. First, the pupae resulting from the four highest levels of larval feeding (.150, .125, .062 and .031 gm./20 larvae) were significantly heavier than those from the two lower feeding rates (.015 and .0078 gm./20 larvae) at the one per cent level of

significance. Second, the pupae from the two heaviest levels of larval feeding (.150 and .125 gm./20 larvae) were significantly heavier at the five per cent level than those from the next two feeding levels (.062 and .031 gm./20 larvae). Third, pupae from larvae fed at the .031 level were significantly heavier than those from the two lowest levels (5% for .015 and 1% for .0078 gm./20 larvae). Fourth, there was no significant difference in pupal weights between those reared on the two lowest levels of larval food, i.e., .015 and .0078 gm./20 larvae.

Table 5b. Total weights of 30 Aedes aegypti L pupae reared on five different levels of yeast as food.

Level of food	Volumes of liquid medium:		
	2 ml./larva	3 ml./larva	Total
.150 gm./20 larvae	.0172 gm.	.0168 gm.	.0340 gm.**
.125 gm./20 larvae	.0166 gm.	.0178 gm.	.0344 gm.**
.062 gm./20 larvae	.0151 gm.	.0166 gm.	.0317 gm.**
.031 gm./20 larvae	.0135 gm.	.0136 gm.	.0271 gm.**
.015 gm./20 larvae	.0102 gm.	.0084 gm.	.0186 gm.
.0078 gm./20 larvae	.0073 gm.	.0070 gm.	.0143 gm.
Total	.0799 gm.	.0862 gm.	.1661 gm.

** significant at .01 level

LDS .05 level = .0034236 gm. LDS .01 level = .0073970 gm.

The results for Aedes aegypti L. presented in Table 5b are in general accord with those for Culex pipiens L. presented in Table 5a and discussed above. First, the four highest feeding rates gave pupae which were significantly heavier at the one per cent level than those from the two lowest larval feeding rates. Second, the pupae from larvae fed at the two highest levels (.150 and .125 gm./20 larvae) were significantly heavier than those fed at the fourth highest level (.031 gm./20 larvae) at the five per cent level of significance (actually these differences approached the one per cent level). Third, there was no significant difference in pupal weights in the two lowest larval feeding rates (.015 and .0078 gm./20 larvae).

Larval mortalities in the nutrition experiments are presented graphically in Figures 1 and 2. The general range was from about five per cent at the highest feeding rate to about twenty per cent at the lowest level of larval feeding. The increase in mortality from the highest to the lowest larval feeding level was remarkably consistent for both species.

The final phase of the larval feeding studies dealt with the food consumed at the six different feeding levels in which the records were kept by instars. These results are presented in Tables 6a and 6b.

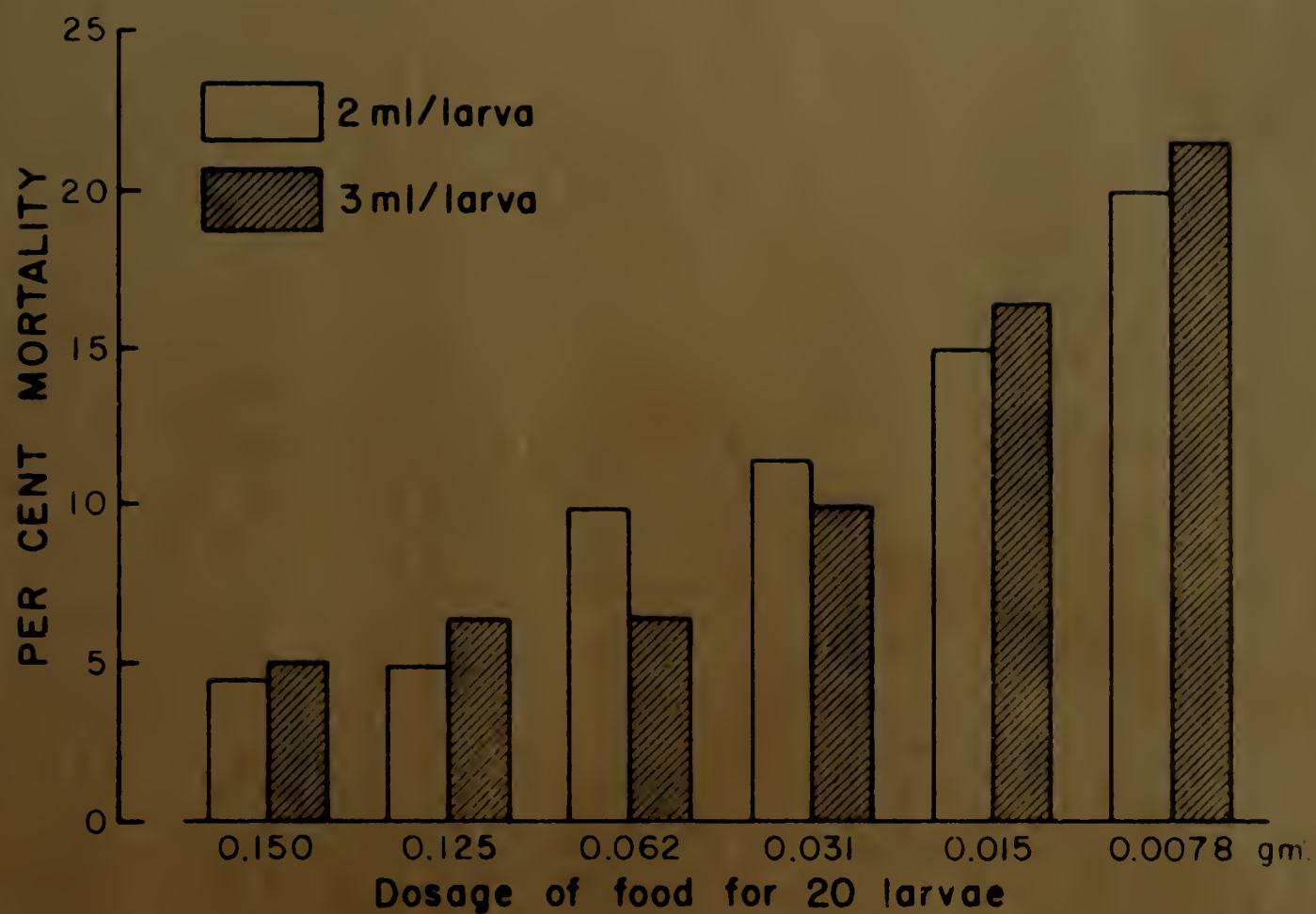


Fig.-1. Larval mortalities of Culex pipiens (L) at different feeding levels.

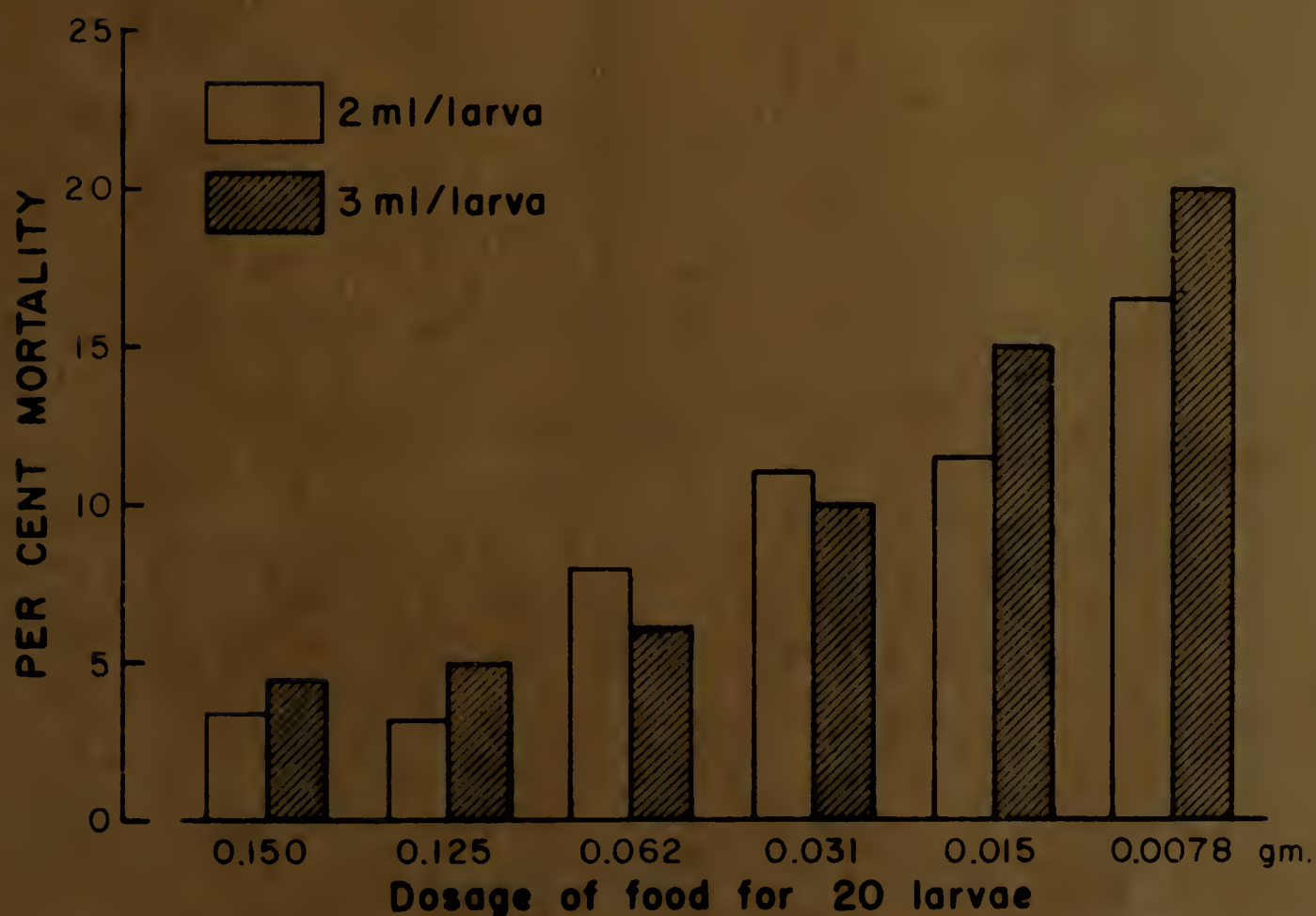


Fig.-2. Larval mortalities of Aedes aegypti (L) at different feeding levels.

Table 6a. Calculated food (dried brewer's yeast) consumption per larva of Culex pipiens L. by instars (expressed in milligrams per larva).

Level of food	Volume of liquid medium:				
	2 ml./larva:		3 ml./larva:		
	Instars:				Total
	1	2	3	4	
.150 gm./20 larvae	2.4	2.6	2.7	2.9	10.6
.125 gm./20 larvae	1.5	2.5	2.6	2.8	9.4
.062 gm./20 larvae	1.8	2.6	2.7	2.7	9.8
.031 gm./20 larvae	1.2	1.3	1.4	1.4	5.3
.015 gm./20 larvae	.53	.70	.73	.71	2.67
.0078 gm./20 larvae	.39	.39	.39	.39	1.46
	Instars:				Total
	1	2	3	4	
	2.0	2.4	2.6	2.8	9.8
	1.9	2.3	2.6	2.7	9.5
	1.8	2.4	2.7	2.9	9.8
	1.2	1.3	1.4	1.4	5.3
	.71	.73	.75	.75	2.94
	.39	.39	.39	.39	1.46

The following generalizations can be made as a result of the larval feeding experiments reported in Tables 6a and 6b, and summarized graphically in Figures 1 and 2.

1. Under the conditions of these experiments the three highest larval feeding rates (.150, .125 and .062 gm./20 larvae) were adequate for good growth and apparently normal development.
2. The larval feeding rate of .031 gm./20 larvae appeared to be close to the breaking point between adequate and inadequate nutrition, but the rate of development was not reduced at this rate in comparison with the higher feeding levels.
3. The larvae at the two lowest feeding rates, which were considered inadequate (.015 and .0078 gm./20 larvae), were found to be smaller in size than those on the higher feeding levels. Also these larvae were observed to be more active than the larvae that were more adequately fed.

Surface and Volume Experiments

The object of the second set of experiments was to determine the approximate minimum requirements of mosquito larvae for both volume and surface area of water. In other words, how little water and/or how small a surface area per larva can be tolerated without appreciably prolonging the larval period, decreasing the size of the larvae or reducing

the weight of the pupae? Again a preliminary experiment was carried out which was followed by a more comprehensive main experiment.

In the preliminary experiment 4-dram homeopathic vials were used as containers. The volumes employed were 1, 5, and 10 ml. per vial respectively, and the larval numbers were 1, 2, 3, and 5 per vial respectively. Four replications were used for each combination of volume and larval number. Dried brewer's yeast was used as food, and distilled water as the medium. The following amounts of food were provided for the four instars:

First instar	3.0 mg.
Second instar	3.5 mg.
Third instar	4.0 mg.
Fourth instar	4.5 mg.

The tabulation below shows the four surface areas per larva employed and the volume of water per larva at each of the three levels.

No. larvae per vial	Surface area per larva	Volume of water per larva:		
		1 ml./vial	5 ml./vial	10 ml./vial
1	201 mm ²	1.00 ml.	5.00 ml.	10.0 ml.
2	100 mm ²	.50 ml.	2.50 ml.	5.0 ml.
3	67 mm ²	.33 ml.	1.67 ml.	3.3 ml.
4	40 mm ²	.20 ml.	1.00 ml.	2.0 ml.

The data for this surface-volume experiment were collected so as to show the numbers of larvae which reached the pupal stage. These results are presented in Table 7.

Table 7. Larval mortality of Culex pipiens L. and Aedes aegypti L., reared in different combinations of surface area and volume of water (expressed in per cent).

Volume and surface per larva		Larval mortalities:					
Volume	Surface	Aedes aegypti L:			Culex pipiens L:		
10.0 ml.	201 mm ²	0	0	0	0	0	0
5.0 ml.	100 mm ²	0	0	0	0	0	0
3.3 ml.	67 mm ²	0	0	0	0	0	0
2.0 ml.	40 mm ²	0	0	0	0	0	0
5.0 ml.	201 mm ²	0	0	0	0	0	0
2.5 ml.	100 mm ²	0	0	0	0	0	0
1.66 ml.	67 mm ²	0	0	0	0	0	0
1.0 ml.	40 mm ²	0	0	0	0	0	0
1.0 ml.	201 mm ²	0	0	0	0	0	0
5.0 ml.	100 mm ²	0	0	0	0	0	0
.33 ml.	67 mm ²	33.3	33.3	33.3	66.6	66.6	33.3
.02 ml.	40 mm ²	80.0	60.0	80.0	80.0	80.0	80.0

Table 7 shows that a surface area of only 40 square millimeters is sufficient for larval survival if the volume is adequate (1.0 ml. or more per larva). The only other mortalities shown in Table 7 were with a surface of 67 square millimeters where the volume was inadequate (only .33 ml. per larva).

In the main experiment on volume and surface relationships the same general procedure was followed, but petri dishes were used as containers in order to increase the number of larvae. The larval numbers used were 57, 76, and 92; the surface areas per larva were 40, 30, and 25 square millimeters; and the volumes per larva 1.0 and 0.5 milliliters. Yeast was used at the same levels as in the first experiment, and both food and water were changed at 24-hour intervals. The experiments were run at room temperature, i.e. about 25°C. All containers were cleaned with soap and hot water before they were used again.

The main experiments on surface-volume relationships yielded the same kind of data on successful pupation as did the earlier experiments. The fully formed (pigmented) pupae were preserved in 75 per cent alcohol until the experiments were completed, after which they were air dried and weighed to obtain average pupal weights.

In the more detailed experiment on surface and volume relations with larval development, both species were

exposed to three surface areas per larva (40, 30, and 25 square millimeters), and two volumes per larva (0.5 and 1.0 milliliters). The food supply was adequate in all cases. These results are presented in Tables 8a and 8b in the form of air dried pupal weights.

Table 8a. weights of 40 air dried Aedes aegypti L. pupae in experiment on surface and volume (weights expressed in grams).

Surface per larva	Volume of liquid medium:				Total weights		
	0.5 ml./larva		1.0 ml./larva		.5 ml. /larva	1.0 ml. /larva	Total
40 mm ²	.0241	.0250	.0242	.0251	.0976	.0983	.1959**
	.0244	.0241	.0248	.0242			
30 mm ²	.0236	.0234	.0240	.0240	.0939	.0956	.1895**
	.0230	.0239	.0238	.0238			
25 mm ²	.0194	.0197	.0198	.0196	.0774	.0804	.1578
	.0184	.0199	.0206	.0204			
				Totals	.2689	.2743	.5432

** significant difference at .01 level

Table 8b. Weights of 40 air dried Culex pipiens L. pupae in experiment on surface and volume (weights expressed in grams).

Surface per larva	Volume of liquid medium:				Total weights		
	0.5 ml./larva		1.0 ml./larva		.5 ml. /larva	1.0 ml. /larva	Total
40 mm ²	.0257	.0256	.0244	.0250	.1017	.1010	.2027**
	.0250	.0254	.0256	.0260			
30 mm ²	.0240	.0248	.0243	.0246	.0982	.0990	.1972**
	.0251	.0243	.0251	.0250			
25 mm ²	.0215	.0217	.0218	.0214	.0855	.0866	.1721
	.0213	.0210	.0215	.0219			
	Totals				.2854	.2866	.5720

** significant difference at .01 level

The results presented in Tables 8a and 8b indicate that the combination of 40 square millimeters of surface area and 1.0 milliliter of water per larva was the nearest to the optimum ratio tried. It must be remembered, however, that increasing the number of larvae calls for an increase in both the surface area and volume of the medium, in order to avoid disturbances from excessive larval movements. There is also an indication that surface area may be more influential or important to Culex pipiens L. than to Aedes aegypti L. Possibly Culex larvae are more active in searching for food, resulting in more disturbance.

Statistical analysis of the results presented in Table 8a shows significantly different results at the one per cent level for the two larger surface areas (40 and 30 mm²) at both volumes, as compared with a surface area of only 25mm² per larva for Aedes aegypti L. Although the difference between the 40 and 30 mm² surface areas does not appear to be great, the analysis shows that it approaches significance at the five per cent level.

Analysis of the Culex pipiens L. data shown in Table 8b gave results similar to those for Aedes aegypti L. discussed above, except that in Table 8b the difference in pupal weights for the two higher surface areas and the lower one is significant at the five per cent level of probability.

Light and Darkness Experiment

In some of the preliminary experiments already reported, it was observed that Aedes aegypti L. appeared to develop at somewhat different rates when the lighting was not the same for the extra containers as for the experimental containers. Therefore it seemed logical to carry out a third primary experiment with light as the variable factor, using Aedes aegypti L. as the test insect. In this experiment the volume, surface area, and food per larva were set at rates that had been found to be favorable to good growth and normal pupation.

Again four replications of each treatment were employed and the experiment was run at room temperature. Petri dishes were used as containers. The lighting conditions employed were 24, 8 and 0 hours per day respectively. The light source was a 15-watt GE fluorescent tube in a desk lamp. Darkness was maintained by keeping the containers in a cage covered with black polyethylene film which was opened only to change the food and water and to make the necessary observations. The rates of larval growth, per cent pupation, and pupal dry weights were all determined as they had been in the previous experiments. The results of this lighted versus darkness experiment are presented in Figure 3 and in Tables 9 and 10.

Figure 3 clearly shows that pupation was earlier in constant darkness than in either constant light or eight hours of light per day. In constant darkness 51 to 56 per cent of the larvae pupated by the eighth day, whereas under the other conditions only 1 to 18 per cent pupation took place by the eighth day. The difference between constant light and eight hours of light per day was not great, although pupation under the former condition tended to be very slightly advanced over the latter.

In order to analyze the light experiment results reported in Figure 3, the original data were subjected to the square root transformation procedure, the results

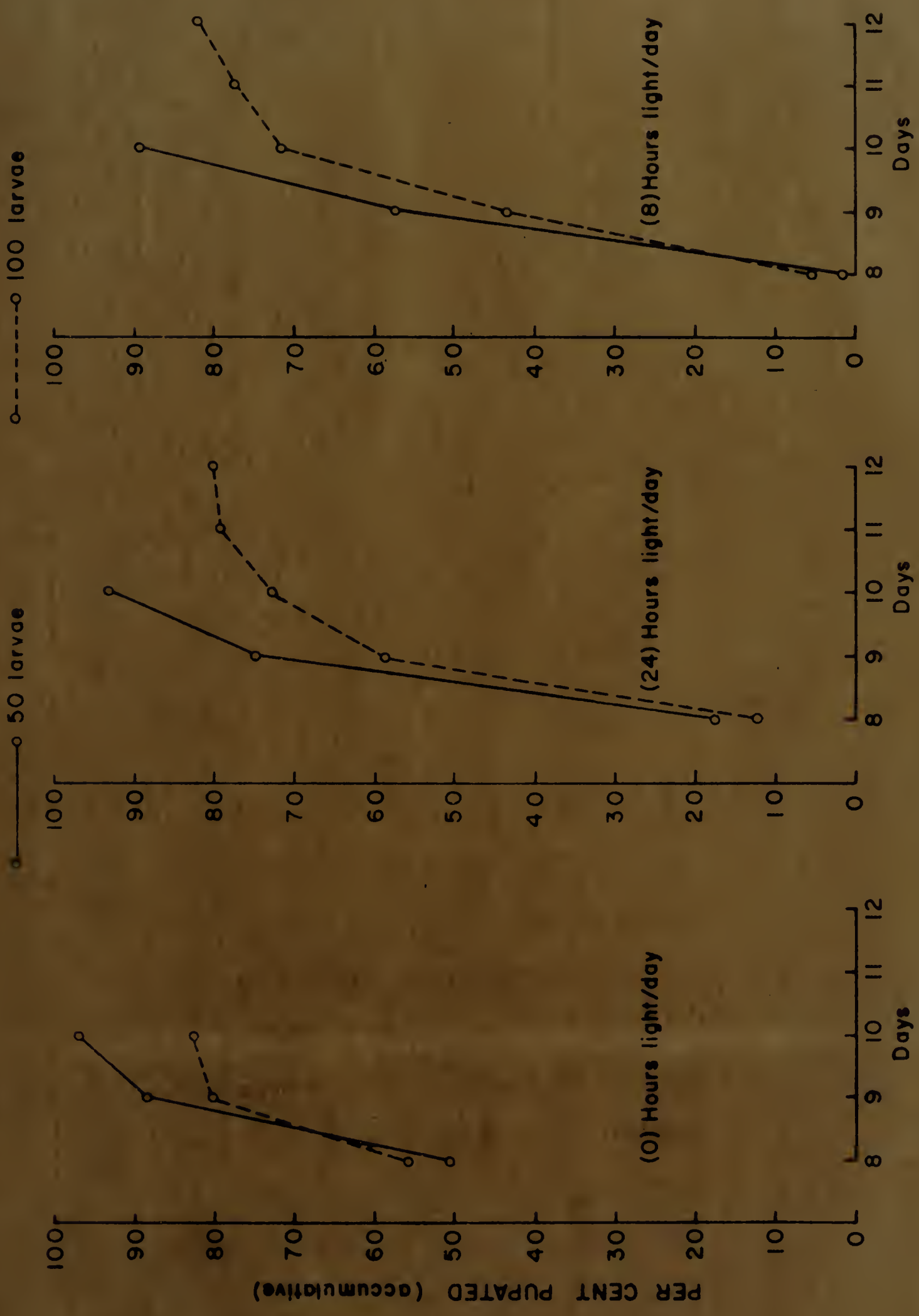


Figure 3. Effects of three different lighting conditions on rate of larval development (days to pupation) of *Aedes aegypti* larvae.

of which are shown in Table 9. The original data on which these values are based are presented in the appendix.

Table 9. Square root transformation values of the numbers of Aedes larvae which pupated in 8, 9, 10, 11 or 12 days.

Day	0 hours light		8 hours light		24 hours light		Totals
	Population		Population		Population		
	50 larvae	100 larvae	50 larvae	100 larvae	50 larvae	100 larvae	
8	28.54	30.10	4.58	9.50	16.85	13.65	103.22
9	24.80	19.52	30.11	24.79	30.20	27.49	156.91
10	11.30	6.83	22.93	21.20	17.06	14.99	94.31
11	2.84	2.84	2.84	10.04	2.84	10.39	31.79
12	2.84	2.84	2.84	9.28	2.84	4.73	25.37
Totals	70.32	62.13	63.30	74.81	69.79	71.25	
	<u>132.45</u>		<u>138.11</u>		<u>141.04</u>		411.60

The differences in the time periods required for pupation were highly significant when the constant darkness treatment was compared with the other two treatments. However, there were no significant differences between the total number of larvae which finally pupated under the three lighting conditions.

Table 10. Dry weights of Aedes pupae in experiment to determine the influence of light on larval development.

Pupal dry weights in grams:									
Hours/day of light	Replicates for 50 larvae:			Replicates for 100 larvae:					
	1	2	3	Subtotal	1	2	3	Subtotal	Grand Total
24	.0289	.0286	.0290	.0865	.0251	.0253	.0257	.0761	.1626
8	.0291	.0293	.0289	.0873	.0255	.0258	.0256	.0769	.1642
0	.0317	.0305	.0307	.0929	.0274	.0281	.0279	.0834	.1763**

** indicates significance at the one per cent level of probability

The pupal dry weights presented in Table 10 confirm Figure 3 and Table 9 in showing little difference between constant light and eight hours of light per day, but a highly significant difference in pupal weights where the larvae were kept in constant darkness, as compared with those reared in either eight hours or twenty-four hours of light per day.

SUMMARY AND CONCLUSIONS

Mosquitoes are important pests of man due to the direct annoyance and irritation they cause, as well as the various diseases they transmit. Mosquito control is complicated and expensive, and any information that might have a practical bearing on the control of these pests is therefore of interest. The present study was carried out to gain additional information on the ecology of the larval stages of two important species of mosquitoes.

The experimental work described was started in July, 1965, and terminated in April, 1966. Most of it was carried out in the laboratory but some of the initial work was done in the field. Three different aspects were considered in the following sequence:

1. Larval food requirements of Culex pipiens L. and Aedes aegypti L.
2. Water volume and surface area requirements of Culex pipiens L. and Aedes aegypti L. larvae.
3. Influence of light on larval development of Aedes aegypti L.

Nutrition Experiments

The feeding experiments were carried out at room temperature, using dried brewer's yeast as food. The levels used in the main experiments were .150, .125, .062, .031, .015 and .0078 grams per 20 larvae. The data collected

showed: (1) speed of development, (2) per cent of larval mortality, and (3) average pupal weight in each of four replicates.

Dried brewer's yeast at the .031 gm./20 larvae level appeared to be near the borderline between adequate and inadequate nutrition under the conditions of these experiments. At this level the larval development of both species was fairly successful, but still not as good as that observed at the higher feeding rates. The larval mortalities at this level were intermediate (Figures 1 and 2), and the rate of larval development at .031 grams per 20 larvae was only slightly longer than for the three higher levels, (Tables 4a and 4b); however the average pupal weights were substantially higher at the heavier feeding levels than at .031 grams per 20 larvae (Tables 5a and 5b). On the other hand, all three of these criteria showed that the two lowest feeding levels (.015 and .0078 grams per 20 larvae) were inferior. Details are available in Tables 4a, 4b, 5a, and 5b, and in Figures 1 and 2.

The nutritional studies on mosquito larvae were carried a step farther in an attempt to approximate the food requirements by instars. Dried brewer's yeast was used as food and changed after each molt so as to reduce the effects of bacterial contamination. These results are shown in Tables 6a and 6b. They show less difference between the amounts of food consumed by the different larval instars

than one might predict, and also less than reported by Casanges, McGovran and Chiles (1947). Otherwise all of the results on larval nutrition appear to be in general accord with the information which was available from the literature.

Water Volume and Surface Area Experiment

The surface area and water volume experiments also involved both species of mosquitoes and were carried out at room temperature. The containers used were 4-dram homeopathic vials where these were suitable, and petri dishes where larger surface areas were required. The volumes used were 10.0, 5.0, 3.3, 2.5, 2.0, 1.6, 1.0, 0.5, 0.33 and 0.2 milliliters of distilled water per larva. The surface areas investigated were 201, 100, 67, 40, 30, and 25 square millimeters per larva. Table 8a and Table 8b present the combinations of volume and surface area in which larvae lived and developed. The most restricting combination shown was 0.2 milliliters of volume and 40 square millimeters of surface area per larvae. Substantial larval mortalities are also shown for a combination of 0.33 milliliter and 67 square millimeters, but at all of the less restricted combinations shown, larval survival was 100 per cent. Apparently a volume as low as one milliliter per larva, combined with a surface area of at least 40 milliliters per larva, is adequate for

successful development of both species. These results are presented in Table 9 and seem to be in general accord with the limited information found in the literature.

Light Experiment

The light experiment involved only Aedes aegypti L. larvae which were exposed to zero, eight, and twenty-four hours per day of artificial light respectively at room temperature. Dried brewer's yeast again served as food, and water volume and other factors were favorable for larval development. Aedes aegypti L. was selected for this part of the study because it appeared to show responses to light which were not observed for Culex.

The results of the light experiment are presented in Figure 3 and in Table 10. Obviously pupation came much earlier in darkness than with either eight or 24 hours of light per day. Also, the pupae produced in darkness were somewhat heavier than those reared under either of the other conditions. These results are contrary to those of Jobling (1937) who reported that light had little or no effect on the development of Aedes aegypti L. larvae. We have no basis for believing that this response to light is typical of mosquito larvae in general, or even of the larvae of any other species.

APPENDIX

ORIGINAL DATA: SPEED OF DEVELOPMENT OF MOSQUITO LARVAE OF
AEDES AEGYPTI L.

		Hours light						
		0		8		24		
		<u>Population</u>		<u>Population</u>		<u>Population</u>		
<u>Replicates</u>	<u>Day No.</u>	<u>50</u>	<u>100</u>	<u>50</u>	<u>100</u>	<u>50</u>	<u>100</u>	<u>Total</u>
1	8	29	52	1	5	6	12	105
	9	18	29	21	33	30	49	180
	10	1	1	18	30	11	11	72
	11				10		6	16
	12				3		1	4
								377
2	8	25	63	0	10	9	3	110
	9	21	19	30	38	22	50	180
	10	2	3	14	22	14	22	77
	11				5		6	11
	12				4		1	5
								383
3	8	22	53	0	5	12	18	110
	9	19	31	32	41	26	42	191
	10	8	3	16	27	8	15	77
	11				4		6	10
	12				6		2	8
								396
4	8	25	57	1	2	8	15	108
	9	18	16	30	40	36	46	186
	10	6	3	17	32	4	8	70
	11				5		7	12
	12				7		0	7
								383
		<u>194</u>	<u>330</u>	<u>180</u>	<u>329</u>	<u>186</u>	<u>320</u>	
Totals		<u>524</u>	<u>509</u>	<u>506</u>	<u>1539</u>			

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