FEASIBILITY OF USING *Toxorhynchites rutilus rutilus* (COQ.) IN THE CONTROL OF CONTAINER-BREEDING MOSQUITOES

By

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1977

ACKNOWLEDGEMENTS

The author wishes to acknowledge his sincere appreciation to Dr. J.A. Seawright, his research advisor, for his patience, assistance, support, and critical review of the dissertation. Special thanks are extended to Dr. D.W. Hall, his Committee Chairman, for advice and encouragement during the course of this study. To all the members of his Ph.D. Committee, the author expresses appreciation for their critical appraisal of the dissertation and their helpful comments.

The author wishes to express his gratitude to the staff of the U.S. Department of Agriculture Insects Affecting Man Laboratory, Gainesville -- in particular, Drs. D.E. Weidhaas and D. Haile, and C.E. Schreck, J.H. Gackson and M.Q. Benedict.

The author thanks Dr. R. Darsie, Center for Disease Control, USPHS, Atlanta, Georgia, for assistance on adult identification and J.S. Haeger, Vero Beach Laboratories, Inc., Vero Beach, Florida, for assistance with the colonization.

For his cooperation during the field trials, thanks are given to B.C. Lemont of the University of Florida Pest Control Auxillary.

Finally, deep gratitude is extended to his wife Debby, for her patience, encouragement, and aid during both the course of study and the preparation of this manuscript.

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FEASIBILITY OF USING Toxorhynchites rutilus rutilus (COQ.) IN THE CONTROL OF CONTAINER-BREEDING MOSQUITOES

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March 1977

Chairman: D.W. Hall
Major Department: Entomology and Nematology

Toxorhynchites rutilus rutilus (Coq.) was successfully colonized and studied in the laboratory to determine the potential usefulness of this predatory species of mosquito as a biological control agent for container-breeding mosquitoes. When Tx. r. rutilus larvae were reared at 28 ± 1°C in individual containers with a surplus of larvae of Aedes aegypti (L.) as prey, the duration of the immature stages averaged 1.6, 15.6, and 6.0 days for eggs, larvae, and pupae, respectively. Contrarily, with mass rearing conditions, the duration of the larval stage was significantly reduced to 11.1 days and pupation was more uniform than in individual containers. Adult females survive for 7 wk in laboratory cages and oviposit an average of 1 egg/day. Fourth-instar larvae of Tx. r. rutilus can survive for about 2 months without food. Adult females preferred to lay their eggs in water previously used to rear Ae. aegypti.

For the first time, Tx. r. rutilus was reared on a non-living diet of TetraMin-Staple Food^{R}, a commercially available food for tropical fish. Larvae reared on TetraMin required an average development time of 107.5 days from egg to pupa compared to 15.6 days for larvae that were fed a diet of larvae of Ae. aegypti. The daily survival rate of larvae reared on the non-living diet was 0.9901 ± 0.0098, a value slightly less than the survival rate of 0.9973 ± 0.0075 for larvae fed Ae. aegypti. Pupae reared on the non-living diet were small (29.3 ± 3.6 mg) compared to a control group (50.0 ± 2.0 mg), but surprisingly, adult longevity and fecundity of the small adults did not differ significantly from the control. The significance of the above findings are discussed relative to the use of Tx. r. rutilus as a biological control agent for container-breeding mosquitoes.

Three hundred and fifty, 6-day old laboratory-reared Tx. r. rutilus adults were released into a sparsely wooded 13-acre residential area in Gainesville, Florida. Oviposition was monitored for 14 days using a grid of 64 oviposition traps located within the residential area and surrounding woods. Eighty percent of the ovitraps received eggs, and despite the migration of the females into the surrounding woods at a rate of about 7% per day, 64% of the eggs recovered were laid in the residential area. Equations were derived that allowed estimating the daily adult survival (S_a) and lifetime egg production per female released (F)

to be 0.79 and 3.99 eggs/female, respectively.

A deterministic computer model is presented detailing the interaction of the container-breeding mosquito Ae. aegypti and the larval predator Tx. r. rutilus. Results of simulation runs involving the release of Tx. r. rutilus adults indicate that predator releases resulting in 1 predator larva per container are sufficient to reduce Aedes adult density 75% in 20 days. The slow rate of immature predator development enables control to be maintained for several months. Simulations of predator release and the use of adulticides indicate that it is possible to obtain zero adult densities. Finally, the model indicates that the most important parameter determining the degree of control established is the distribution of predator eggs.

INTRODUCTION

Increased impetus to reconsider mosquito control methodologies involving biological control agents is being provided by the phenomenon of pesticide resistance. Toxorhynchites (Theob.) is a genus of large, brilliantly colored, non-biting mosquitoes that are predacious during the larval stage on certain mosquitoes which breed in discarded cans, bottles, tires, water cisterns and tree holes. The research reported herein was undertaken to evaluate the feasibility of using Toxorhynchites rutilus rutilus (Coq.) as a biological control agent against the yellow fever and dengue hemorraghic fever vector Aedes aegypti (L.). Each of the 4 subsequent chapters are papers currently being submitted to journals; they are complete in themselves. Each introduces itself, reviews the relevant literature, presents results of the work done and reports the significance of these findings relative to the use of Tx. r. rutilus in mosquito control.

LABORATORY COLONIZATION OF *Toxorhynchites rutilus rutilus* (COQ.)

Abstract

Toxorhynchites rutilus rutilus (Coq.) was successfully colonized and studied in the laboratory to determine the potential usefulness of this predatory species of mosquito as a biological control agent for container-breeding mosquitoes. When *Tx. r. rutilus* larvae were reared at 28 ± 1°C in individual containers with a surplus of larvae of *Aedes aegypti* (L.) as prey, the duration of the immature stages averaged 1.6, 15.6, and 6.0 days for eggs, larvae, and pupae, respectively. Contrarily, with mass rearing conditions, the duration of the larval stage was significantly reduced to 11.1 days and pupation was more uniform than in individual containers. Adult females survive for 7 wk in laboratory cages and oviposit an average of 1 egg/day. Fourth-instar larvae of *Tx. r. rutilus* can survive for about 2 months without food. Adult females preferred to lay their eggs in water previously used to rear *Ae. aegypti*.

Introduction

Extensive use of synthetic pesticides has resulted in the phenomenon of resistance to them. The ramifications of

this problem are providing increased impetus to reconsider mosquito control strategies involving biological control agents. Several of the more important mosquito vectors of human disease breed in discarded cans, bottles, tires, water cisterns and tree holes. Control of larvae of these species is difficult because the larval habitats are small, dispersed and often inaccessible. Mosquitoes of the genus Toxorhynchites are larval predators of container-breeding mosquitoes, and possibly could be highly effective control agents because the female Toxorhynchites might more efficiently find these breeding sites than the mosquito-control worker (Brown 1973). However, the literature is replete with examples where Toxorhynchites spp. failed to control prey species (Newkirk 1947; Paine 1934; Swezey 1930 and 1931; Williams 1931). This has been attributed to intrinsic factors such as long life cycles, low fecundity, and survival rate (Nakagawa 1963). Gerberg (1974) and Muspratt (1951) contend these shortcomings can be overcome with inundative releases of Toxorhynchites, a situation that upsets the normal predator-prey relationship.

Currently we are conducting research on the feasibility of using Toxorhynchites rutilus rutilus (Coq.) as a biological control agent against container breeding mosquitoes. The present paper summarizes our progress in laboratory studies and describes the colonization, mass rearing, and other aspects of the biology of this species.

The genus, Toxorhynchites, in North America north of

Mexico is represented by two subspecies (Jenkins 1949) and perhaps a third, the status of which is uncertain (Zavortink 1969). Tx. rutilus septentrionalis (Coq.) is known to occur in the Eastern United States north to New Jersey and Pennsylvania and west to the great plains of Kansas, Oklahoma, and Texas. Tx. r. rutilus is known only from the extreme Southeastern United States in peninsular Florida, southern and coastal Georgia and coastal South Carolina north to Myrtle Beach (Carpenter and LaCasse 1955). Intergrades occur in the zone of overlap of the ranges of these two subspecies (Jenkins 1949). The egg, larva, pupa, adult stages (Carpenter and LaCasse 1955; Dodge 1964), oviposition habits (Olinger 1957) and habitats (Seabrook and Duffey, 1946; Basham et al. 1947) of Tx. r. rutilus have been described.

Colonization

We collected Tx. r. rutilus eggs from cavities in various trees in Alachua County, Florida, during September and October 1975. Larvae were reared to the pupal stage individually in 8-dr glass vials. Each egg was individually set with approximately 250 first stage larvae or eggs of Aedes aegypti (L.); additional prey were added as required. The prey larvae fed on 5-10 mg of TetraMin^{R} (a commercially available tropical fish food) supplied every other day. Subterranean well water was used in all rearing. The photoperiod was 14 hr of subdued light : 10 hr dark. Reared

at 28 ± 1°C, the development time from egg to pupation for 156 individuals was 17.2 ± 3.3 days and total mortality was 8% (Table 1).

The first cohort of 150 pupae reared in the above fashion was placed in a 1 m x 1 m x 2 m high screened aluminum cage covered with a clear plastic film to maintain humidity. Water on sponge wicks, honey, apple slices and black, 0.5-liter glass jars half filled with water for oviposition sites were provided. The cage was within an environmentally controlled room lacking windows. Light was provided by 8 40-watt fluorescent tubes. The conditions were: photoperiod 14 hr light : 10 hr dark, temperature 24 ± 5°C, relative humidity (RH) 85 ± 10%. No attempt was made to simulate twilight. No mating was observed and no eggs were produced. No mortality was observed for the first 6 wk, but 75% died within the next 2 wk and no individual lived longer than 10 wk.

A second cohort replaced the first under similar temperature and humidity conditions and a number of things were tried in an attempt to induce mating. J.S. Haeger (personal communication) has had success in colonizing difficult species by using either singly or in combination the following: (1) plants within the cage to act as swarm markers; (2) twilight simulation using incandescent lights on a rheostat; (3) the addition of a second species (Ae. aegypti); and (4) casting shadows across the interior of the cage. Using the above techniques Haeger has been able to stimulate

Table 1. Duration of eggs, larval and pupal stages of Tx. r. rutilus and total numbers of Ae. aegypti larvae[a] eaten.

Stage	No. ($\bar{X}$ ± SD) prey devoured	Development time (days)
Egg		1.6 ± 0.55
Larvae		
1st	6.4 ± 4.2	1.2 ± 0.45
2nd	6.6 ± 2.8	2.6 ± 0.42
3rd	8.0 ± 2.0	3.8 ± 0.76
4th	73 ± 14	8.0 ± 1.58
Pupae		6.0 ± 0.82
Total	94 ± 15	23.2 ± 1.36

[a]1st stage larval predators offered 1st and 2nd instar prey; 2nd, 3rd and 4th instars offered 3rd and 4th instar prey.

mating and oviposition in Tx. r. rutilus. However, we tried his techniques with no success. Varying the numbers of Tx. r. rutilus within the cage (n = 25, 50, 100, or 150) was also unsuccessful.

A third cohort of about 30 adults was placed in a 0.5 m x 0.5 m x 0.5 m plexiglass (acrylic plastic) cage. The conditions were as for the first cohort. Mating was observed on the second day after emergence, and oviposition was observed 6 days postemergence. Females fly in a vertical circle several inches above the oviposition jar and eject the eggs singly onto the surface of the water (Olinger 1957). Sixteen females observed for a period of 3 wk after oviposition began to produce an average of 1.23 eggs/female/day. All the eggs were fertile but no ovarian cycle was immediately apparent. Using the techniques described above, Tx. r. rutilus has been maintained in the laboratory for 9 generations.

Rearing in outdoor cages was attempted during July and August 1976 in an attempt to increase egg production. The same 1 m x 1 m x 2 m high cage described previously but without the plastic covering was used. This cage was located within a longer 5.5 m x 7.3 m x 4.3 m screened building. The enclosures were situated under large oak trees which provided shade during the middle of the day. An average daily temperature of 29°C and RH of 85% were recorded inside the smaller cage. Various numbers of Tx. r. rutilus (n = 18, 30, 150, 250, or 450) were placed in the

inner cage with water wicks, honey, apple slices and oviposition jars (half filled with well water). At each density of adults, first matings and oviposition were observed at 2 and 6 days, respectively.

During the outdoor cage work, 15 females were removed after 12 days of being with 15 males and confined in individual containers supplied with honey, water wicks and oviposition jars. The containers, held at 28°C, RH of 85 ± 10% and a photoperiod of 14 hr light : 10 hr dark, were checked daily for oviposition over a period of 25 days. Thirteen (or 87%) of the females laid eggs. Fecundity was 0.83 ± 0.66 eggs/female/day, a value not significantly different ($p = 0.05$) from 1.23 eggs/female/day reported above for the indoor cage. The oviposition cycle can best be described as highly irregular. Three of the females laid 1-3 eggs daily, and 5 of them oviposited 5-10 eggs on successive days with 5-15 day intervals of no oviposition. A third group of 5 females was intermediate in behavior. As evidenced by the standard deviation, differences in total egg production between females were great. The egg fertility was virtually 100% suggesting that females do not oviposit unless they have mated.

Before colonization procedures were established for Tx. r. rutilus, induced mating was used to maintain laboratory stocks. Techniques described by Gerberg (1970) and Trimble and Corbet (1975) were successfully employed with the exception of ethyl ether being used as the anesthetic.

fertile eggs were laid 2 days after forced mating. Typically, however, oviposition stopped after only a few days.

The amount of time larvae of Tx. r. rutilus can withstand fasting may be an important parameter in biological control considerations. To investigate this, variously aged larvae and eggs were placed in individual vials containing clean well water and observed until death by starvation occurred. Larvae were fed the usual diet of Ae. aegypti before the test began. From the results in Table 2, one can readily see that the ability of Tx. r. rutilus larvae to survive fasting will indeed be of value in the use of this predator for control. The first two larval stages survived for about a week and third stage larvae lived for 18 days without food. The fourth larval stage was exceptional in its ability to withstand fasting, and this noteworthy fact is all the more important because this stage also is capable of eating more prey than the first 3 instars.

The existence of an oviposition stimulant, e.g., the presence or past presence of a prey species could be important in biological control by Tx. r. rutilus. A simple 2-choice test between well water and water which had been used in rearing Ae. aegypti from egg to pupa (hereafter referred to as colony water) was conducted to determine whether Tx. r. rutilus preferred one or the other as an oviposition media. Four pairs of 0.5-liter black oviposition jars were placed in the 1 m x 1 m x 2 m high cage under

Table 2. Length of larval life of Tx. r. rutilus when deprived of food.

Larval stage in which starvation began	Age at beginning of test (days)	Days before death	No. individuals observed
1st	0	6.9 ± 0.3	9
2nd[a]	4&5	8.4 ± 0.5	10
3rd[b]	9&10	18.0 ± 6.5	10
4th[b]	13	59.0 ± 22.4[c]	8

[a]Temperature = 27°C.

[b]Temperature 27°C during first 11 days then raised to 30°C.

[c]One individual fasted 88 days.

the previously described outdoor conditions. Each pair consisted of 1 jar half full of well water and 1 jar half full of colony water. During the test, the colony water did not contain any Ae. aegypti. Each pair was placed in a corner of the cage floor. Daily, the positions of the jars within each pair were alternated to nullify any positional effects. At 3-day intervals, the water in each of the 8 jars was replaced with new well or colony water as appropriate. The cage contained approximately 750 adults (ca. 375 females). The number of eggs in each type of water was recorded daily for 6 days. The mean daily oviposition in the jars containing well water and colony water was 74 ± 47 eggs/day and 247 ± 87 eggs/ day, respectively. The difference between the 2 means is highly significant (t-test).

The preference of Tx. r. rutilus females to oviposit in the polluted water can be quite important in regard to using this predator for control. Although the need for more work is indicated, it appears that the females prefer to oviposit in water similar to that found in natural settings (treeholes, discarded trees, etc.) rather than in containers (water bottles, rain barrels, and cisterns) commonly employed to hold water for household uses.

Mass Rearing

Since the rearing of Tx. r. rutilus in individual vials is a laborious, impractical process, experiments were

conducted to determine the feasibility of mass production. Cannibalism is the principle problem encountered in mass rearing Tx. r. rutilus in the same container; therefore, all of these trials were conducted in complete darkness. Fourteen plastic trays (38 cm x 51 cm x 10 cm) were filled with well water to a depth of 4 cm, and the water temperature was maintained at 28 ± 0.3°C. To these trays were added 110 Tx. r. rutilus eggs (less than 24 hr old), ca. 10,000 Ae. aegypti eggs and 1.7 g TetraMin. The same day the Tx. r. rutilus eggs are set, a second identical tray is set with ca. 10,000 Ae. aegypti eggs and 1.7 g TetraMin. Each tray receives an additional 1.7 g TetraMin on alternate days. The second tray containing Ae. aegypti is added to the first tray containing Ae. aegypti and Tx. r. rutilus 8 days after the trays were set. Overcrowding and underfeeding the Ae. aegypti result in a mixture of third and fourth instars being added to the Tx. r. rutilus. Seventy ± 6% of the Tx. r. rutilus survived to the pupal stage with cannibalism being the most likely cause of mortality. The initial larval density of 18 cm^2/larva changed to 25 cm^2/larva at pupation. Reared in this manner, the average development time from egg to pupation was 12.66 ± 1.22 days for 547 larvae. The difference between length of development (egg to pupa) when 156 larvae were reared individually (17.99 ± 3.31 days) and when reared in mass is highly significant (t-test), but the reason for this observation is not readily apparent.

LABORATORY REARING OF *Toxorhynchites rutilus rutilus* (COQ.) ON A NON-LIVING DIET

Abstract

For the first time, *Toxorhynchites rutilus rutilus* (Coq.), a predatory species of mosquito, was reared on a non-living diet of TetraMin-Staple Food®, a commercially available food for tropical fish. Larvae reared on TetraMin required an average development time of 107.5 days from egg to pupa compared to 15.6 days for larvae that were fed a diet of larvae of *Aedes aegypti* (L.). The daily survival rate of larvae reared on the non-living diet was 0.9901 ± 0.0098, a value slightly less than the survival rate of 0.9973 ± 0.0075 for larvae fed *Ae. aegypti*. Pupae reared on the non-living diet were small (29.3 ± 3.6 mg) compared to a control group (50.0 ± 2.0 mg), but surprisingly, adult longevity and fecundity of the small adults did not differ significantly from the control. The significance of the above findings are discussed relative to the use of *Tx. r. rutilus* as a biological control agent for container-breeding mosquitoes.

Introduction

Toxorhynchites (Theob.) is a genus of large, non-biting

mosquitoes that are predacious during the larval stages on certain mosquitoes which breed in discarded cans, bottles, tires, water cisterns and treeholes. Brown (1973) considers the genus to be sufficiently promising as a biological control agent of artificial-container breeding mosquitoes to warrant continued research toward this end. In this connection, inundative release of adult Toxorhynchites has been proposed as necessary to upset the normal predator-prey relationship, thus effecting control (Gerberg, 1974; Muspratt 1951). Inundative releases imply the mass rearing of large numbers of mosquitoes and in determining the practical utility of Toxorhynchites as a biological control agent, the cost of mass rearing could be as important a parameter as the biological aspects of the mosquito. In a separate report, Focks and Seawright (1977) wrote that one Tr. r. rutilus larva requires ca. 100 Ae. aegypti as food; thus, a rather large colony of Ae. aegypti would be required for the mass production of Tx. r. rutilus. In view of the expense involved in maintaining a large colony of mosquitoes as a prey species, the author is currently involved in a study directed toward the development of alternative food sources for Tx. r. rutilus. In the present paper the author presents the results of investigations into the feasibility of using a non-living diet. The fecundity, daily adult survival, development time of immature stages and pupal weights are reported for Tx. r. rutilus reared solely on TetraMin^{R} and compared with observations on

larvae fed solely on larvae of Aedes aegypti (L.).

Materials and Methods

'TetraMin Staple Food' (manufactured in West Germany) is a dried, flaky material consisting of fish and shrimp meal, oat flour, fish liver, squid, fish roe, kelp, mosquito larvae, brine shrimp, aquatic plants, agar agar, chlorophyll and carotene.[1] By weight it is 45% crude protein, 5% fat, 6% fiber and 44% water. It was selected for these experiments for the following reasons: (1) Considering the diversity of substances making up TetraMin, it was deemed reasonable that TetraMin contained the required nutrients for a predator; therefore, if Tx. r. rutilus failed to develop on TetraMin, the feasibility of rearing this predator on a non-living diet would be dubious. (2) TetraMin has been used as the sole food source in rearing other mosquitoes, e.g., Culex spp., Aedes spp. and Anopheles spp. (Pappas, 1973). (3) TetraMin does not promote as much scum formation in water as is found with other materials used as food for mosquitoes. (4) Since TetraMin is a commercially available, fairly inexpensive material, the cost of using it for rearing Tx. r. rutilus would not be prohibitive.

The Tx. r. rutilus used in these experiments were taken from the fifth and sixth generations of a colony maintained

[1]Distributed in the U.S.A. by Tetra Sales of Hayward, CA.

in the laboratory. The original material was collected as eggs from treeholes in Alachua County, Florida.

Tx. r. rutilus eggs were individually set in 8 dram glass vials which were held in a water bath at 28 ± 1°C and a photoperiod of 14 hr light : 10 hr darkness. One group of 83 larvae was fed 5-10 mg of pulverized TetraMin at 3-day intervals, and the control group of 48 larvae was fed larvae of Ae. aegypti as required. At intervals of 10 days, the Tx. r. rutilus larvae were transferred to clean water.

Notes were kept on the development time required by each larval instar and on the comparative weights of pupae for the mosquitoes reared on the living and non-living diets.

Adult fecundity (eggs/female/day), longevity and daily survival were recorded in an outdoor cage. The cage was a 1 m x 1 m x 2 m screened cage located within a larger 5.5 m x 7.3 m x 4.3 m screened building. These enclosures were situated under large oak trees which provided shade during the middle of the day. The inner cage was protected from rainfall. The cage contained water wicks, honey, apple slices and black 0.5-liter oviposition jars half-filled with water. Eggs were removed and counted on a daily routine to provide information on fecundity and percent hatch of the two groups of adults.

Results

The development time for Tx. r. rutilus larvae reared on TetraMin and Ae. aegypti are shown in Table 3. Larvae maintained on a diet of TetraMin required an average of 107.5 ± 19.8 days compared to a relatively short 15.6 ± 1.4 days for larvae reared on Ae. aegypti. Even this comparison is not complete, for after 160 days when the test was terminated, 12% (or 10) of the 83 larvae reared on TetraMin were in the 4th larval instar. These 10 remaining larvae were offered (on day 190) Ae. aegypti as prey and pupated within 10.4 ± 3.0 days after consuming 87 ± 34 1st and 2nd stage larvae. During the 160 days, only 24% of the larvae receiving TetraMin pupated, and 64% of the larvae died. In comparison, 95.8% of the larvae fed Ae. aegypti pupated. A daily survival rate (S_i) for the larval stages was calculated for the two groups of larvae by averaging the ratios obtained by dividing the number of larvae alive on a given day by the number of larvae alive on the previous day. The S_i values were 0.9901 ± 0.0098 and 0.9973 ± 0.0075 for larvae reared on TetraMin and Ae. aegypti, respectively.

A comparison of the average weight of the two groups revealed that pupae from the larvae reared on TetraMin were 40% lighter. Weights of the pupae were 29.3 ± 3.6 mg ($n = 21$) and 50.0 ± 2.0 mg ($n = 8$) for TetraMin and Ae. aegypti, respectively.

Daily survival (S_a) of the adults was calculated by the same method used in calculating S_i. Using this method,

Table 3. Duration of larval instars of individually reared Tx. r. rutilus at 28 ± 1°C when fed a diet of Ae. aegypti larvae or TetraMin.

Instar	Mean Development Time (days)[a]		
	Ae. aegypti fed (n = 48)	TetraMin fed (n = 83)	% Increase
I	1.2	8.6	720
II	2.6	5.2	200
III	3.8	14.4	380
IV	8.0	79.3	990
Totals ± S. Dev.	15.6 ± 1.4	107.5 ± 19.8	690

[a]According to the arithmetic method of Southwood (1972); each entry is the time required for half the population to moult to the next instar.

estimates of S_a were 0.988 ± 0.051 and 0.979 ± 0.020 for adults from larvae reared on TetraMin and Ae. aegypti, respectively. There was no significant difference in adult survival.

For convenience in comparing the fecundity (F) of the two groups of adult females, fecundity was expressed as eggs/female/day and was calculated by dividing the total egg production by the number of female days. Using this method, the fecundity averaged 0.98 ± 0.23 and 1.03 ± 0.28 eggs/female/day for adults reared on TetraMin and Ae. aegypti, respectively. Given $S_a = 0.979$, F = 1.00 egg/female/day and total egg production = $nF \sum_{t=0}^{35} S_a^t$, a cage of n = 100 females would be expected to produce ca. 2500 eggs during a 5 week period. Percentage hatch for eggs from the two groups of females was the same, 98%.

Discussion

The results presented herein document for the first time that Tx. r. rutilus is not an obligate predator, albeit the mortality of 64% of the larvae fed the non-living diet would preclude the use of this technique for mass production of this species. In consideration of the biological parameters measured, the undesirable aspects of using TetraMin centers on the extremely long development time and the reduced size and weight of the pupae. Obviously the larvae were not receiving an adequate diet, but whether

the malnutrition was due to the lack of essential nutrients or ingestion is unknown. In his observations, the author noted that the Tx. r. rutilus never consumed all the TetraMin offered to them and this fact indicated that the larvae simply do not feed vigorously on a non-moving diet. The other parameters we measured, which included larvae survival (S_i), adult survival (S_a) and fecundity (F) were not different from those of the control group. However, in the context of mass-rearing, the extremely long development time overshadows these bright points. For example, the larval mortality exceeded 60% and pupation was highly asynchronous due to the length of development. The cost of rearing larvae with a 3 to 4 month development time would be prohibitive.

One important fact concerning the results herein is the ability of Tx. r. rutilus to survive over long periods without prey. This aspect of the durability and flexibility of this predatory species could be of some importance during a biological control program. It may be possible to release adult females and obtain a fairly high population of larvae of Tx. r. rutilus in the environment preceding the expansion of the prey population (Trpis 1972).

Further attempts to reduce the cost of mass production of Toxorhynchites could center on using a prey species which does not require an obligate blood meal in its life history. The elimination of maintaining animals would reduce the expenses incurred during culturing of the prey.

FIELD SURVIVAL, MIGRATION AND OVIPOSITIONAL CHARACTERISTICS OF LABORATORY-REARED *Toxorhynchites rutilus rutilus* (COQ.)

Abstract

Three hundred and fifty, 6-day old laboratory-reared adult *Toxorhynchites rutilus rutilus* (Coq.) mosquitoes were released into a sparsely wooded 13-acre residential area in Gainesville, Florida. Oviposition was monitored for 14 days using a grid of 64 oviposition traps located within the residential area and surrounding woods. Eighty percent of the ovitraps received eggs, and despite the migration of the females into the surrounding woods at a rate of about 7% per day, 64% of the eggs recovered were laid in the residential area. Equations were derived that allowed estimating the daily adult survival (S_a) and lifetime egg production per female released (F) to be 0.79 and 3.99 eggs/female, respectively.

Introduction

Past attempts to use *Toxorhynchites* spp. for control of container-breeding species of mosquitoes failed to achieve favorable results in Fiji (Paine, 1934), Hawaii (Bonnet et al., 1951) and American Samoa (Peterson, 1956).

In these efforts, the method used involved the release of small numbers of adult Toxorhynchites in the hope that these adults would establish populations of the predator sufficient to effect control of the prey species in containers. These releases failed to reduce populations of the prey species because the numbers released produced too few eggs (Muspratt, 1951) and the subsequent progeny were not numerous enough for control (Nakagawa, 1963; Newkirk, 1947).

In analyzing these attempts to use Toxorhynchites as a control agent, the most obvious mistake was the failure to consider the normal relationship between predator and prey species. For example, Trpis (1972) reported a situation in East Africa where naturally occurring densities of Tx. brevipalpus are sufficient to control Aedes aegypti (L.), but only at the end of the rainy season. Trpis attributed the lack of control early in the rainy season to the slow population growth rate of Tx. brevipalpus. The most recent attempt to use a species of Toxorhynchites as a control agent was conducted on the island of St. Maarten by Gerberg (1974). He was able to eliminate Ae. aegypti from houses by placing Tx. brevipalpus eggs in containers, and albeit this is a laborious method the degree of control was satisfactory.

The observations of Trpis (1972) and Gerberg (1974) clearly indicate that the effective utilization of Toxorhynchites spp. as a control agent will require multiple,

inundative releases of the Toxorhynchites adults or eggs.

The author is currently involved in an investigation to study the feasibility of using Tx. rutilus rutilus, a species endemic to the Southeastern United States, as a control agent. In separate reports the author has reported observations on the life history (Chapter 2) and rearing on an artificial diet (Chapter 3), and this present paper contains the results of a release of adults in a residential area. Herein, the author reports observations of oviposition, migration, and adult survival for females of Tx. r. rutilus.

Materials and Methods

The Tx. r. rutilus used in this experiment were mass-reared in the laboratory on a diet of Ae. aegypti larvae by methods described in Chapter 2. Pupae were transfered to an outdoor screened cage 1 m x 1 m x 2 m located within a screened building. The adults were supplied with water wicks, honey, apple slices and black oviposition jars and held in the outdoor cage for 6-8 days prior to release.

The adults were held for at least 6 days prior to the release for 3 reasons: (1) the females do not begin ovipositing until they are 6 days old, (2) by holding them until they were ready to oviposit, a better estimate could be obtained on migration out of the release area, and (3) maximum oviposition would be obtained from the released

females. On the evening of July 26, 1976, 175 males and 175 females were released at 3 sites within the experimental area.

The approximately square experimental area (Fig. 1) covered about 31 acres and was located on the campus of the University of Florida and a neighboring residential section. The release was done in the center of a student housing project that occupied a 13-acre plot more or less centrally located in the experimental area. This housing project plot was interspersed with various hardwood and pine trees, shrubs, and open expanses of lawn. On 3 sides the surrounding area was more densely wooded, and on the fourth side there was a lake.

Oviposition by Tx. r. rutilus females was monitored by means of 64 ovitraps placed at ca. 60 m intervals. Thirty of the ovitraps were within the student housing project and the remainder were in the densely wooded areas.

The ovitraps were similar to those used previously in determining the distribution and density of Ae. aegypti during the U.S. Aedes aegypti Eradication Program (Tanner, 1969; Jakob and Bevier, 1969). Briefly, the ovitrap consists of a pint (ca. 0.5 l) glass jar sprayed with flat black enamel paint on the outside. The jar is 13 cm high and the opening is 6 cm in diameter. The jars were filled with ca. 200 ml of water which had been used in the routine rearing of Ae. aegypti in the laboratory. The jars were placed in wooden shelters designed to afford protection

Figure 1. Aerial view of the experimental area showing the student housing project and the surrounding woods. (North at top of photograph; date February, 1977.)

from rainfall. The shelter consisted of a narrow shelf to support the jar and a 15 cm^2 roof 15 cm above the mouth of the jar. The shelters were attached about 2 m (6-8 ft) above the ground on tree trunks and posts to minimize tampering by children.

The ovitraps were checked daily for the presence of Tx. r. rutilus eggs which were discarded after counting. Water levels in the ovitraps were adjusted twice weekly.

Results and Discussion

Just prior to the release of the laboratory-reared adults, the 64 ovitraps were monitored for 4 days to detect the presence of indigenous Tx. r. rutilus adults. The average number of eggs laid per day within the experimental area was 1.75. Therefore, the best estimate of the contribution of indigenous females to the total oviposition observed during the 14 days subsequent to the release of the laboratory material is 24.5 eggs (i.e., 1.75 eggs/day x 14 days). A total of 407 eggs were recovered from the experimental area during the 14 days: thus, indigenous adults were responsible for about 6% of the total oviposition observed.

The distribution and number of eggs recovered subsequent to the release are shown in Fig. 2 and Table 4 and 5. The release of slightly less than 6 females/acre (175 females/31 acres) resulted in 51 (80%) of the ovitraps

Figure 2. Map of the experimental area showing the 3 release points, ovitrap locations, and the distribution and number of eggs recovered subsequent to the release of 350 Tx. r. rutilus adults.


NORTH
0 50 100
meters
— RELEASE POINTS
LAKE ALICE

Table 4. Tx. r. rutilus oviposition observed after the release of 350 adults into the central area of the experimental area.

Days Since Release	Number of Eggs Recovered Daily from 64 Ovitraps			Percent	
	Central Area	Outer Area	Total for Release Area	Central	Outer
1	69	7	76	91	9
2	24	20	44	55	45
3	40	4	44	91	9
4	49	15	64	77	23
5	36	14	50	72	28
6	19	5	24	78	21
7	6	36	42	14	86
8	0	8	8	0	100
9	6	6	12	50	50
10	7	19	26	27	73
12	0	7	7	0	100
14	1	9	10	10	90
Totals	259	148	407		

Table 5. The number and distribution of eggs recovered within the experimental area.

Area	Number of Eggs Recovered	Percent of Total	Number of Bottles	Number of Eggs per Bottle ($\bar{x} \pm$ S.D.)[a]	Number of Bottles Receiving No Eggs
Central	259	64	30	8.6 ± 12.0	8
Outer	148	36	34	4.4 ± 4.8	5
Total	407	100	64	6.4 ± 9.1	13

[a]These values are not significantly different.

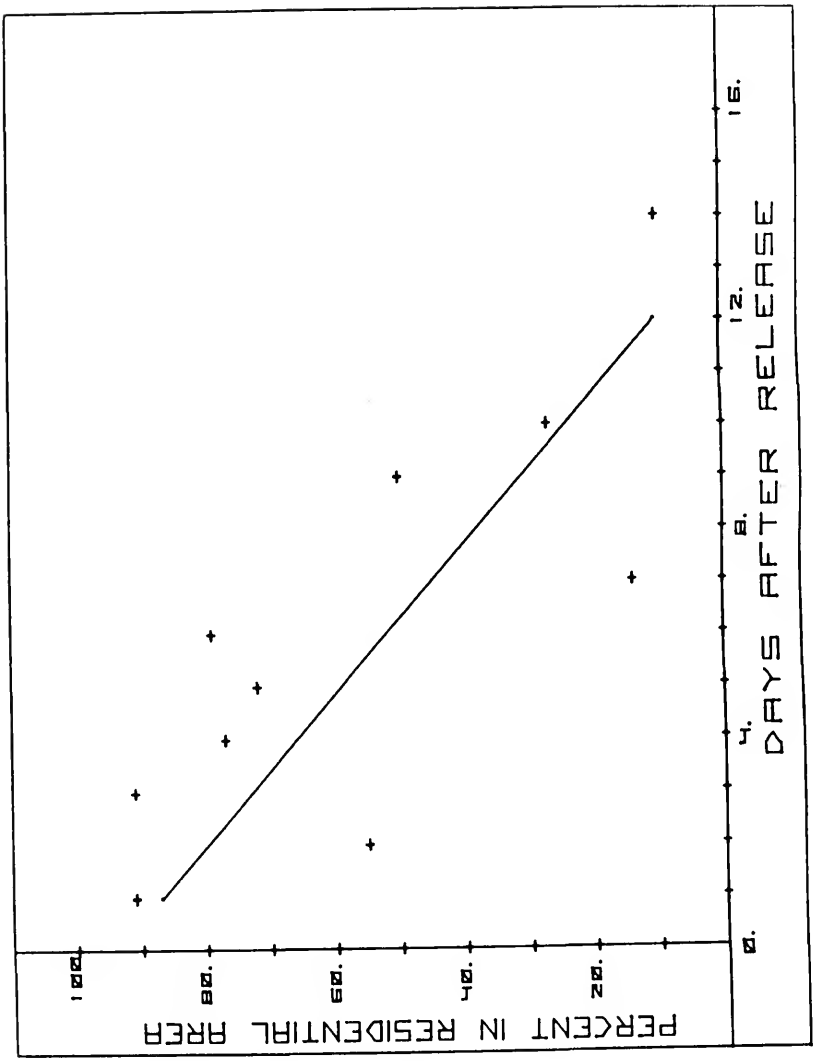
within the experimental area receiving eggs.

Of the 30 ovitraps within the housing project where the adults were released, 73% of the traps were positive for eggs. Some of the ovitraps nearest the release points received the greatest number of eggs (Fig. 2). It might have been possible to reduce the number of ovitraps receiving no eggs by releasing smaller numbers of adults at more locations.

Prior to this experiment, the author was concerned with the possibility of Tx. r. rutilus ovipositing the bulk of its eggs in just a few containers. Examination of Fig. 2 visually demonstrates not a clumped, but rather a random (or Poisson) distribution of eggs among the 64 ovitraps. Mathematically, this is borne out by noting that the mean and variance are approximately equal for the number of eggs per container (4.40 and 4.69, respectively) when the 4 ovitraps immediately adjacent to the release points are omitted from consideration and by a X^2-test for goodness of fit for a Poisson model.

Because Ae. aegypti and Tx. r. rutilus are typically domestic and sylvan species, respectively, the author expected to observe migration from the release site into the surrounding wooded area. During the 14 days after the release, the percentage of the total oviposition which occurred within the housing project decreased steadily from an initial 91% on day 1 to 10% on day 14. A linear regression analysis (Fig. 3) of the daily percent of oviposition

Figure 3. A regression of the daily percent of oviposition occurring within the housing project area on days after release. (R^2 = .63)


PERCENT IN RESIDENTIAL AREA
100.
80.
60.
40.
20.
0.
4.
8.
12.
16.
DAYS AFTER RELEASE

occurring within the housing project area (column 5, Table 4) on days after release showed this trend to be significant (R^2 = .63). Moreover, the difference between the means for the percent oviposition within that area for the 2 periods, day 1-6 and day 7-14 (0.78 $\pm$ 0.13 and 0.17 $\pm$ 0.19, respectively) are highly significant (t-test). The slope of the regression equation would indicate an overall rate of migration out of the residential area and into the forest of about 7% daily. Notice, that if the dispersion of adults within the release area was due entirely to random movement, the percent oviposition occurring within the housing project area would have been expected to tend toward 50% and not zero. Notice, also, that even though the exodus of females was nearly complete, 64% of all the eggs were recovered within the housing project area.

The determination of the daily adult survival (S_a) is important because knowing S_a enables one to calculate the expected number of eggs per lifetime per female released (F). Following is the derivation of some equations useful in the estimation of S_a and lifetime fecundity (F) from oviposition data.

If S_a is the probability of surviving from one day to the next and this does not change with the age of the mosquito, then the proportion of adults alive on any particular day t is S_a^t. It follows then, that the number of females alive on day t can be represented by the expression

$$N_t = N_o S_a^t \quad , \qquad (I)$$

where N_o is the initial number of females and N_t is the number alive on day t. Alternatively, since the numbers dying are proportional to the numbers alive, the change in numbers over time can be expressed as

$$dn/dt = -k\ N_t \qquad \text{(II)}$$

which upon integration yields

$$N_t = N_o\ e^{-kt} \quad . \qquad \text{(III)}$$

Taking the natural logarithms of equations (I) and (III) yields the respective linear equations

$$\ln N_t = \ln N_o + t \ln S_a \qquad \text{(IV)}$$

and

$$\ln N_t = \ln N_o - kt \quad . \qquad \text{(V)}$$

Here, $\ln N_o$ is the y-intercept, and $\ln S_a$ and k are the slopes of (IV) and (V), respectively. Notice that setting equation (IV) equal to (V) and simplifying yields

$$S_a = e^{-k} \text{ or } k = -\ln S_a \quad .$$

Assuming that (1) the number of eggs recovered is directly related to the number of surviving females, (2) S_a in the field is constant over time[a] and (3) the

[a]Estimates from outdoor cage studies of daily fecundity (f) and S_a for Tx. r. rutilus are constant over the time period being discussed (Chapter 3).

pretreatment indigenous population is stable, an estimate of S_a ($= e^{-k}$) can be made by regressing the daily egg production on days after release. The value calculated (Fig. 4) for Tx. r. rutilus in this experiment was 0.785 with 95% confidence limits of 0.845 and 0.720 ($R^2 = 0.79$). This value of S_a compares favorably with estimates made for other mosquitoes (Seawright et al., 1977; Sheppard et al., 1969).

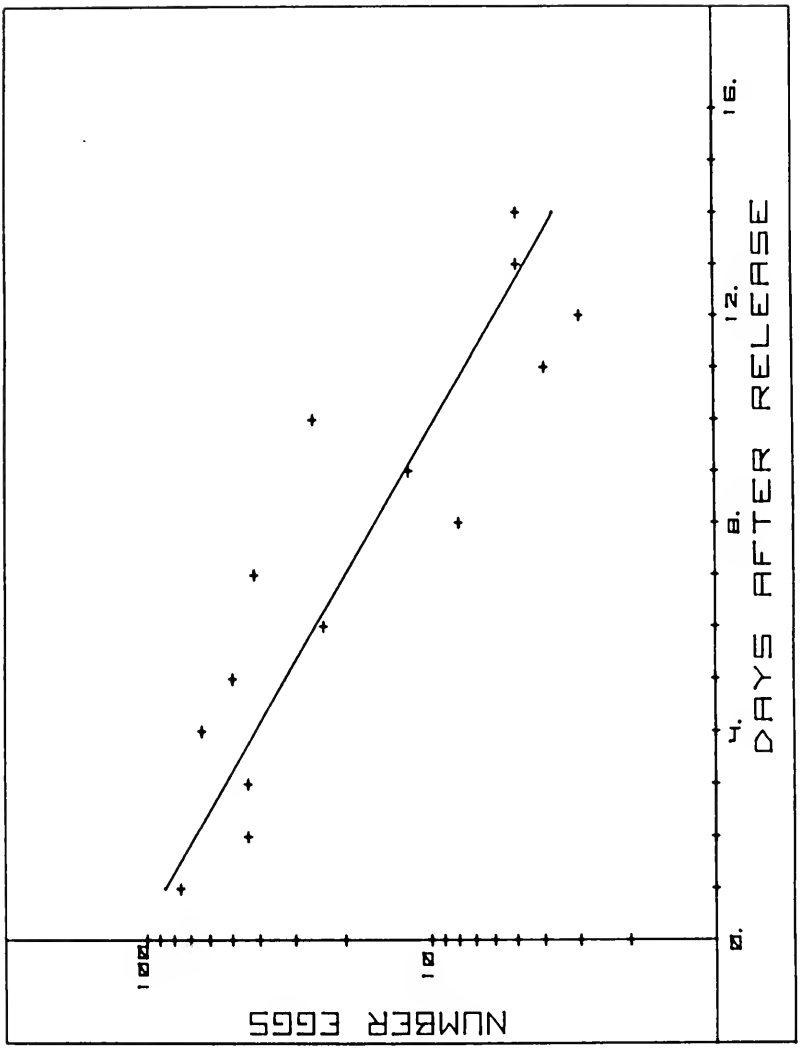
It should be obvious, given the foregoing assumptions, that the regular decline in egg production in the study area was due to daily adult mortality ($1 - S_a$) and migration out of the experimental area. Since the data gathered in this experiment does not permit us to partition out the decline in oviposition that is due to migration, $S_a = 0.785$ represents a minimum value of adult survival. In larger release areas where the effects of migration would be minimized, the S_a term as derived here would more accurately reflect the true adult survival.

The daily egg production (f) of Tx. r. rutilus females in an outdoor cage is ca. 1.0 egg/female. By multiplying equations (II) or (III) by f and then integrating, one can obtain an estimate of the expected total egg production, i.e.,

$$f N_o \int_{t=0}^{\infty} S_a^t \qquad \text{(VIa)}$$

or

Figure 4. A regression of daily egg production on days after release. (R^2 = .79)


NUMBER EGGS
100
50
0
0.
4.
8.
12.
16.
DAYS AFTER RELEASE

$$f N_o \int_{t=0}^{\infty} e^{-kt} . \qquad \text{(VIb)}$$

The expected egg production over the period of the release (day 0 thru 14) obtained over the interval 0 to 14 is 699.8 eggs per 175 females released. Notice that the approximation of total egg production

$$f N_o \sum_{t=0}^{14} S_a^t \quad (= 787.7 \text{ eggs})$$

overestimates the above integrals by 13%.

Of the expected oviposition, only 58% (407/699.8) was recovered in ovitraps. Part of this may be attributed to other oviposition sites within the experimental area. Five days after the release, a tree stump flush with the ground containing rain water was discovered in the densely wooded area. During the remainder of the experiment, i.e., day 5 thru 14, this one natural site received eggs equal to 17% of all the eggs recovered in the entire experimental area during that period (36/215). This suggests that a good deal of the egg production went to sites other than the 64 ovitraps.

In the context of using Tx. r. rutilus as a biological control agent against container breeding mosquitoes, the results reported herein are encouraging. Although migration out of the area was observed, the preponderence of eggs were laid within the urban area. The values of female

daily survival (S_a = 0.785) and lifetime fecundity (F = 3.99 eggs) are high enough that the cost of area treatment by inundative release of adults should not prove prohibitive. Finally, demonstrated here is the ability of Tx. r. rutilus females to locate oviposition sites typical of Ae. aegypti habitats and to randomly distribute their eggs among them. Using these preliminary data, the author is planning an adult release where the control of a prey species will be monitored.

A DETERMINISTIC MODEL FOR SIMULATING THE PREDATION OF *Toxorhynchites rutilus rutilus* (COQ.) ON *Aedes aegypti* (L.)

Abstract

A deterministic computer model is presented detailing the interaction of the container-breeding mosquito *Aedes aegypti* (L.) and the larval predator *Toxorhynchites rutilus rutilus* (Coq.). Results of simulation runs involving the release of *Tx. r. rutilus* adults indicate that predator releases resulting in 1 predator larva per container are sufficient to reduce *Aedes* adult density 75% in 20 days. The slow rate of immature predator development enables control to be maintained for several months. Simulations of predator release and the use of adulticides indicate that it is possible to obtain zero adult densities. Finally, the model indicates that the most important parameter determining the degree of control established is the distribution of predator eggs.

Introduction

Historically, mosquito control involved source reduction and insecticides. Predicting the outcome of these measures was simple and straightforward. Currently, additional methods of mosquito control are being studied, some

of which involve the interaction of 2 species. Difficulty in understanding the dynamics of the interaction between the 2 species makes predicting the outcome of new methods complicated. Attempting to optimize a strategy utilizing 2 different methods in conjunction, e.g., predators and insecticides, is particularly difficult. Therefore, computer simulation models are becoming increasingly popular as a tool in the development and evaluation of new control strategies.

The purpose of this paper is to: 1) present a deterministic computer model examining the interaction of Aedes aegypti (L.) and a larval predator, Toxorhynchites rutilus rutilus (Coq.); and 2) simulate the population dynamics of Ae. aegypti under different control strategies involving the release of Tx. r. rutilus adults and/or the use of adulticides. Pertinent to this discussion is an explanation of methods used in life-history analysis by Mertz (1970). The utility and rationale of simulation in developing control strategies is presented by Conway (1970), Haile and Weidhaas (1976), and Weidhass (1974).

It is desirable to emphasize here, that while many of the values for Toxorhynchites parameters are from laboratory experiments conducted under conditions obviously different from those expected in the field, there remains a very real value in the model building exercise. Characterizing the interaction of the 2 species highlights areas where better information is needed. Additionally, simulating with the

present model gives us insight into how best to approach the first large-scale release experiment where prey control is to be attempted. Finally, the preliminary model gives us a framework within which to interpret the resulting, experimental field data.

Model Description

The model described herein may be called a compartment model (Miller et al., 1973). Each stage of each species is represented by a number of storage registers within an array (an array is a group of registers). The registers represent 1-day age classifications of the various stages; a particular array represents the age distribution of a particular stage or instar. Except for the Toxorhynchites larval stage, the length of the various stages and instars is determined by the number of registers within a particular array. The model is made to cycle on a daily basis by replacing the contents of the next storage register with the contents of the previous register multiplied by the daily survival for that particular stage and species; the output of the terminal register of one array (stage) is the input to the next array (stage). Most values of daily survival for both species are fixed during any particular simulation run (Table 6); the daily survival for Ae. aegypti 1st and 2nd instars is a density dependent variable. The length of larval life of Tx. r. rutilus is also a variable -- it being a function of the amount of prey available. Details of

Table 6. Ae. aegypti and Tx. r. rutilus survival and oviposition distribution parameters.

Name	Value	Description
Aedes		
SA	0.88	Adult daily survival.
F	93	Fecundity (eggs/oviposition).
P	0.90	Proportion of pupation which is successful.
SE	0.98	Daily egg survival.
S	0.95	Daily survival for 3rd & 4th instars, and pupae.
C	0.01	Density dependent coefficient for calculating SI.[a]
SI	0-1.0	Density dependent daily survival for 1st & 2nd instars.
AEDIST	0.53	Proportion of containers positive for Aedes immatures.
Toxorhynchites		
SAT	0.88	Daily adult survival.
FT	1.00	Fecundity (eggs/female/day).
SIT	0.99	Daily survival for larvae and pupae.
DISTBN	0.5-0.9	Proportion of containers positive for Toxorhynchites larvae.

[a]See Fig. 5 for details.

these variables will be presented in detail presently.

The data enabling the modeling of Ae. aegypti were largely derived from Southwood et al. (1972) (immatures) and Sheppard et al. (1969) (adults). These life-table and ecological studies were conducted in Bangkok, Thailand in hopes of correlating Ae. aegypti population dynamics with the known seasonal incidence of dengue haemorrhagic fever. Surprisingly absent from both studies was any information on fecundity and ovipositional patterns; estimates for these parameters were derived from data on Ae. aegypti in northern coastal Florida (J.A. Seawright, personal communication). Data for Tx. r. rutilus came from laboratory, outdoor cage, and field experiments conducted by the author (Chapters 2, 3 and 4).

Briefly, the Ae. aegypti situation to be modeled is as follows (Figure 5): according to Sheppard et al., the main source of all Ae. aegypti breeding in Bangkok is the earthen or ceramic water storage jar (ca. 100-200 l capacity) which is found in association with all types of housing. They usually contain water throughout the year and are replenished with rainwater, tapwater or riverwater. Southwood et al., state the water storage jars number about 150 per acre and about 53% of these are positive for aegypti immatures at any particular time. Interestingly, both studies reported remarkably stable densities and distributions of all stages from season to season. Adult densities of 1100 per acre (or 7.3 adult/container) were reported; Table 7 presents

Figure 5. Ae. aegypti subroutine. (Letters within parentheses refer to daily survival values listed in Table 6.)

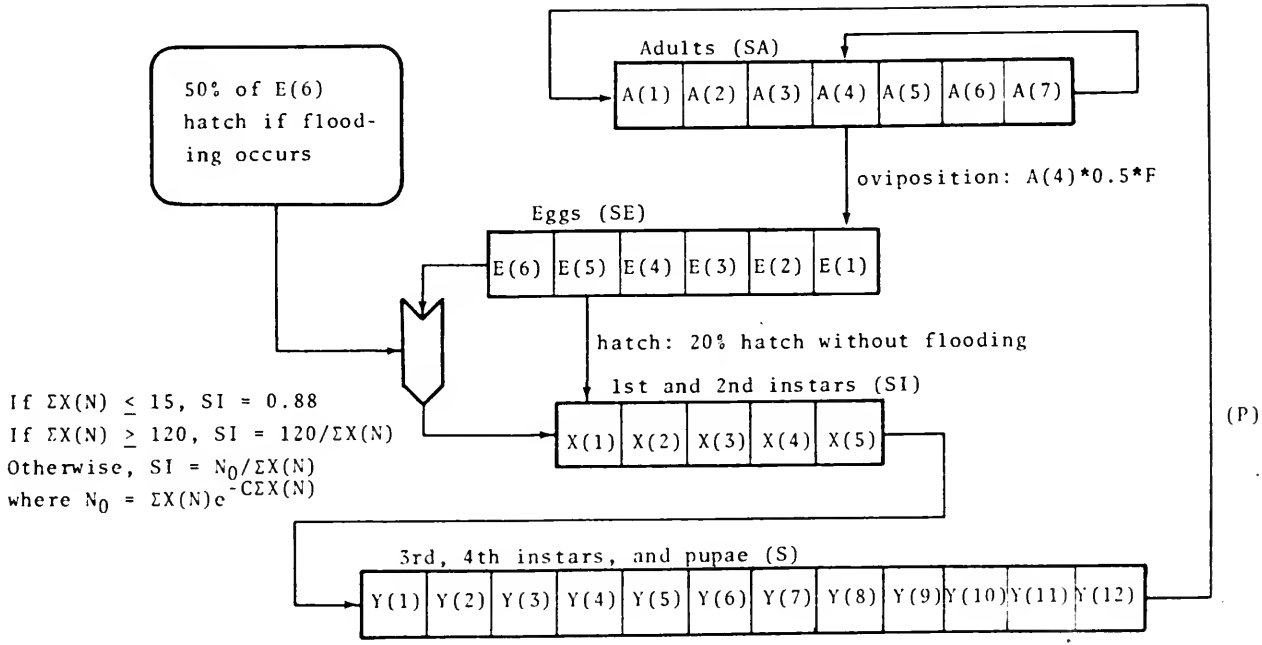

50% of E(6) hatch if flooding occurs
Adults (SA)
A(1) A(2) A(3) A(4) A(5) A(6) A(7)
oviposition: A(4)*0.5*F
Eggs (SE)
E(6) E(5) E(4) E(3) E(2) E(1)
hatch: 20% hatch without flooding
1st and 2nd instars (SI)
X(1) X(2) X(3) X(4) X(5)
3rd, 4th instars, and pupae (S)
Y(1) Y(2) Y(3) Y(4) Y(5) Y(6) Y(7) Y(8) Y(9) Y(10) Y(11) Y(12)
(P)
If ΣX(N) ≤ 15, SI = 0.88
If ΣX(N) ≥ 120, SI = 120/ΣX(N)
Otherwise, SI = N0/ΣX(N)
where N0 = ΣX(N)e^-CΣX(N)

Table 7. Ae. aegypti immature development times and average numbers (and proportions) per water storage jar positive for Ae. aegypti (Southwood et al., 1972).

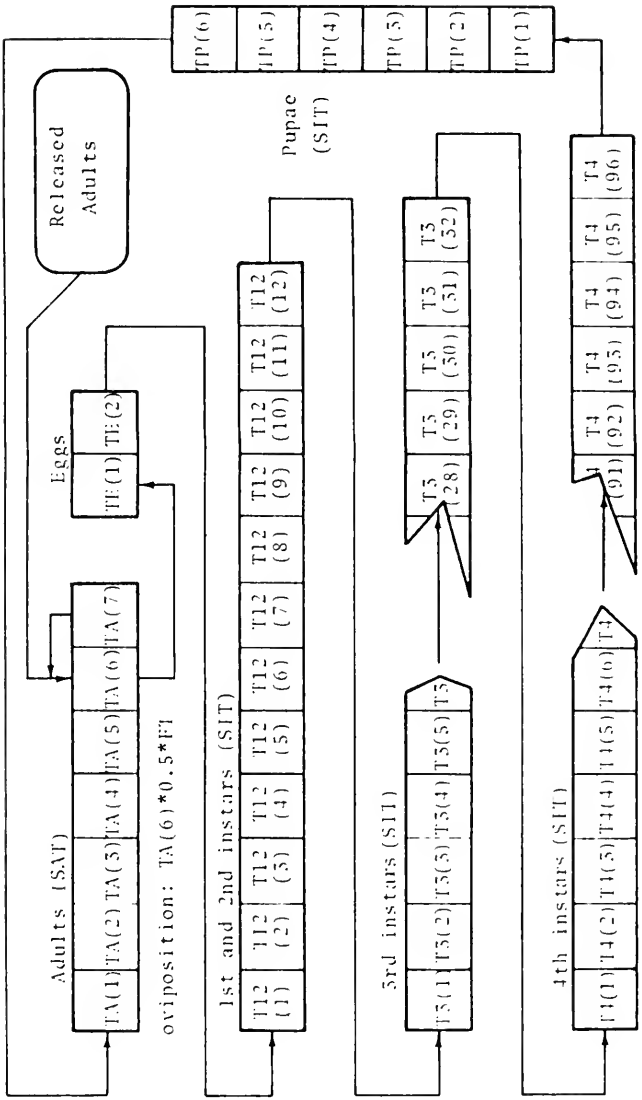
Stage	Mean Development Time (days)	Mean Number/Container	Proportions
Egg	4	---	---
Larvae			
I + II	5.2	20.0	0.51
III	3.2	9.5	0.24
IV	6.5	8.0	0.20
Pupa	2.2	2.0	0.05
Totals (excluding eggs)	17.1	39.5	1.00

immature development times and average numbers of immatures per container. Southwood et al. note that 20% of the embryonated eggs hatch without the flooding stimulus described by Christophers (1960), and that approximately 50% of the remaining eggs hatch with each subsequent reflooding. Since they recorded no data on the frequency of flooding for the jars, the computer model was simulated for no-rain and daily rain situations. A final feature of the population dynamics of Ae. aegypti in Bangkok is the density dependent survival during the first 2 larval instars. The stability of the mean number of immatures/container (Table 7) appears to stem from a paucity of larval food; the number of new 3rd instar larvae is a consequence of the available food and not the number of newly hatched 1st instars. An algorithm for producing the observed larval densities which is largely independent of oviposition or frequency of hatch is detailed in Figure 5.

The subroutine describing Tx. r. rutilus, with several exceptions, is similar to that for Ae. aegypti. Reference to Figure 6 reveals: 1) the 3 last immature stages are separated into 3 arrays; 2) that eggs hatch on the second day after oviposition and independently of rainfall; and, 3) that oviposition begins 6 days after eclosion and occurs daily thereafter. Notice that Figure 6 shows laboratory-reared adult Toxorhynchites being released at 6 days of age (Chapter 4).

The nature and mechanics of modeling the predation of

Figure 6. Tx. r. rutilus subroutine. (Letters within parentheses refer to daily survival values listed in Table 6.)


Released Adults
Adults (SAT)
TA(1) TA(2) TA(3) TA(4) TA(5) TA(6) TA(7)
oviposition: TA(6)*0.5*FI
Eggs
TE(1) TE(2)
1st and 2nd instars (SIT)
T12 (1) T12 (2) T12 (3) T12 (4) T12 (5) T12 (6) T12 (7) T12 (8) T12 (9) T12 (10) T12 (11) T12 (12)
Pupae (SIT)
TP(6) TP(5) TP(4) TP(3) TP(2) TP(1)
3rd instars (SIT)
T3(1) T3(2) T3(3) T3(4) T3(5) T3 T3 (28) T3 (29) T5 (30) T5 (31) T5 (32)
4th instars (SIT)
T4(1) T4(2) T4(3) T4(4) T4(5) T4(6) T4 T4 (91) T4 (92) T4 (93) T4 (94) T4 (95) T4 (96)

Tx. r. rutilus on Ae. aegypti are presented below. Table 8 presents development times and the numbers of prey devoured when the various instars are offered either early (1st and 2nd instar) or late (3rd, 4th instar, and pupae) Ae. aegypti immatures. Table 2 details how long the various Tx. r. rutilus instars can fast before death and Table 3 presents the duration of larval instars when fed on organic, non-living diet. The situation to be modeled is as follows: 1) Tx. r. rutilus 3rd and 4th instars develop faster on a diet of late instars and pupae than when fed early instar prey; 2) all larvae develop at a rate proportional to the amount of prey available until the amount of prey exceeds that given in Table 8; 3) all predator instars can survive without prey for a period of time and if provided detritus, can develop at a very slow rate through to eclosion; and 4) the daily immature survival (SIT) is constant, irrespective of diet (Chapter 4).

In the model, 1st and 2nd instar predators (array T12) are assumed to eat before 3rd and 4th instar predators (T3 and T4, respectively) on any particular day. This assumption simplifies the programming of predation, and does not, considering the relative amounts of prey consumed by each instar, introduce significant errors. The number of storage registers within the arrays T12, T3 and T4 (16, 32 and 96, respectively) do not correspond directly to 1 day each. This is because the larvae are moved incrementally within an array as a function of prey size and numbers of prey

Table 8. Larval development times for Tx. r. rutilus in the laboratory when fed early (I & II) or late (III, IV & pupa) instar Ae. aegypti.

Instar	Diet -- early instars			Diet -- late instars		
	Time (days)	No. devoured	Daily consumption	Time (days)	No. devoured	Daily consumption
I	1.2	6.4	5.3	1.2	6.4[b]	5.3
II	2.6	6.6	2.5	2.6	6.6[b]	2.5
III	8.0	60[a]	7.5	3.8	8.0	2.1
IV	32.0	600[a]	18.8	8.0	73	9.1
Totals	43.8 days	673 larvae	---	15.6 days	94 larvae	---

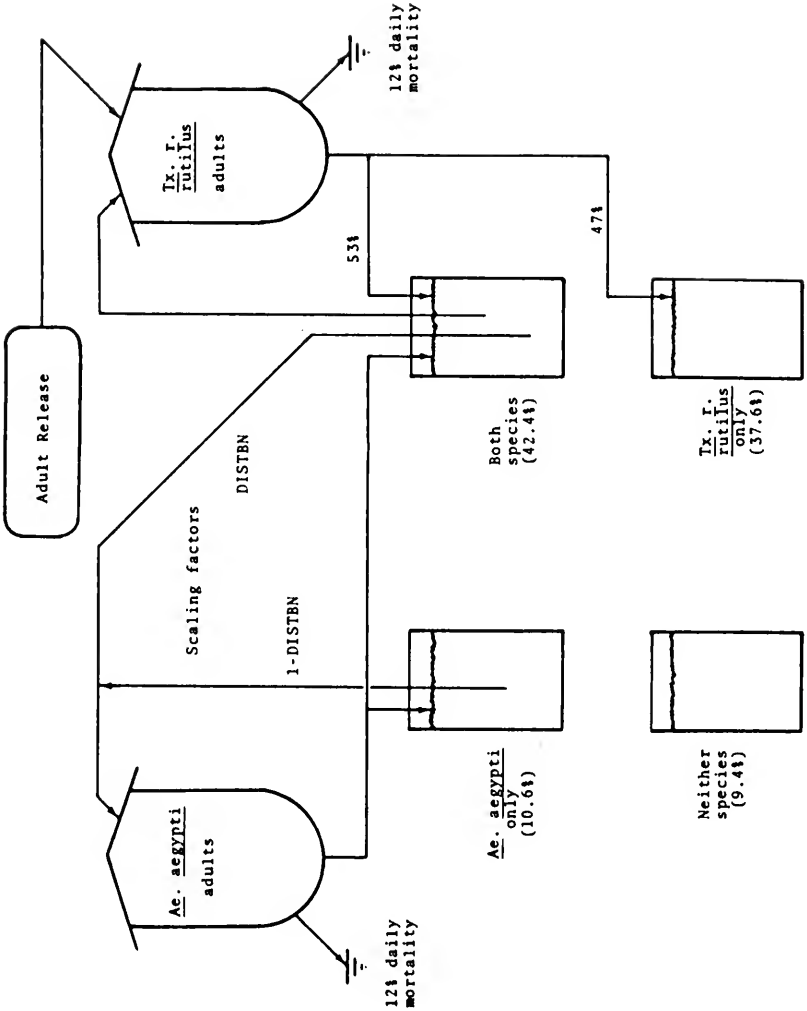
[a]Estimated from 3 replicates.

[b]The numbers reported consumed are I & II instar prey.

available. If no prey are available, all the predators are incremented 1 register forward. If the number of prey equals or exceeds the numbers presented in Table 8, the larvae are incremented 4, 8 and 12 registers forward in arrays T12, T3 and T4, respectively. Intermediate amounts of prey result in incrementing the predator arrays an amount proportional to the available prey. The Ae. aegypti immature arrays in containers positive for Tx. r. rutilus (XA and YA) are updated daily for the effects of predation. Since cannibalism does not occur among the predators at the densities considered here, even when there is no prey, cannibalism is not modeled.

Following a release of Tx. r. rutilus adults, one would expect on the basis of the distribution of Ae. aegypti larvae (53% positive) and an assumed predator distribution of 80% (from Chapter 4) to obtain 4 types of containers: 42.4% positive for both species, 10.6% positive for Ae. aegypti only, 37.6% positive for Tx. r. rutilus only, and 9.4% containing neither species. Since it is assumed that no Toxorhynchites adults result from oviposition into containers devoid of prey, and the 4th type of container produces neither species, the latter 2 types of containers are not specifically modeled in the program but are accounted for with scaling factors (Figure 7). To represent the salient features of the interaction in the field and to represent the resulting Aedes adult density, 2 containers both positive for Ae. aegypti are included in the model,

Figure 7. Distribution of container types. (Numbers in parentheses refer to frequency of each type when 53% are positive for Ae. aegypti and 80% are positive for Tx. r. rutilus.)


Adult Release
Ae. aegypti adults
Tx. f. rutilus adults
12% daily mortality
12% daily mortality
Scaling factors
DISTBN
1-DISTBN
53%
47%
Both species (42.4%)
Ae. aegypti only (10.6%)
Tx. f. rutilus only (37.6%)
Neither species (9.4%)

only 1 of which is interfaced with the predator subroutine. Ae. aegypti eclosion and Tx. r. rutilus oviposition are scaled by factors of 'DISTBN' and 0.53, respectively, to depict the frequency of the 2 types of containers in the field. Notice, that because of the scaling factors, the number of Aedes and Toxorhynchites adults are in terms of 1 container and that multiplying the output of adults by the number of containers per area gives the absolute population estimate.

Results of Simulation

Figures 8 and 9 represent model generated Ae. aegypti adult densities on a per container basis for the 'no rain' and 'daily rain' into every container situation, respectively. Figures 10 and 11 depict the corresponding total number of Aedes immatures/container for the 2 rainfall situations. In each instance and in all subsequent simulation runs, the model was initialized on day 1 with 7.4 1-day old adult Ae. aegypti. The resulting numbers within each stage are largely independent of the number and stages used in initializing the model. The graphs have been smoothed by plotting 4-day moving averages. Notice that the cyclic nature of the 'daily rain' situation in Figure 9 dampens with time.

Table 9 presents a comparison of the number of Ae. aegypti individuals per stage per container observed by Southwood et al., and the number of individuals per stage

Figure 8. Ae. aegypti adult density on a per container basis with no control measures applied. (No rain)

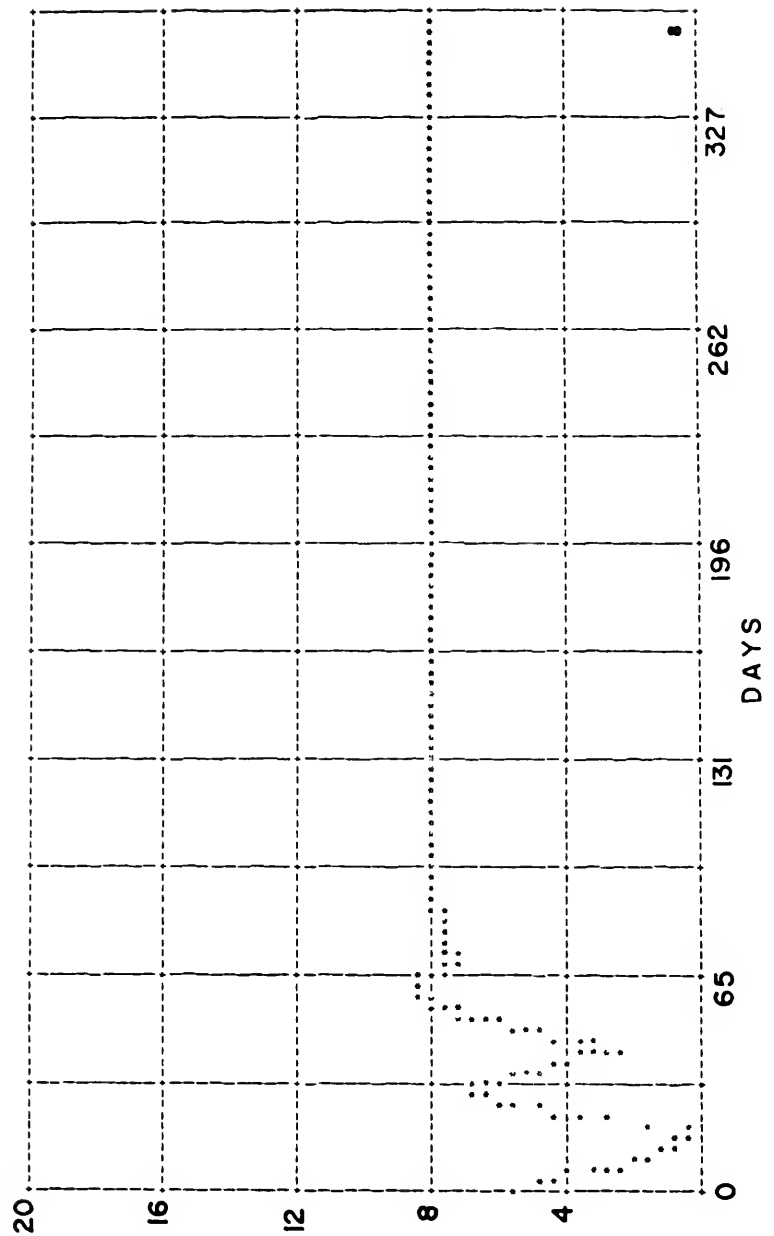

20
16
12
8
4
0
65
131
196
262
327
DAYS

Figure 9. Ae. aegypti adult density on a per container basis with no control measures applied. (Daily rainfall into every container)

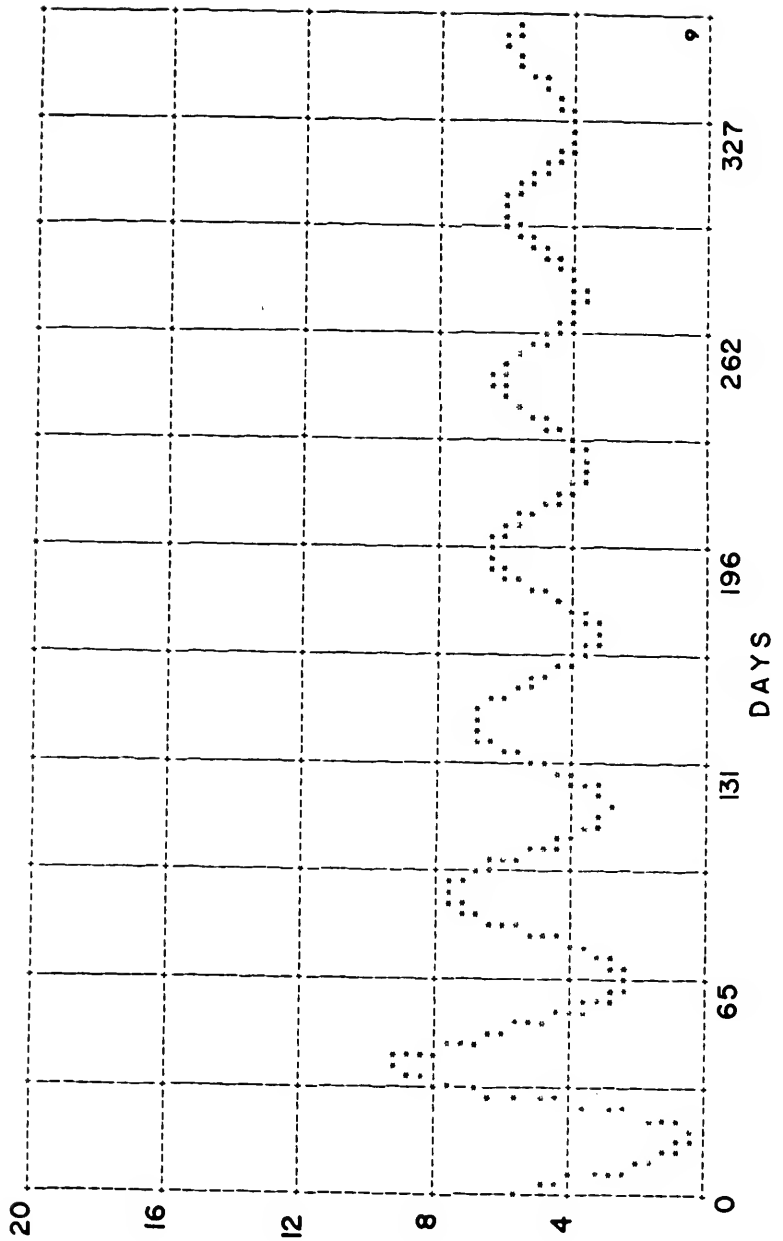

20
16
12
8
4
0
65
131
196
262
327
DAYS

Figure 10. Total number of Ae. aegypti immatures/container. (No rain)

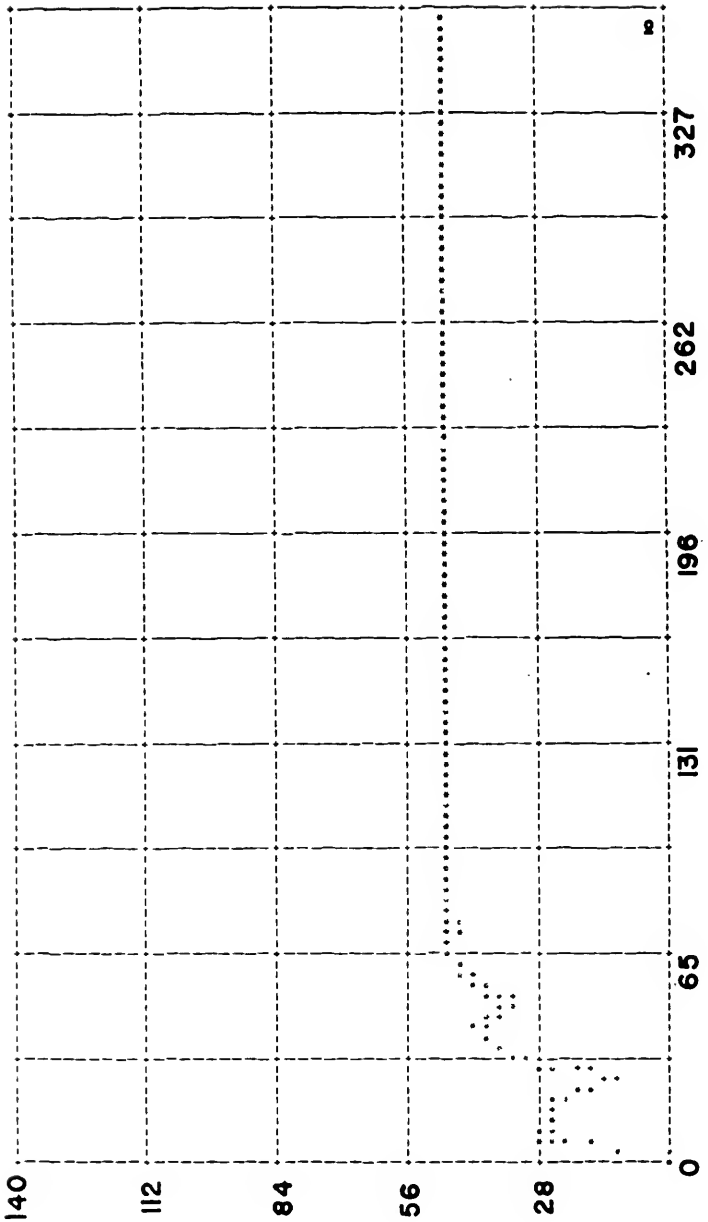

140
112
84
56
28
0
65
131
196
262
327

Figure 11. Total numbers of Ae. aegypti immatures/container. (Daily rainfall into every container)

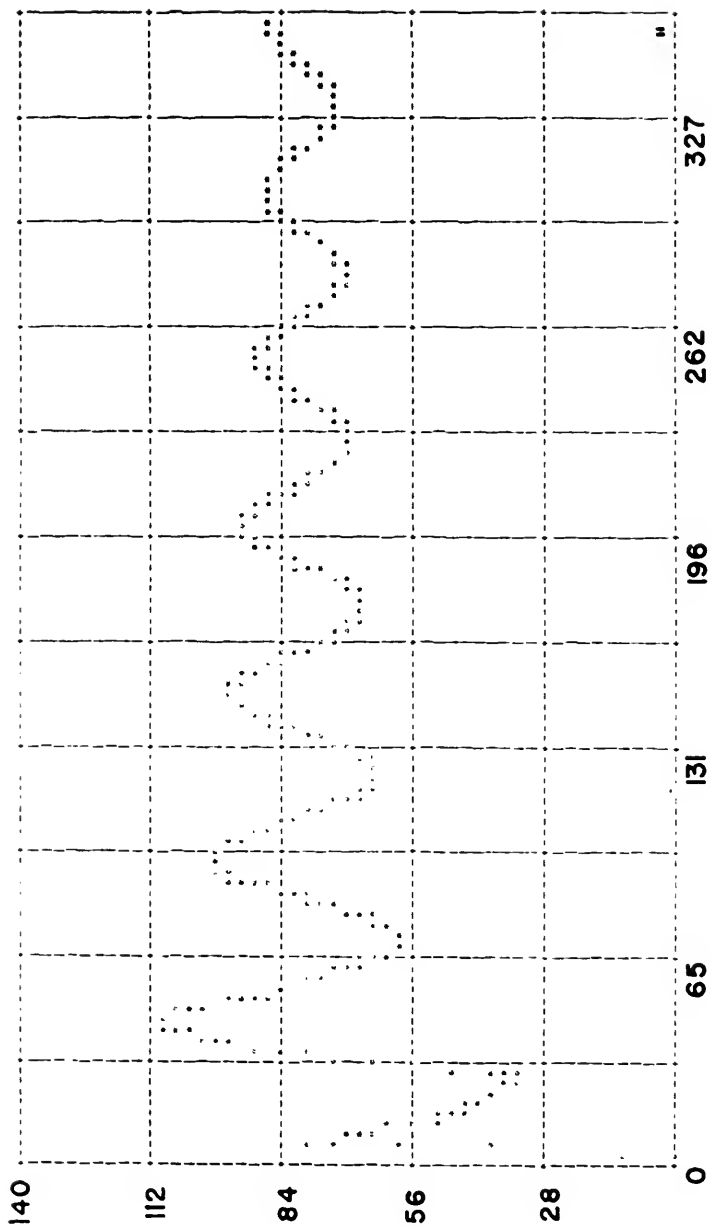

140
112
84
56
28
0
65
131
196
262
327

Table 9. Comparison of the number of Ae. aegypti individuals per stage per container as reported by Southwood et al., with model output.

Stage	Southwood et al.	Model Output		
		No rain	Rain[a]	Average
Larvae				
I & II	20	30	70	50
III	9.5	8.0	5.0	6.5
IV	8.0	8.0	5.0	6.5
Pupae	2.0	2.2	1.5	1.9
Total	39.5	49.0	81.5	64.9
Adults	7.3	8.0	5.0	6.5

[a]Situation modeled is that all containers receive daily rain.

per container generated by the model when no control measures are applied. Notice that the model generated total number of immatures/container exceeds the total reported by Southwood et al. This disparity was allowed to remain because the difference was not large (especially in the 'no rain' situation) and would make the model more conservative in subsequent control simulations. Density-dependent 1st and 2nd instar daily survival (SI) for the 'no rain' and 'daily rain' situation averaged ca. 0.75 and 0.50, respectively.

The effects on Ae. aegypti population dynamics of contact adulticide application causing 95% mortality among the adults with no residual action are presented in Table 10 and Figures 12-14. Notice in Figure 12a, as a consequence of 1 application, the overshoot of adults 1 generation later. Notice also (Figures 13a and 14a) the increased frequency of application required to maintain an increasingly lower adult density. Because of this, population suppression has usually involved the use of larvicides in addition to adulticides (Gould et al., 1970; Gould et al., 1971).

Figure 15a shows the effects of a predator release resulting in 1 predator egg in 80% of the containers on day 98 during a period of no rainfall. Notice that a 75% reduction in Ae. aegypti adult density occurs within 20 days. The Aedes adult blip beginning about day 170 results from the eclosion of the predator. The effects of the subsequent

Table 10. Effects of contact adulticide application causing 95% mortality with no residual action on Ae. aegypti. (Numbers reported are on a per container basis.)

Effects	Frequency of Application			
			Treatment applied when adult density exceeds . . .	
	No treatment	Once	7 adults/container	4 adults/container
Mean no. adults	8	8	3	2
Mean no. immatures	49	49	28	21
SI	0.75	0.75	0.84	0.88
No. applications/year	0	1	15	21

Figure 12a. Effects on Ae. aegypti adults of a contact adulticide application at day 90 causing 95% adult mortality with no residual effects. (No rain)

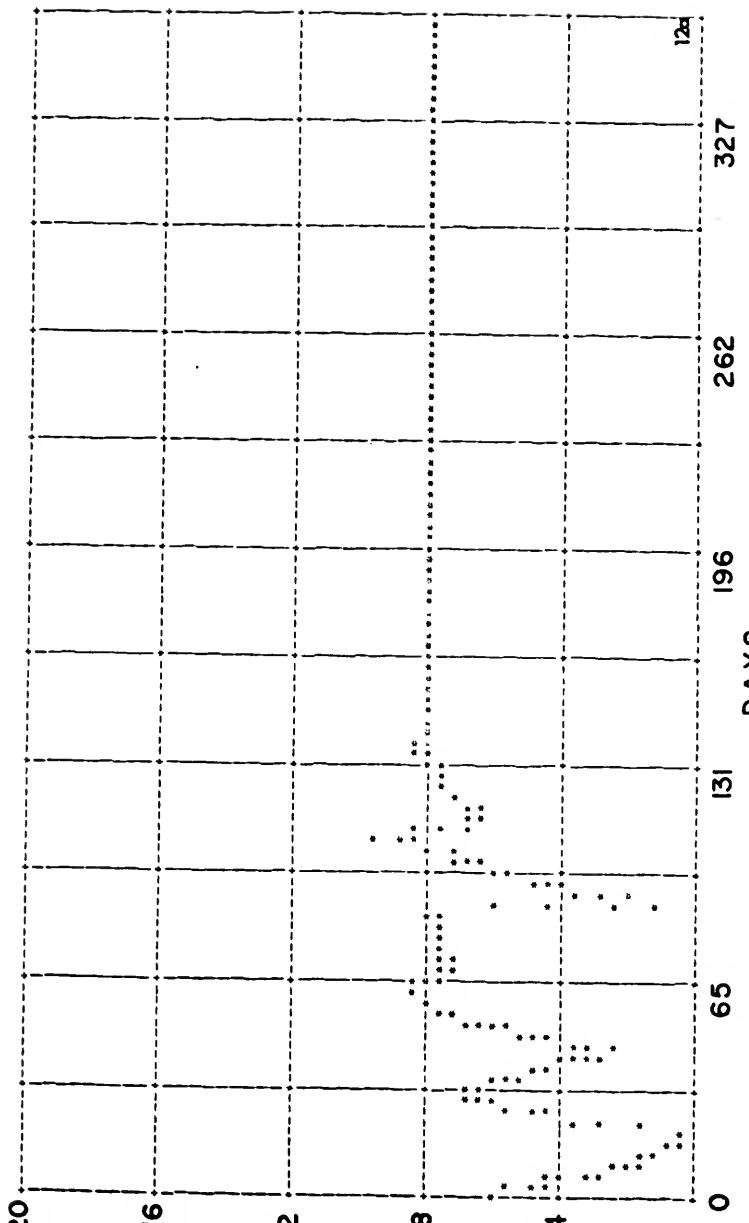

20
16
12
8
4
0
65
131
196
262
327
DAYS
12a

Figure 12b. Effects on Ae. aegypti immatures when adulticide applied as in Fig. 12a.

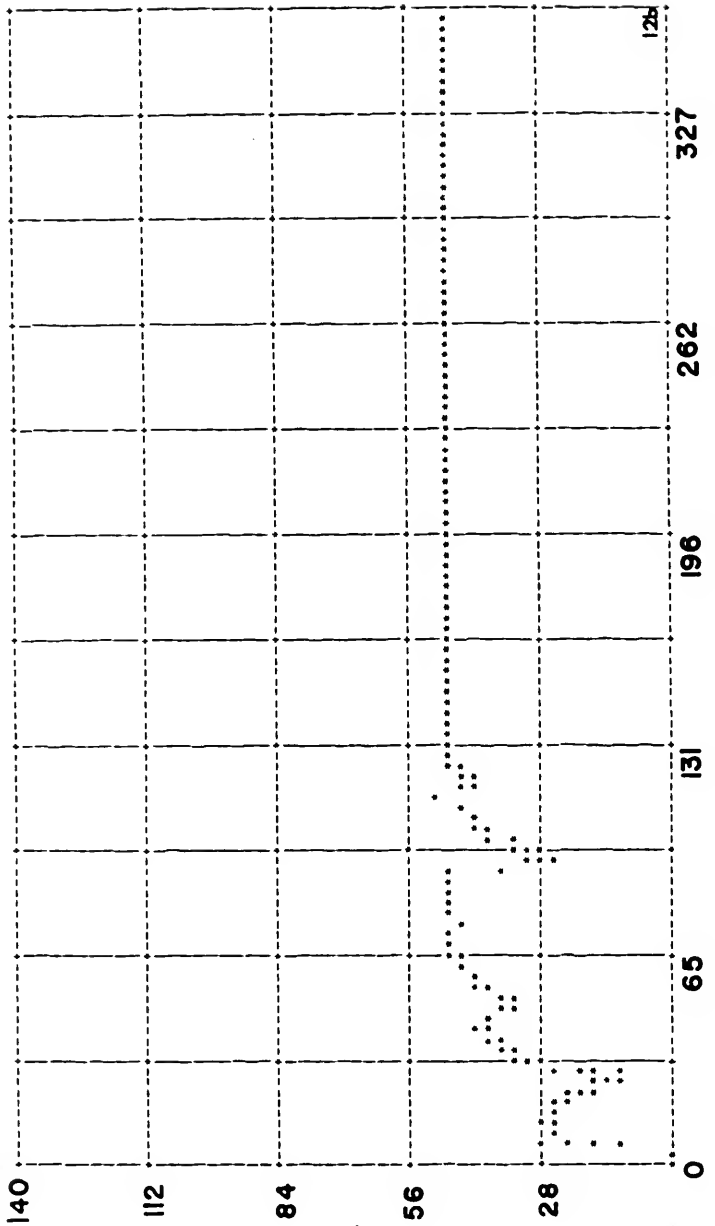

140
112
84
56
28
0
65
131
196
262
327

Figure 13a. Effects on Ae. aegypti adults of a contact adulticide application applied when Aedes adult density exceeded 7 adults/container. Spraying at this density resulted in 15 applications in 262 days. (No rain)

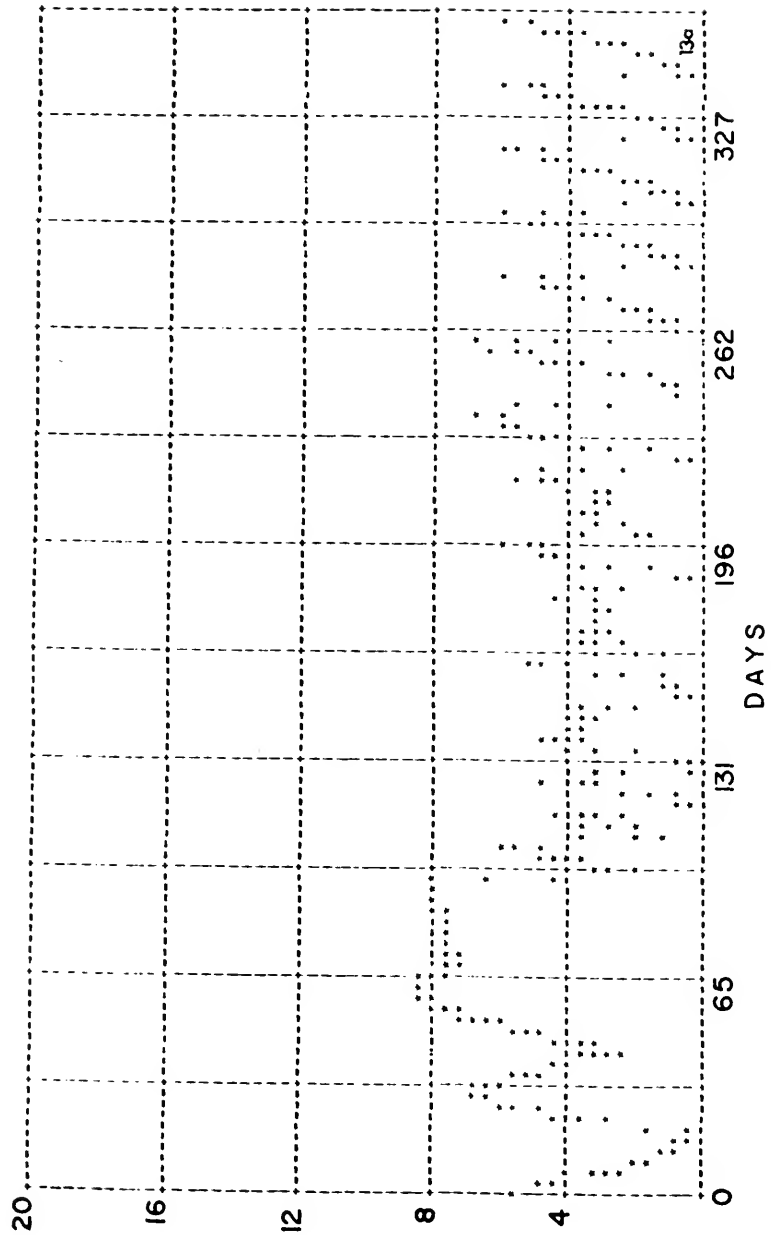

20
16
12
8
4
0
65
131
196
262
327
DAYS
13a

Figure 13b. Effects on Ae. aegypti immatures when adulticide applied as in Fig. 13a.

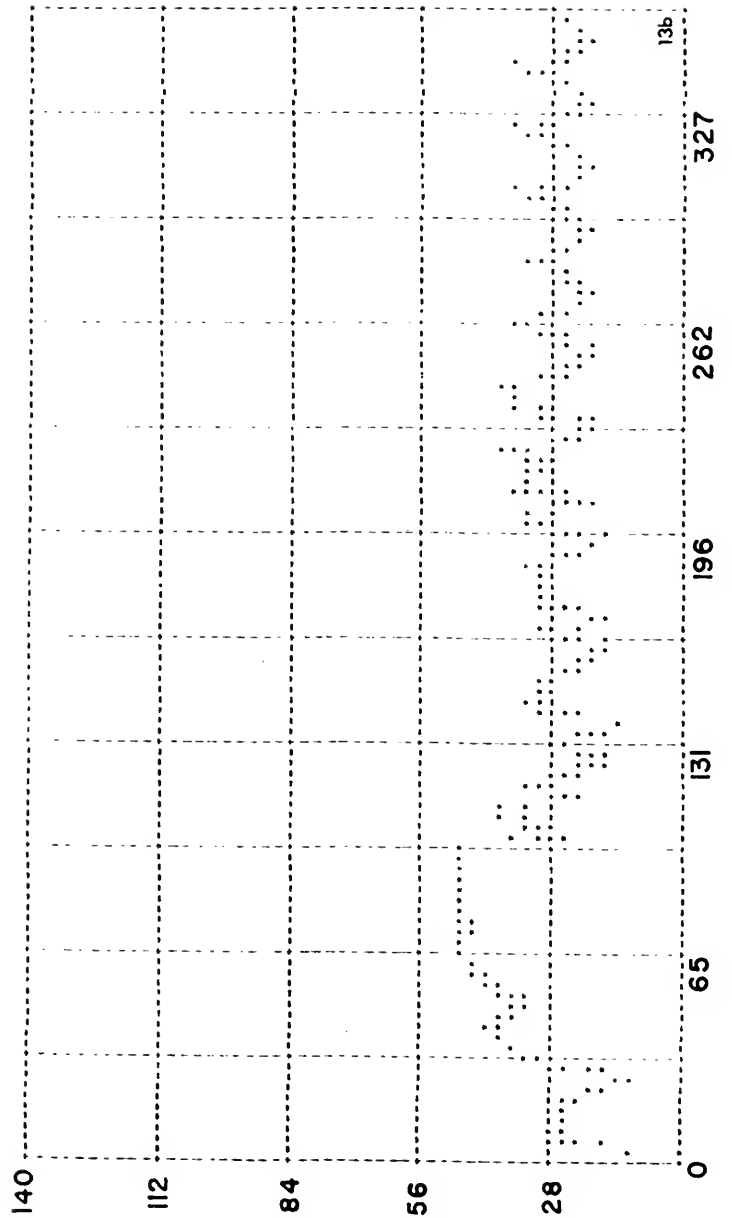

140
112
84
56
28
0
65
131
196
262
327
13b

Figure 14a. Effects on Ae. aegypti adults of a contact adulticide application applied when Aedes adult density exceeded 4 adults/container. Spraying at this density resulted in 21 applications during the period 98-360 days. (No rain)

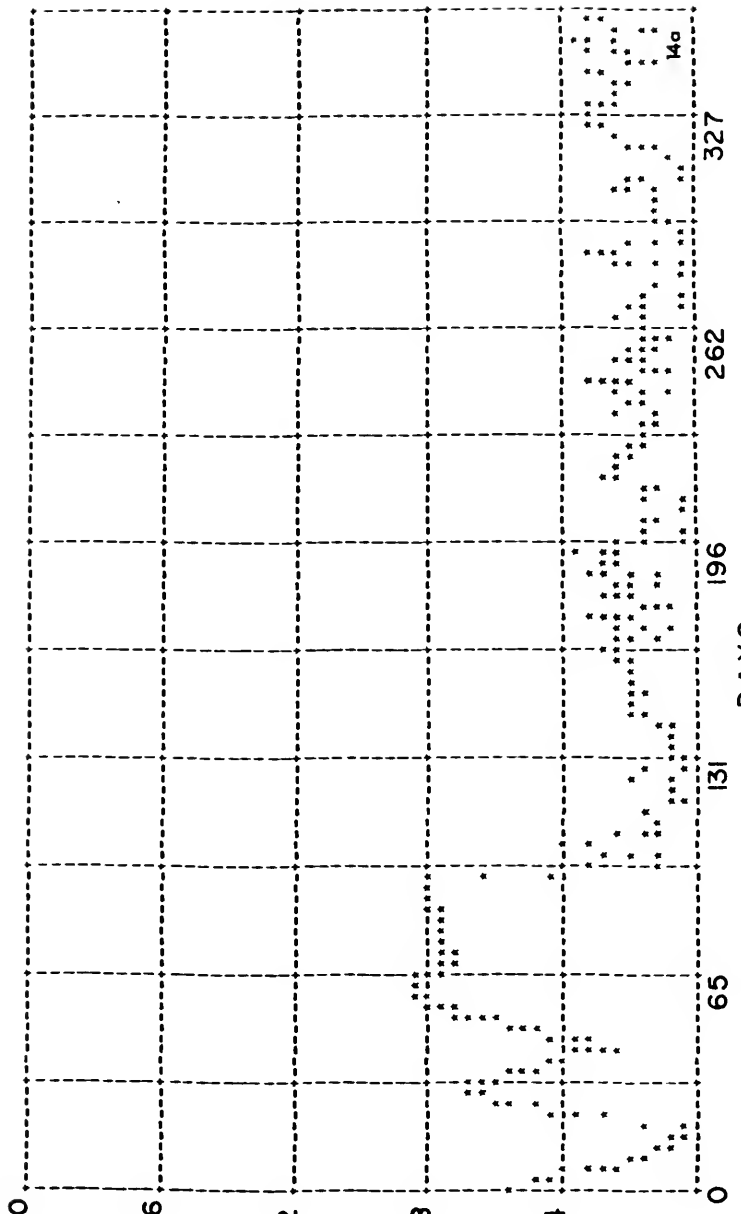

20
16
12
8
4
0
65
131
196
262
327
DAYS
140

Figure 14b. Effect on Ae. aegypti immatures when adulticide applied as in Fig. 14a.

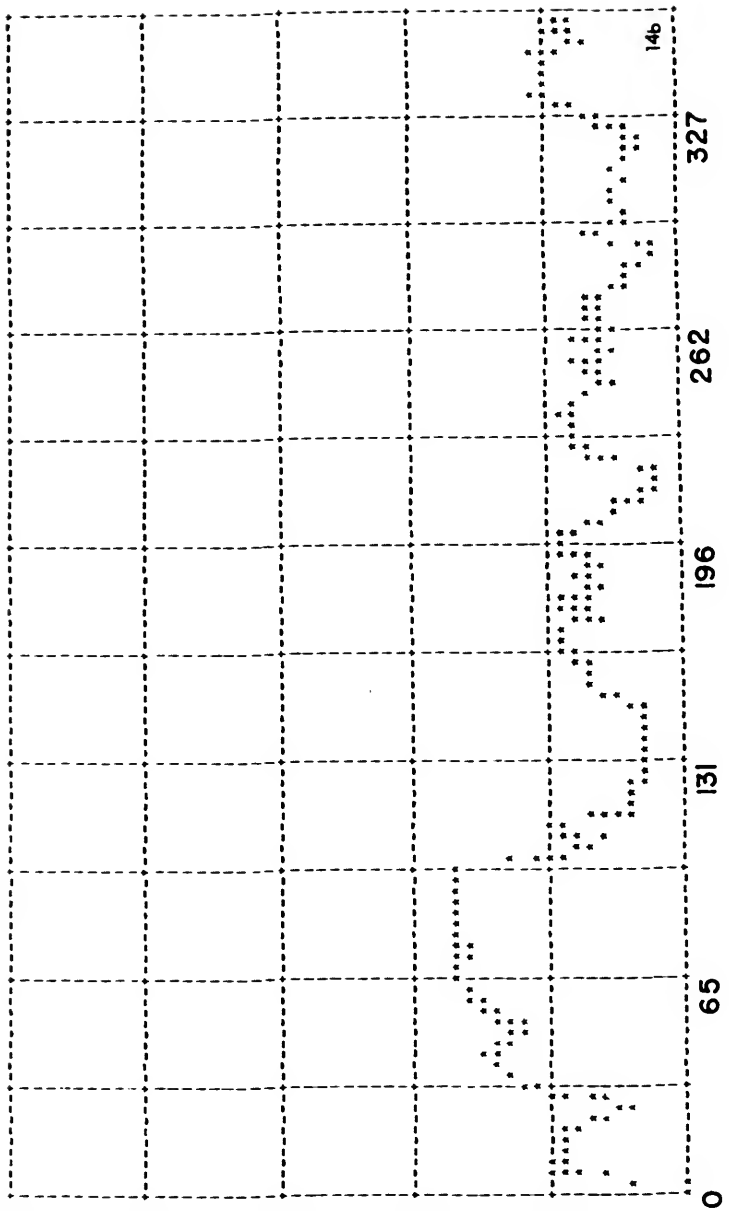
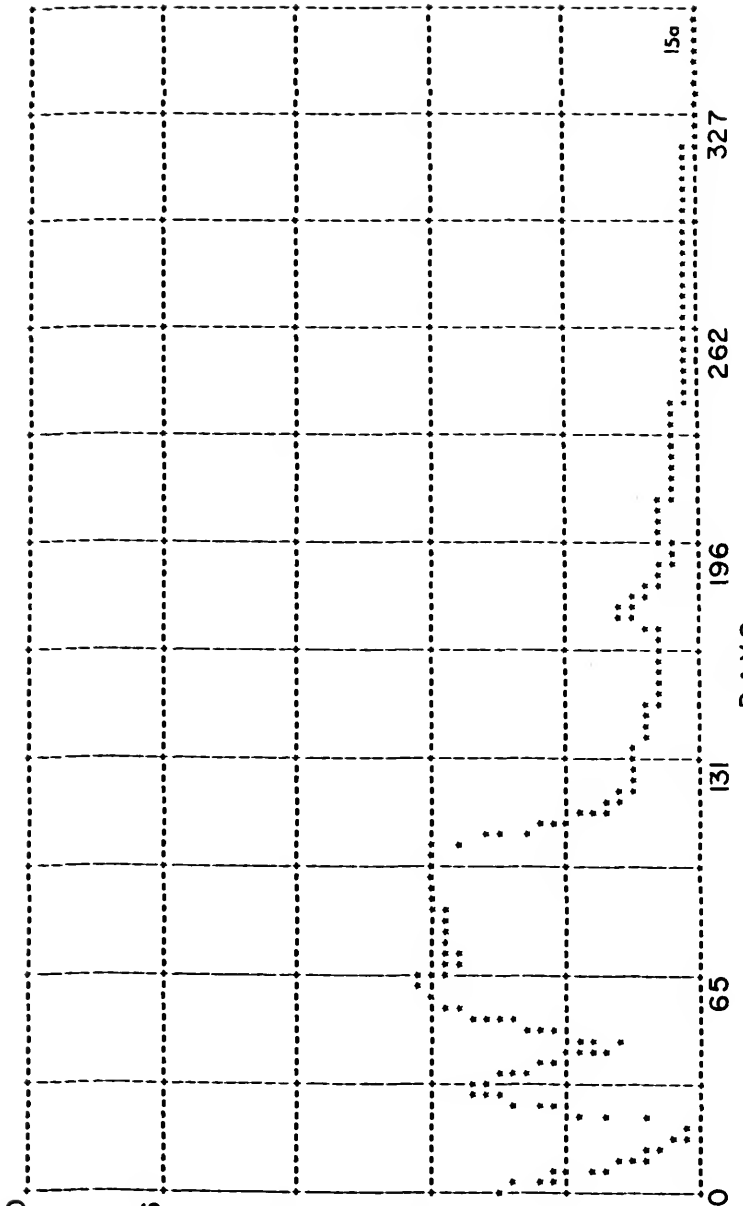

140
112
84
56
28
0
65
131
196
262
327
14b

Figure 15a. Ae. aegypti adult density following an adult Tx. r. rutilus release on day 98 producing 1 predator egg in 80% of the containers positive for prey. (No rain)


20
16
12
8
4
0
65
131
196
262
327
DAYS
150

2nd generation of predators resulting from the release are shown in Figure 15a after day 185. Until actual field trials are made, the reality of the effects of a 2nd generation will remain uncertain. The model does depict, however, smaller and smaller subsequent generations of predator -- results which are consistent with known equilibrium densities of predator and prey in nature. Figure 15b reveals that 1 predator larva is sufficient to deplete a water storage container in 6-8 days. The model further shows continued oviposition by declining numbers of Ae. aegypti provides food allowing the predator to develop and eclose in approximately 53 days.

Simulations involving larger adult releases producing greater numbers of predator eggs/container did not appreciably alter the Ae. aegypti decline. This is reasonable in that 1 predator/container is sufficient to stop prey breeding in that particular container, and additional predators are superfluous. Larger numbers of predators/container further exacerbates the prey shortage producing even longer development times. In the field, the variable number of predators/container would likely result in asynchrony of predator eclosion.

Since rainfall increases the number of young prey larvae, 5 predators/container are required to eliminate aegypti breeding in containers positive for Toxorhynchites. Figure 16 refers to the failure of 3 predators/container to establish control. Furthermore, the increased food supply

Figure 15b. The effect of 1 predatory larva/container in 80% of the containers positive for Ae. aegypti. The blip at 165 days results when the predator ecloses from the container. Predator release on day 98.

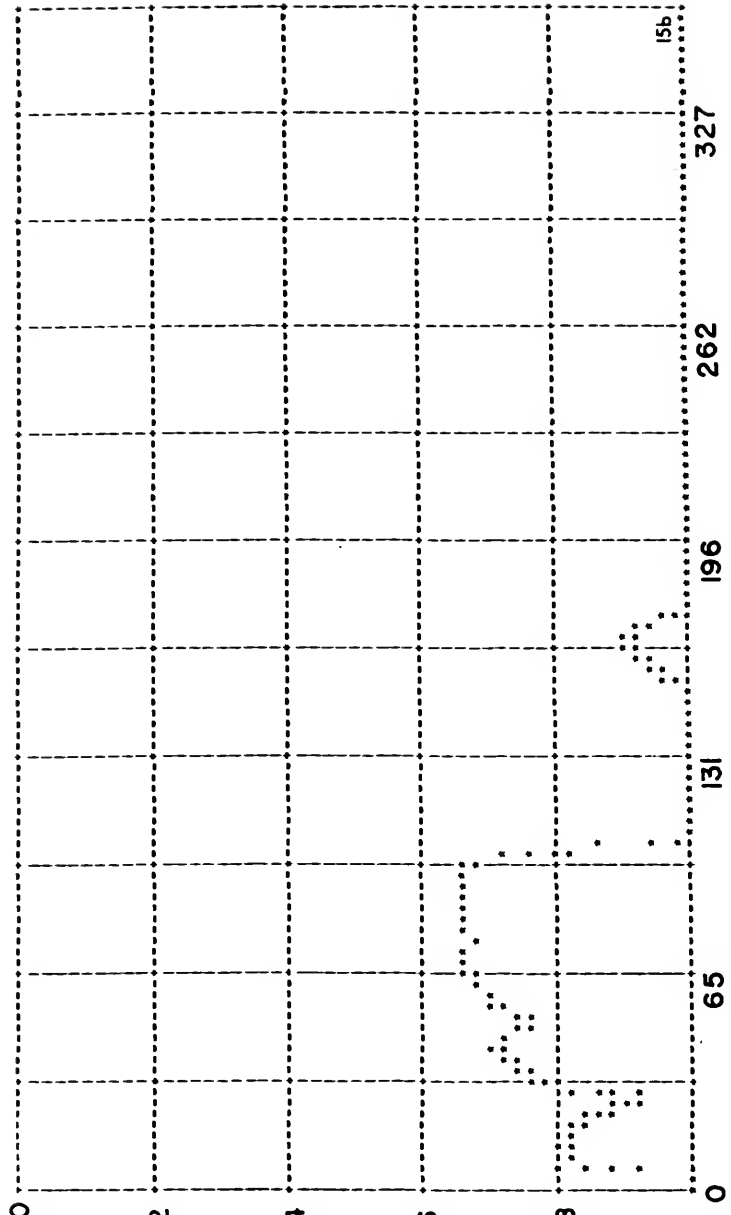
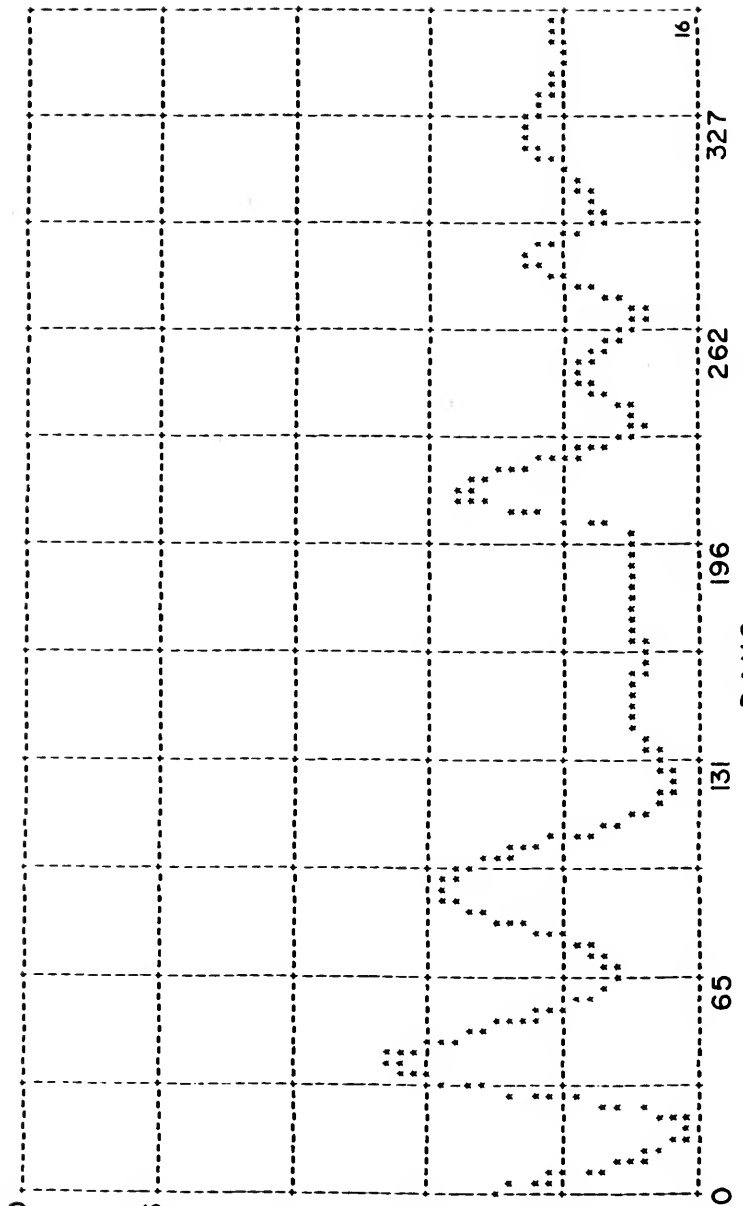

140
112
84
56
28
0
65
131
196
262
327
15b

Figure 16. The effect on Ae. aegypti adult density from an adult predator release on day 98 resulting in 3 predator larvae/container in 80% of the containers positive for Ae. aegypti. (Daily rain)


20
16
12
8
4
0
65
131
196
262
327
DAYS
16

results in shorter predator development times resulting in shorter periods of control from the first cohort of predators. The ramifications of this depend on the results of subsequent generations of predators resulting from the original release. At any rate, the lower numbers of predators required in the dry season to initiate control indicate the logical time to attempt control.

Figures 17a, b and c represent expected Ae. aegypti adult densities when the proportion of containers receiving 1 predator egg/container is 0.6, 0.8 and 0.9, respectively. The preceeding discussion and these figures lend support to the idea that the most important parameter determining the degree of control established is the distribution of predator eggs (= DISTBN). It seems likely that energy expended to improve predator distribution by releasing smaller numbers of adults at more sites would be well spent.

Simulations involving the use of adulticides and predators reveal the following: 1) One adulticide application shortly before or after a predator release does not significantly decrease the resulting prey density nor increase the rate at which it is achieved (Figure 18). 2) Several applications 2 or 3 days apart after a predator release should result in immediate and sustained prey densities near zero (Figure 19). The maintenance of control is again, a function of the efficacy of the resulting subsequent predator generations.

Figure 17a. The effect on Ae. aegypti adults of a predator release on day 107 resulting in 1 predator egg/container in 60% of the containers positive for Ae. aegypti immatures. (No rain)

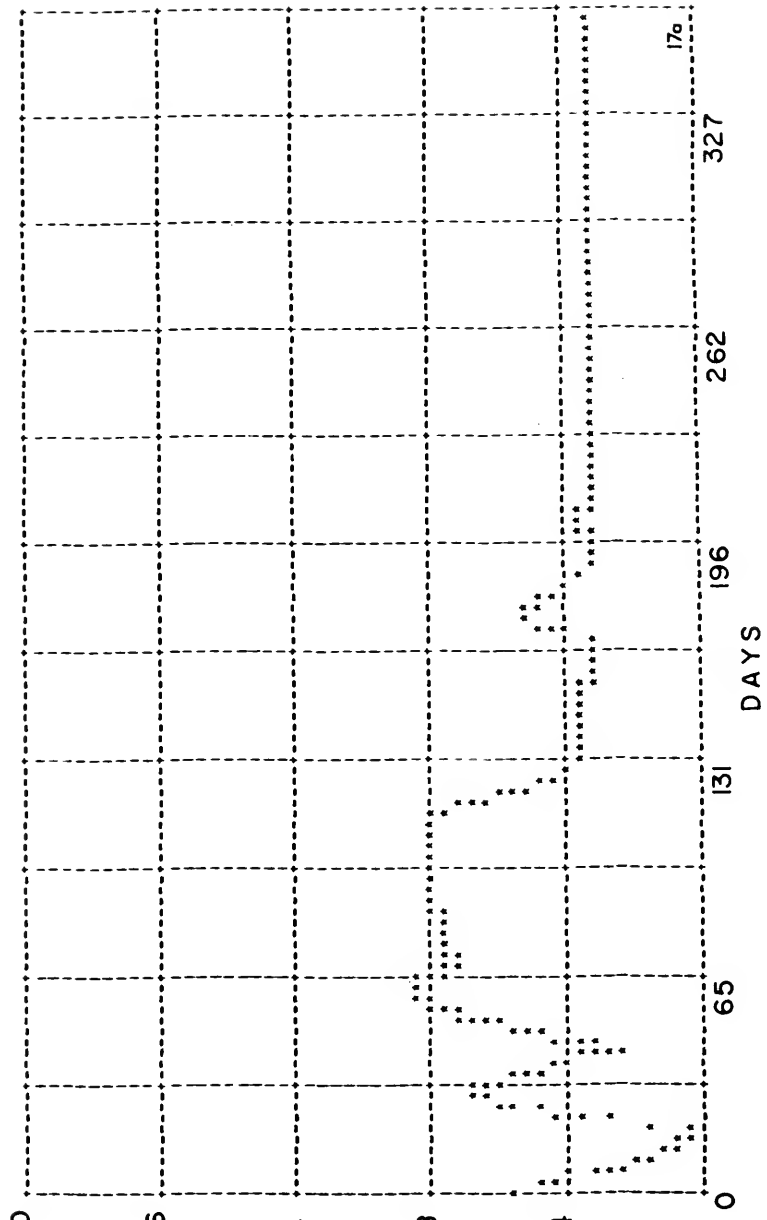

20
16
12
8
4
0
65
131
196
262
327
DAYS
17a

Figure 17b. The effect on Ae. aegypti adults of a predator release on day 107 resulting in 1 preador egg/container in 80% of the containers positive for Ae. aegypti immatures. (No rain)

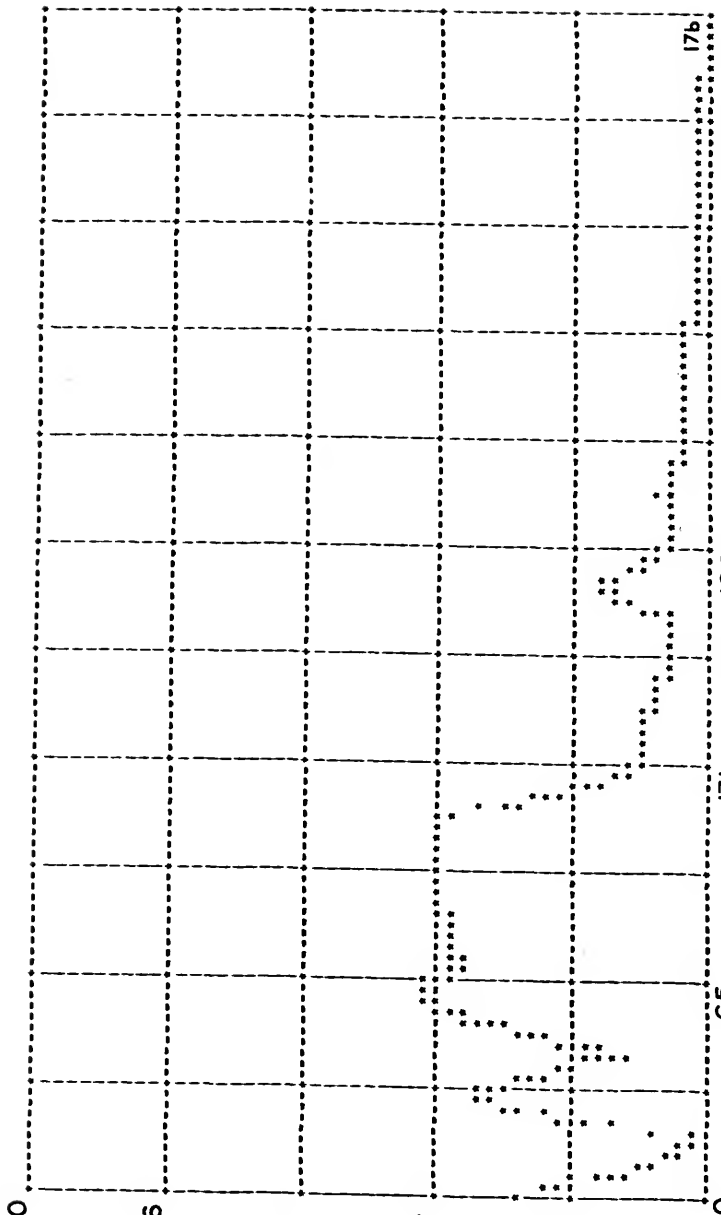

20
16
12
8
4
0
65
131
196
262
327
DAYS
176

Figure 17c. The effect on Ae. aegypti adults of a predator release on day 107 resulting in 1 predator egg/container in 90% of the containers positive for Ae. aegypti immatures. (No rain)

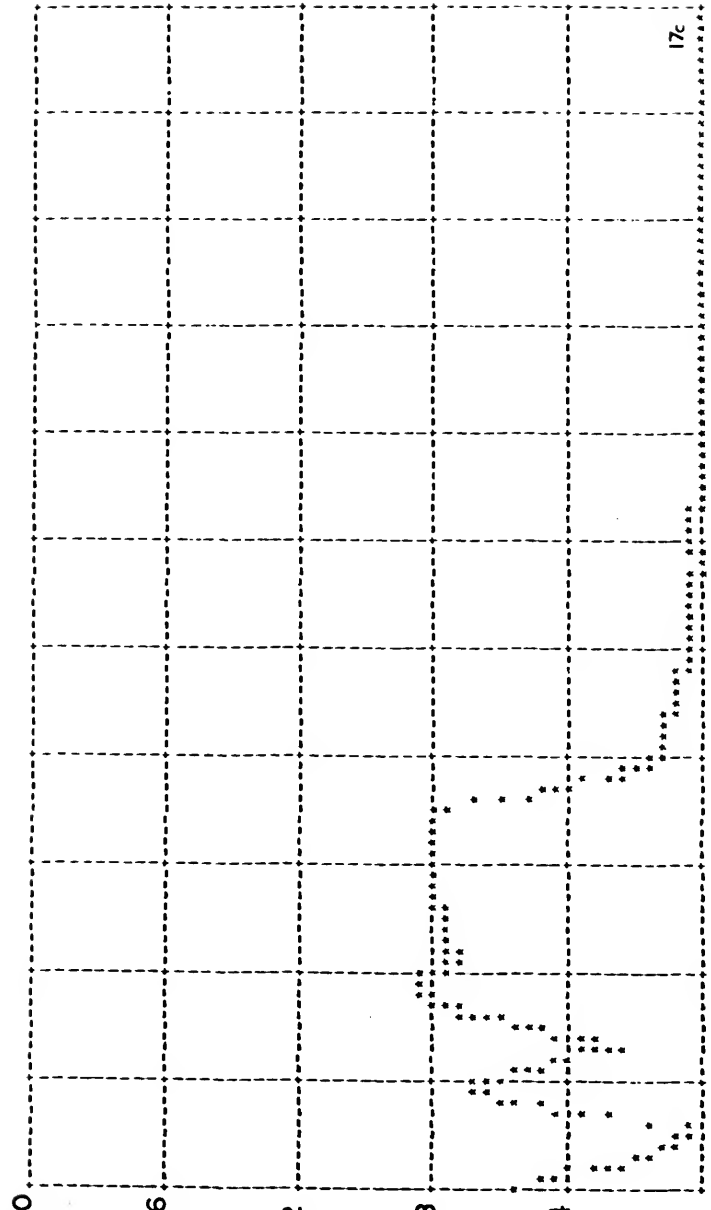

17c
20
16
12
8
4
0
65
131
196
262
327
DAYS

Figure 18. Adult Ae. aegypti densities resulting from an adulticide application at day 90 followed by a predator release on day 98. Compare with Figures 15a and 12a. (No rain)

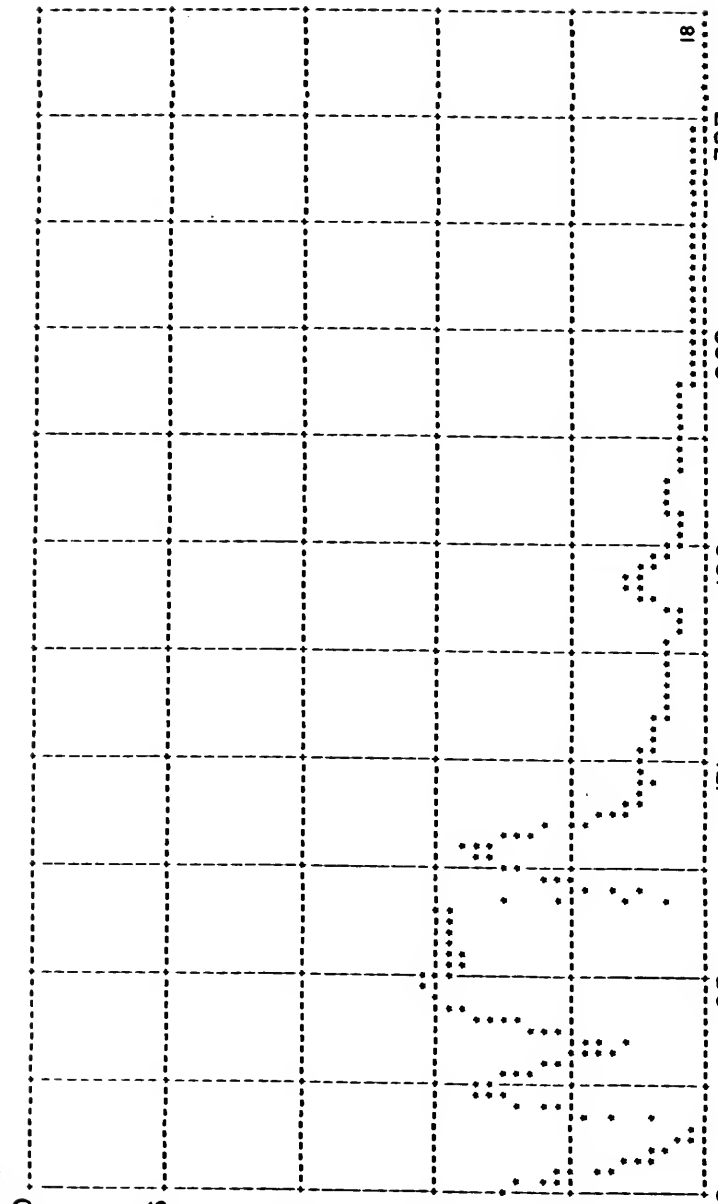
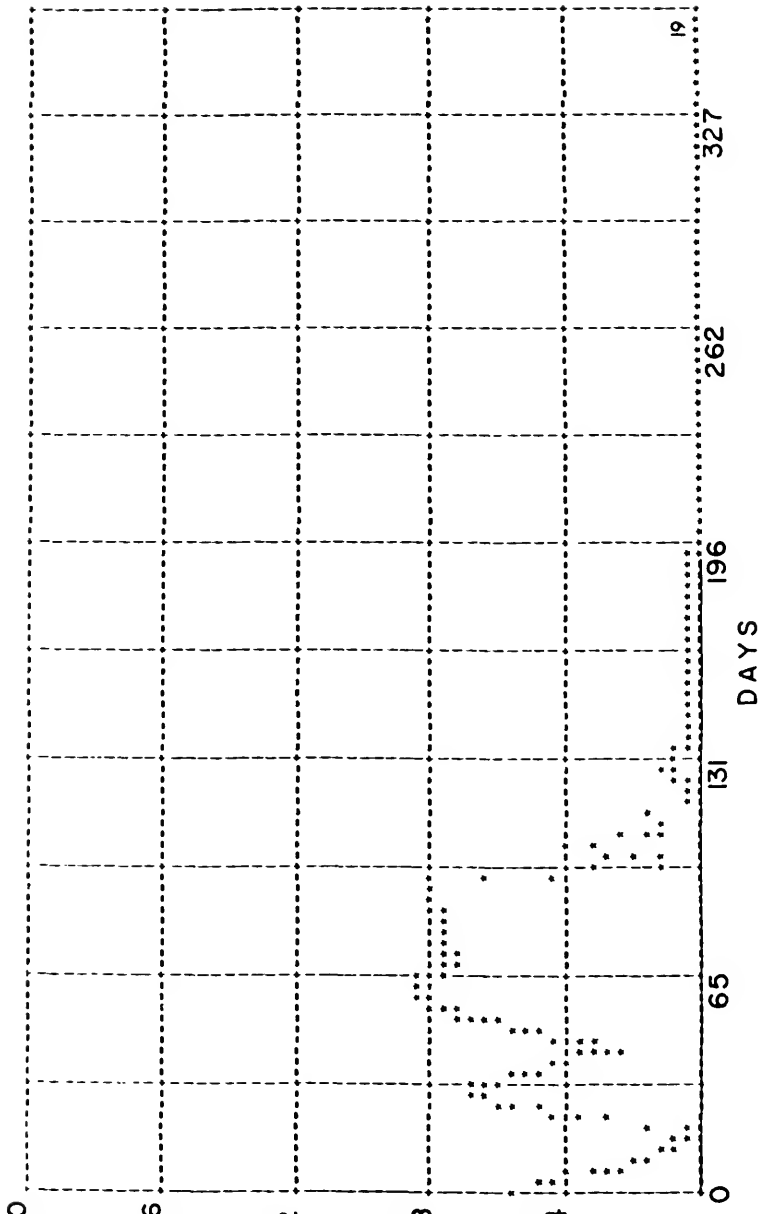

20
16
12
8
4
0
65
131
196
262
327
DAYS
18

Figure 19. Adult Ae. aegypti densities resulting from a predator release and 5 subsequent adulticide applications on days 98, 101, 109, 113 and 117. Applications were made only when Aedes adult density exceeded 4 adults/container. Compare with Figure 15a. (No rain)


20
16
12
8
4
0
65
131
196
262
327
DAYS
19

Conclusions

As stated previously, the computer model is a tool useful in evaluating and developing new control strategies. The utility of Toxorhynchites in controling container-breeding mosquitoes will be demonstrated in the field and not in a computer. Demonstrated here is the feasibility of control and a justification for larger scale field work.

Field data on predator distribution, the effects of second and third generation predators resulting from an initial release, and field survival values for the immature predator stages, will make it possible to develop an accurate stochastic model enabling the evaluation of a strategy involving insecticides, predators and the sterile-male technique.

APPENDIX

FORTRAN MODEL OF *Aedes/Toxorhynchites* POPULATION DYNAMICS

```
      REAL GRAPH(3000), DAY(360), SUMA(360), SUMTA(360),
     1SUM1(360), SUM2(360), SUM3(360), X1(360), X2(360),
     1X3(360), X4(360), X5(360), X6(360), X7(360)
      DATA DAY, SUMA, SUMTA, SUM1, SUM2, SUM3, X1, X2,
     1X3, X4, X5, X6, X7/ 360*0., 360*0., 360*0., 360*0.,
     1360*0., 360*0., 360*0., 360*0., 360*0., 360*0., 360*0.,
     1360*0.     /
      REAL B1(360), B2(360), B3(360), B4(360)
      DATA B1,B2,B3,B4/360*0.,360*0.,360*0.,360*0./
      REAL C1(360),C2(360),C3(360),C4(360)
      DATA C1,C2,C3,C4/ 360*0.,360*0.,360*0.,360*0./
      REAL D1(360)
      DATA D1/360*0./
      REAL B5(360), C5(360)
      DATA B5,  C5  /360*0.,360*0./
      REAL A(8),TA(7)
C           A & TA REPRESENT AEDES & TOX. ADULTS
C
      REAL E(6),X(5),Y(12)
C           AEDES IMMATURES IN CONTAINERS WITHOUT TOX.
C           WHERE E=EGGS, X=STAGES 1&2,& Y=STAGES 3,4&PUPA.
C
      REAL TEMP12(24), TEMP3(40), TEMP4(108)
      DATA  TEMP12, TEMP3, TEMP4 /24*0., 40*0., 108*0.  /
      REAL ET(6),XA(5),YA(12)
C           AEDES IMMATURES IN CONTAINERS POSITIVE FOR TOX.
C           ET=AE.EGGS, XA=AE.STAGES 1&2 & YA REPRESENTS STAGES 3-PUPA
C
      REAL  T12(24),T3(40),T4(108),TP(6), TE(3)
C           TOXORHYNCHITES IMMATURE ARAYS, T12=STAGES 1&2, T3=STAGE 3
C           T4=STAGE-4, TP=PUPA, &TE=EGGS
C
      DATA  A,TA/ 8*0.,7*0.    /
```

```
      DATA  E,X,Y/ 6*0.,5*0.,12*0. /
      DATA  ET,XA,YA/ 6*0.,5*0.,12*0.  /
      DATA  T12,T3,T4,TP/ 24*0.,40*0.,108*0.,8*0. /
      DATA  TE/ 3*0.   /
      I=0
C
C           AEDIST=PROPORTION OF CONTAINERS POSITIVE FOR AEDES LARVAE
      SA=0.88
C           SA=ADULT DAILY SURVIVAL
      F=93
C           F=FECUNDITY (EGGS/BATCH)
      P=0.90
C           P=PROPORTION OF PUPATION WHICH IS SUCCESSFUL
      SE=0.98
C           SE=DAILY EGG SURVIVAL
      S=0.95
C           S=DAILY SURVIVAL FOR STAGES 3,4 & PUPA
      C=0.01
C           C=DENSITY DEPENDENT COEFFICIENT FOR CALCULATING
C           S1,WHERE S1=DAILY LARVAL SURVIVAL,STAGES 1 &2.
C
C
C     ***TOX. PARAMETERS***
      SAT=0.88
C           SAT=DAILY ADULT SURVIVAL.
      FT=1.0
C           FT=DAILY EGG PRODUCTION.
      SIT=0.99
C           SIT=DAILY IMMATURE SURVIVAL, STAGES 1-PUPA.
C           DISTBN=PROPORTION OF CONTAINERS POSITIVE FOR TOX. EGGS.
      DISTBN=0.50
C     ***************************************************************
      IRAIN=1
```

```
 1000 I=I+1
C              INITIALIZE AEDES WITH 7.4 ADULTS/CONTAINER
      IF(I.EQ.1) A(1)=7.4
C     BELOW REPRESENTS AN ADULTICIDE APPLICATION CAUSING 95% MORTALITY
      IF(I.LT.98) GOTO 1501
C     R=ADULT DENSITY WHEN OLV IS APPLIED
      R=1000
      SA=0.05
      IF (SUMA(I-1).GT.R) SA=0.05
      IF(SA.EQ..05) GOTO 1500
      GOTO 1501
 1500 WRITE (6,1502) I
 1502 FORMAT (1X,I3)
 1501 CONTINUE
      IF (I.EQ.107) TE(1)=1
C           ***AEDES ADULTS***
      A(8)=A(7)*SA
      A(7)=A(6)*SA
      A(6)=A(5)*SA
      A(5)=A(4)*SA
      A(4)=A(3)*SA+A(8)*SA
      A(3)=A(2)*SA
      A(2)=A(1)*SA
      A(1)=(Y(12)*P*(1-DIST8N))+(YA(12)*P*DIST8N)
C           ***AEDES LARVAE IN CONTAINERS WITHOUT TOX. LARVAE***
C
C     FOLLOWING MOVES STAGES 3-PUPA
  150 Y(12)=Y(11)*S
      Y(11)=Y(10)*S
      Y(10)=Y(9)*S
      Y(9)=Y(8)*S
      Y(8)=Y(7)*S
      Y(7)=Y(6)*S
```

```
      Y(6)=Y(5)*S
      Y(5)=Y(4)*S
      Y(4)=Y(3)*S
      Y(3)=Y(2)*S
      Y(2)=Y(1)*S
C     FOLLOWING CALCULATES SI FOR STAGES 1 AND 2
      SUM=X(1)+X(2)+X(3)+X(4)+X(5)
      IF(SUM.EQ.0) SI=1.0
      IF(SUM.EQ.0) GOTO 30
      IF(SUM.LT.15) GOTO 10
      IF(SUM.GT.120) GOTO 20
      XNT= SUM*(EXP(-C*SUM))
      SI=XNT/SUM
      GOTO 30
   10 SI=0.85
      GOTO 30
   20 SI=120/SUM
C     FOLLOWING MOVES STAGES 1 AND 2
   30 Y(1)=X(5)*SI
      X(5)=X(4)*SI
      X(4)=X(3)*SI
      X(3)=X(2)*SI
      X(2)=X(1)*SI
C               THE FOLLOWING CONTROLS EGG HATCH OVIPOSITION
      E(6)=(L(5)+E(6))*SL
      E(5)=E(4)*SL
      E(4)=E(3)*SL
      E(3)=E(2)*SL
      E(2)=E(1)*SL
C
C               AEDES OVIPOSITION INTO TOX-FREE CONTAINERS
      L(1)=A(4)*F*0.5
C               EGG HATCH AS FUNCTION OF RAINFALL (IRAIN)
```

```
      IF(IRAIN.EQ.1) GOTO 100
      A(1)=E(5)*0.2
      E(5)=E(5)*0.8
      GOTO 200
  100 A(1)=E(6)*0.5+(E(5)*0.2)
      E(6)=E(6)*0.5
      E(5)=E(5)*0.8
  200 CONTINUE
C           ***AEDES LARVAE IN CONTAINERS WITH TOX. LARVAE***
C
C     FOLLOWING MOVES STAGES 5=PUPA
  151 YA(12)=YA(11)*S
      YA(11)=YA(10)*S
      YA(10)=YA(9)*S
      YA(9)=YA(8)*S
      YA(8)=YA(7)*S
      YA(7)=YA(6)*S
      YA(6)=YA(5)*S
      YA(5)=YA(4)*S
      YA(4)=YA(3)*S
      YA(3)=YA(2)*S
      YA(2)=YA(1)*S
C     FOLLOWING CALCULATES SIX FOR STAGES 1 AND 2
      SUMA=XA(1)+XA(2)+XA(3)+XA(4)+XA(5)
      IF(SUMA.EQ.0) SIX=1.0
      IF(SUMA.EQ.0) GOTO 31
      IF(SUMA.LT.15) GOTO 11
      IF(SUMA.GT.120) GOTO 21
      XNTA= SUMA*(EXP(-C*SUMA))
      SIX=XNTA/SUMA
      GOTO 31
   11 SIX=0.66
      GOTO 31
```

```
   21 SIX=120/SUMA
C     FOLLOWING MOVES STAGES 1 AND 2
   31 YA(1)=XA(5)*SIX
      XA(5)=XA(4)*SIX
      XA(4)=XA(3)*SIX
      XA(3)=XA(2)*SIX
      XA(2)=XA(1)*SIX
C              THE FOLLOWING CONTROLS EGG HATCH& OVIPOSITION
      ET(6)=(ET(5)+ET(6))*SE
      ET(5)=ET(4)*SE
      ET(4)=ET(3)*SE
      ET(3)=ET(2)*SE
      ET(2)=ET(1)*SE
C
C              AEDES OVIPOSITION INTO CONTAINERS POSITIVE FOR TOX.
      ET(1)=A(4)*F*0.5
C              EGG HATCH AS FUNCTION OF RAINFALL (IRAIN)
      IF(IRAIN.EQ.1) GOTO 101
      XA(1)=ET(5)*0.2
      ET(5)=ET(5)*0.8
      GOTO 201
  101 XA(1)=ET(6)*0.5+(ET(5)*0.2)
      ET(6)=ET(6)*0.5
      ET(5)=ET(5)*0.8
  201 CONTINUE
C             ***BELOW REPRESENTS TOX. IMMATURES & THEIR PREDATION***
C                    ON AEDES LARVAE IN THE ABOVE SECTION
C
C              THE FOLLWLING INTERFACES 182-STAGE AEDES
C              WITH 182-STAGE TOX.
      SMXA1=0
C     SMXA1=NO. 182-STAGE AE. LARVAE IN AE./TX. CONTAINER
      DO 15 KN=1,5
```

```
   15 SMXAI=XA(KN)+SMXAI
      SMTI2=0
C     SMTI2=NO. 182-STAGE TOX. LARVAE
      DO 71 M=1,18
   71 SMTI2=SMTI2+TI2(M)
      IF(SMXAI.GT.SMTI2*4) GOTO 29
      IF(SMXAI.EQ.0) IZ=1
      IF(SMXAI.EQ.0) GOTO 39
      IF(SMTI2.EQ.0) GOTO 661
      IZ= INT(SMXAI/SMTI2)
      IF(IZ.EQ.0) IZ=1
      DO 61 L=1,5
   61 XA(L)=0
      GOTO 39
   29 IZ=4
      ATE=(4*SMTI2)
      IF(ATE.EQ.0) GOTO 39
      BE=ATE/SMXAI
      CC=1-BE
      DO 52 K=1,5
   52 XA(K)=XA(K)*CC
   39 CONTINUE
      DO 41 J=1,18
      TEMPI2(J+IZ)=TI2(J)*SIT
   41 CONTINUE
      DO 999 MM=1,24
  999 TI2(MM)=TEMPI2(MM)
  661 CONTINUE
C
C             THE FOLLOWING INTERFACES 364-STAGE TOX. WITH ALL STAGES
C             OF AEDES IMMATURES. MODEL ASSUMES AEDES 182-STAGE LARVAE
C             ARE EATEN FIRST FOLLOWED BY 3,4&P-STAGE AEDES. NOTICE
C             THAT FOR EACH DAY,THE 182-STAGE TOX. EAT FIRST.
```

```
      T3(1)=T12(17)+T12(18)+T12(19)+T12(20)
      DO 400 K30=17,20
      T12(K30)=0
  400 CONTINUE
C                 SMXA2=NO. AEDES LARVAE,STAGE 1&2,AVAILABLE AS FOOD.
      SMXA2=0
      DO 33 N=1,5
   33 SMXA2=SMXA2+XA(N)
C                 SMY=NO. 3,4& PUPAL-STAGE AEDES AVAILABLE AS FOOD.
      SMY=0
      DO 32 LL=1,12
   32 SMY=SMY+YA(LL)
      TOTO=SMXA2+(SMY*7.1111)
C                 NOTE-1 3,4 OR PUPA-STAGE AEDES IS EQUIVLENT TO 7.111
C                 1 OR 2-STAGE AEDES LARVAE. TOTO=AMOUNT OF FOOD AVAILABLE.
C                 ST3=NO. 3-STAGE TOX., ST4=NO. 4-STAGE TOX.
      ST3=0
      DO 42 II=1,32
   42 ST3=ST3+T3(II)
      ST4=0
      DO 85 JJ=1,96
      ST4=ST4+T4(JJ)
   85 CONTINUE
C                 REQ3&4 ARE THE NO. OF AEDES EQUIV. NECESSARY TO MOVE 1
C                 REGULAR DAY.
      REQ3=ST3*14
      REQ4=ST4*64
      TREQ=REQ3+REQ4
      IF(TOTO.EQ.0) IT3=1
      IF(TOTO.EQ.0) IT4=1
      IF(TOTO.EQ.0) GOTO 105
      IF(TREQ.EQ.0) GOTO 106
      IF(TREQ.LE.TOTO) GOTO 115
```

```
      DO 22 KK=1,5
      XA(KK)=0
   22 CONTINUE
      DO 12 KI=1,12
      YA(KI)=0
   12 CONTINUE
      G=TOTO/TREQ
C     IT3&4 INCREMENT THEIR RESPECTIVE ARAYS WHEN LESS THAN MIN. FOOD
C           IS AVAILABLE.
      IT3=INT(8*G)
      IT4=INT(12*G)
      IF (IT3.EQ.0) IT3=1
      IF (IT4.EQ.0) IT4=1
      GOTO 105
  115 IT3=8
      IT4=12
C     ITERATIONS FOR 3&4-STAGE TOX. WHEN AMPLE FOOD AVAILABLE.
      BAL=TREQ-SMXA2
C     BAL HERE REPRESENTS THE NO. UNITS REQD. FROM YA.
      IF(BAL.GT.0) GOTO 90
      SIAX=ABS(BAL/SMXA2)
      DO 91 MI=1,5
   91 XA(MI)=XA(MI)*SIAX
      GOTO 105
   90 CONTINUE
      DO 92 MJ=1,5
   92 XA(MJ)=0
      BAL=ABS(BAL)
      SIYA=((SMY*7.111)-BAL)/(SMY*7.111)
      DO 125 IN=1,12
  125 YA(IN)=YA(IN)*SIYA
  105 CONTINUE
      DO 106 KJ=1,32
```

```
      TEMP3(KJ+IT3)=T3(KJ)*SIT
  106 CONTINUE
      DO 998 M5=1,40
      T3(M5)=TEMP3(M5)
  998 CONTINUE
      DO 107 KM=1,96
      TEMP4(KM+IT4)=T4(KM)*SIT
  107 CONTINUE
      DO 997 M6=1,108
      T4(M6)=TEMP4(M6)
  997 CONTINUE
      T4(1)=0
      DO 555 LM=33,40
      T4(1)=T4(1)+T3(LM)
  555 CONTINUE
      DO 401 K31=33,40
      T3(K31)=0
  401 CONTINUE
  108 CONTINUE
      TP(7)=TP(6)*SIT
      TP(6)=TP(5)*SIT
      TP(5)=TP(4)*SIT
      TP(4)=TP(3)*SIT
      TP(3)=TP(2)*SIT
      TP(2)=TP(1)*SIT
      TP(1)=0
      DO 996 M7=97,108
      TP(1)=TP(1)+T4(M7)
  996 CONTINUE
      DO 402 I31=97,108
      T4(I31)=0
  402 CONTINUE
      TA(7)=(TA(7)+TA(6))*SAT
```

```
      TA(6)=TA(5)*SAT
      TA(5)=TA(4)*SAT
      TA(4)=TA(3)*SAT
      TA(3)=TA(2)*SAT
      TA(2)=TA(1)*SAT
      TA(1)=TP(7)*SIT
      TE(3)=TE(2)
      TE(2)=TE(1)
C     TOX. OVIPOSITION & EGGS
      TE(1)=TA(7)*0.5*FT*AEDIST
      T12(I)=TE(3)
      C1(I)=Y(1)+Y(2)+Y(3)+Y(4)
      C2(I)=Y(5)+Y(6)+Y(7)+Y(8)+Y(9)+Y(10)
      C3(I)=Y(11)+Y(12)
C     C1,2 &3 ARE THE NO. OF AE. STAGE 3,4 AND PUPA
C          IN CONTAINERS WITHOUT TX.
      C4(I)=SI
      C5(I)=SIX
C     SUMA & SUMTA ARE THE NO. OF AE. AND TOX. ADULTS.
      SUMA(I)=0
       DO 801 J1=1,8
      SUMA(I)=SUMA(I)+A(J1)
  801 CONTINUE
      SUMTA(I)=0
      DO 802 J2=1,7
  802 SUMTA(I)=SUMTA(I)+ TA(J2)
      DAY(I)=I
      D1(I)=0
      DO 803 J3=1,5
  803 D1(I)=D1(I)+X(J3)
      X2(I)=0
      DO 804 J4=1,12
  804 X2(I)=X2(I)+Y(J4)
```

```
      SUM1(I)=B1(I)+X2(I)
C     SUM1=SUM OF AE. IMMATURES IN CONTAINERS WITHOUT TX. LARVAE.
      X3(I)=0
      DO 805 J6=1,5
  805 X3(I)=X3(I)+XA(J6)
      X4(I)=0
      DO 806 J7=1,12
  806 X4(I)=X4(I)+YA(J7)
      SUM2(I)=X3(I)+X4(I)
C     SUM2=SUM OF AE. IMMATURES IN CONTAINERS POSITIVE FOR TX. LARVAE.
      X5(I)=0
      DO 807 J7=1,16
  807 X5(I)=X5(I)+T12(J7)
      X6(I)=0
      DO 808 J8=1,32
  808 X6(I)=X6(I)+T3(J8)
      X7(I)=0
      DO 809 J9=1,96
  809 X7(I)=X7(I)+T4(J9)
      SUM3(I)=X5(I)+X6(I)+X7(I)
C     SUM3=SUM OF TX. LARVAE.
      IF(I.LT.360) GOTO 1000
      DO 308 I=1,357
  308 X1(I)=(B1(I)+B1(I+1)+B1(I+2)+B1(I+3))/4
      DO 304 I=1,357
  304 B1(I)=(C1(I)+C1(I+1)+C1(I+2)+C1(I+3))/4
      DO 305 I=1,357
  305 B2(I)=(C2(I)+C2(I+1)+C2(I+2)+C2(I+3))/4
      DO 306 I=1,357
  306 B3(I)=(C3(I)+C3(I+1)+C3(I+2)+C3(I+3))/4
      DO 307 I=1,357
  307 B4(I)=(C4(I)+C4(I+1)+C4(I+2)+C4(I+3))/4
      DO 501 I=1,357
```

```
  501 SUMA(I)=(SUMA(I+1)+SUMA(I)+SUMA(I+2)+SUMA(I+3))/4
      DO 502 I=1,357
  502 SUMTA(I)=(SUMTA(I)+SUMTA(I+1)+SUMTA(I+2)+SUMTA(I+3))/4
      DO 503 I=1,357
  503 SUM1(I)=(SUM1(I)+SUM1(I+1)+SUM1(I+2)+SUM1(I+3))/4
      DO 504 I=1,357
  504 SUM2(I)=(SUM2(I)+SUM2(I+1)+SUM2(I+2)+SUM2(I+3))/4
      DO 505 I=1,357
  505 SUM3(I)=(SUM3(I)+SUM3(I+1)+SUM3(I+2)+SUM3(I+3))/4
      DO 471 I=1,357
  471 C5(I)=(C5(I)+C5(I+1)+C5(I+2)+C5(I+3))/4
C     BELOW PLOTS AE. ADULTS
      CALL PLOT1 (3.51,10.,111,10)
      CALL PLOT2 (GRAPH,360.,0.,20.,0.)
      CALL PLOT3 (1H*,DAY(1),SUMA(1),357)
      WRITE (6,700)
  700 FORMAT (1H1, 'AEDES ADULTS')
      CALL PLOT4 (12,12HAEDES ADULTS)
      WRITE (6,1200)
C     BELOW PLOTS TX. ADULTS
      CALL PLOT1 (3.51,10.,111,10)
      CALL PLOT2 (GRAPH,360.,0.,20.,0.)
      CALL PLOT3(1H*,DAY(1),SUMTA(1),357)
      WRITE(6,701)
  701 FORMAT(1H1,'TOXORHYNCHITES ADULTS')
      CALL PLOT4(11,11HTOX. ADULTS)
      WRITE (6,1200)
C     BELOW PLOTS AE. IMMATURES IN CONTAINERS WITHOUT TX.
      CALL PLOT1 (3.51,10.,111,10)
      CALL PLOT2(GRAPH,360.,0.,140.,0.)
      CALL PLOT3(1H*,DAY(1),SUM1(1),357)
      WRITE (6,702)
  702 FORMAT (1H1,'AE. IMMATURES IN CONTAINERS WITHOUT TX. IMMATURES')
```

```
      CALL PLOT4(15,15HAEDES IMMATURES)
      WRITE(6,1200)
C     BELOW PLOTS AE. STAGE 1 & 2
      CALL PLOT1 (3.51,10.111,10)
      CALL PLOT2 (GRAPH,360.,0.,140.,0.)
      CALL PLOT3 (1H*,DAY(1),A1(1),357)
      WRITE (6,705)
  705 FORMAT (1H1,'AE. STAGES-1&2 IN CONTAINERS WITHOUT TX. LARVAE')
      CALL PLOT4 (9,9HAEDES 1&2)
      WRITE (6,1200)
C     BELOW PLOTS AE. STAGE 3
      CALL PLOT1 (3.51,10.111,10)
      CALL PLOT2 (GRAPH,360.,0.,50.,0.)
      CALL PLOT3 (1H*,DAY(1),B1(1),357)
      WRITE (6,300)
  300 FORMAT (1H1,'AE. STAGE-3 IN CONTAINERS WITHOUT TX. LARVAE')
      CALL PLOT4 (13,13HAEDES STAGE 3)
      WRITE (6,1200)
C     BELOW PLOTS AE. STAGE- 4
      CALL PLOT1 (3.51,10.111,10)
      CALL PLOT2 (GRAPH,360.,0.,25.,0.)
      CALL PLOT3 (1H*,DAY(1),B2(1),357)
      WRITE (6,301)
  301 FORMAT (1H1,'AE. STAGE-4 IN CONTAINERS WITHOUT TX. LARVAE')
      CALL PLOT4 (13,13HAEDES STAGE 4)
      WRITE (6,1200)
C     BELOW PLOTS AE. PUPAE
      CALL PLOT1 (3.51,10.111,10)
      CALL PLOT2 (GRAPH,360.,0.,10.,0.)
      CALL PLOT3 (1H*,DAY(1),B3(1),357)
      WRITE (6,302)
  302 FORMAT (1H1,'AE. PUPAE IN CONTAINERS WITHOUT TX. LARVAE')
      CALL PLOT4 (11,11HAEDES PUPAE)
```

```
      WRITE (6,1200)
C     BELOW PLOTS SI
      CALL PLOT1 (3.51,10.111,10)
      CALL PLOT2 (GRAPH, 360.,0.,1.,0.)
      CALL PLOT3 (1H*,DAY(1),S4(1),357)
      WRITE (6,303)
  303 FORMAT (1H1,'IMMATURE SURVIVAL (SI) FOR STAGES 1&2 AEDES IN
     XCONTAINERS WITHOUT TX.')
      CALL PLOT4 (2,2HSI)
      WRITE (6,1200)
C     BELOW PLOTS SIX
      CALL PLOT1 (3.51,10,111,10)
      CALL PLOT2 (GRAPH,360.,0.,1.,0.)
      CALL PLOT3 (1H*, DAY(1), S5(1),357)
      WRITE (6,450)
  450 FORMAT (1H1,'IMMATURE SURVIVAL (SIX) FOR AEDES STAGES-1&2 IN
     1CONTAINERS POSITIVE FOR TX.')
      CALL PLOT4 (2,2HSI)
      WRITE (6,1200)
C     BELOW PLOTS AE. IMMATURES IN CONTAINERS WITH TX.
      CALL PLOT1 (3.51,10.111,10)
      CALL PLOT2(GRAPH,360.,0.,140.,0.)
      CALL PLOT3 (1H*,DAY(1),SUM2(1),357)
      WRITE (6,703)
  703 FORMAT (1H1,'AE. IMMATURES IN CONTAINERS POSITIVE FOR TX. LARVAE')
      CALL PLOT4 (15,15HAEDES IMMATURES)
      WRITE (6,1200)
C     BELOW PLOTS TX. LARVAE
      CALL PLOT1 (3.51,10.111,10)
      CALL PLOT2 (GRAPH,360.,0.,20.,0.)
      CALL PLOT3(1H*,DAY(1),SUM3(1),357)
      WRITE (6,704)
  704 FORMAT (1H1,'TOXORHYNCHITES LARVAE')
```

```
      CALL PLOT4 (11,11HTOX  LARVAE)
      WRITE (6,1200)
 1200 FORMAT (1H0,53X,'DAY')
      STOP
      END
$ENTRY
```

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The author was born on July 16, 1948, in Salt Lake City, Utah. He attended secondary schools in Jedda, Saudi Arabia, and Titusville, Florida. After attending one year at a junior college he transferred to the University of Florida where he majored in Zoology and minored in Chemistry. After graduation the author worked as a technician for the Insects Affecting Man Laboratory, USDA, Gainesville and the Department of Entomology and Nematology, University of Florida. In September 1974, he began his graduate studies at the University of Florida in the Department of Entomology and Nematology. While in graduate school the author and his wife operated a small business manufacturing insect sampling devices. The author is a member of the Entomological Society of America, the American Mosquito Control Association and the American Scientific Affiliation. After completing the Ph.D., the author anticipates working for USDA at the Insects Affecting Man Laboratory, Gainesville in medical entomology.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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