

ALGAE, BACTERIA AND DETRITUS IN THE GROWTH, DEVELOPMENT
AND SURVIVAL OF TWO *Aedes* MOSQUITOES

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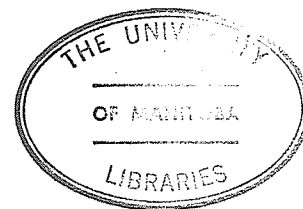
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ABSTRACT

Although mosquito larvae are known to be generally omnivorous in their food habits, little is known about the relative value or effect individual components of the diet have upon the mosquito's growth, development and survival. In this study, several common microflora in the natural diet of mosquitoes were isolated from typical habitats and gut contents and grown in pure culture. *Aedes atropalpus* and *A. vexans* were reared on two species of algae and four species of bacteria. Ground leaf meals of three macrophyte species and pollen were included in some diets to simulate the detritus component. These were compared to various concentrations of a standard diet of a commercial fish food. Growth, development and survival were measured by various means through the larval, pupal and adult stages in order to assess the effects of the different diets.

A wide variation was found between these foods and even between various concentrations of the same food. Survival was generally low and development times longer than normal for most of the diets and none provided a completely satisfactory diet alone although some insects survived to the adult stage on most diets.

The relative effects of these diets on various vital and physical measurements of the mosquito from larvae to adult were discussed.

The feasibility of utilizing such a method for other related studies was also considered.

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INTRODUCTION

Although mosquito larvae are generally considered omniverous, little is known about the relative effects of individual components of natural diets on mosquito growth, development and survival.

The constituents of the larval diet of mosquito larvae in general as determined from gut analysis (Senior-White, 1928; Hinman, 1930; Howland, 1930; Laird, 1956) include algae, bacteria, fungi, protozoa and detritus, both organic and inorganic.

The ability of mosquito larvae in selecting food is of considerable interest. They have generally been considered indiscriminate, (Coggeshall, 1926; Howland, 1930; Becker, 1958; Jones, 1960; Christophers, 1960; Pucat, 1965). Species differences in habitat selection, mouth-part structure and function in feeding behavior (Surtees, 1959) as well as mechanical considerations of particle size and quantity (Shipitsina, 1930, 1935; Pucat, 1965) all bear on the overall feeding selectivity of a species on a particular niche. A species can be considered unselective in its own particular niche, although the influence of sapid factors has been shown to affect the mechanical feeding rate (Dadd, 1968, 1970a, b). Overall comparisons between gut contents and particles present in habitat water suggest that larvae feed indiscriminately (Coggeshall, 1926; Senior-White, 1928a; Howland, 1930a; Jones, 1930).

Nutritional roles of the components of food material have been discussed in a somewhat contradictory and inconclusive manner in the past. Barber (1928) summed up the preceeding literature on Culicine feeding and concluded that algae or bacteria alone could constitute a sufficient source of larval food and gave less emphasis to detritus. Howland (1930a, b) also proposed that algae were of considerable importance in the nutrition of larvae. Others raised doubts concerning the basic value of algae in nutrition on the grounds that many live algae could be found active well back in larval guts as well as being expelled in feces (Hinman, 1930). Hinman (1930, 1932a, c, 1933) and Beclemishev (1930) placed less emphasis on algae than on bacteria, arguing the latter were important because of growth factors bacteria contributed. Hinman (1932a, c, 1933) proposed further that nutrients in true solution and colloidal organics played a considerable role in nutrition. Rozeboom (1935) repeated parts of this work in an attempt to discount the hypothesis that larvae could develop solely on dissolved nutrients. The idea of growth factors present either intra- or extra-cellularly in larval food stimulated work on chemically defined diets, notably by Trager (1942), Goldberg and De Meillon (1948a, b), Lea, Dimond and DeLong (1956), Singh and Brown (1957) and Akov (1962), with the latter two attaining reasonable success.

Little by comparison has been done to investigate the importance of the protozoa or organic detritus. Clements (1963) wrote that: "Although non-living organic matter may also be important, the extent to which it can supplement or replace micro-organisms and its effect as bulk is also little known". Non-organic detritus and its effect as bulk is also little known. Nayar (1966) states that mechanical bulk

is necessary for normal alimentation in *Aedes taeniorhynchus*.

It appears then that larval nutrition in nature is probably drawn from several sources and that the dominance of any one taxonomic group is doubtful. When referring to the food of omnivores and other organisms it would seem reasonable to surmise that not all which is consumed is beneficial and therefore it becomes unrealistic to consider the food role of larger taxa as unit contributors, but rather value and abundance of components are of more interest. Information involving relative values of food component's frequency of occurrence, availability, abundance and seasonal distribution would contribute more to our understanding of larval nutrition. Knowledge of the most nutritious foods and the most detrimental would provide a basis for interpreting influence of natural diets on mosquito populations. Assessment of the value and effect of food component species rather than grouping higher taxa orders as has been done in the past would provide a useful foundation for determining the nutrition of larvae.

Many studies have attempted to determine the food role of various taxonomic groups and to define growth factors and establish the chemical definition of larval nutritional requirements for particular species. Other studies centered on the algae for reasons apart from nutrition, observing that when certain species of this group, notably the *Chara* spp, were present in a pool, oviposition was adversely affected (Hamlyn-Harris, 1928; Matheson and Hinman, 1928, 1929, 1930, 1931; Rudolphs and Laekey, 1929; Matheson, 1930; Levi, 1944; and Gerhardt, 1956).

Plants or their metabolites and extracts affecting animals is

widely known and comprehensively summarized by Jenkins (1964) showing that almost all phyla can be shown to be affected in some way.

One of the most vivid examples of a plant affecting an animal detrimentally can be seen in the case of several species of blue-green algae, which when present in bloom concentrations can be toxic enough to kill or harm livestock or other animals (Prescott, 1948; Gorham, 1960; Round, 1966). Such bloom algae could possibly influence insect larvae since the guts would be predominantly filled with a blooming species because of the indiscriminate filter feeding. Not only phytoplankton but bacteria, protozoa and other micro-organisms are capable of exerting beneficial as well as detrimental or pathogenic effects on insects (Laird, 1960; Henry, 1962; Reeves, 1970).

In view of this it would be of interest to consider the effect various components of the larval diet might have, not only from the standpoint of determining the role of the group or the nutritional value of a species, but also from the consideration of its potential as a biological control agent. Although this had been considered unsuccessfully, it does not preclude the existence of such a potential (Laird, 1960). Such an investigation would involve selectively screening local micro-organisms inhabiting mosquito larval sites followed by selective pure culturing and larval feeding of these to measure the nutritional value or detrimental effects. Needless to say, the species diversity of algae, bacteria and protozoa is immense and such a program would be a laborious and difficult undertaking. However, the magnitude of a screening program cannot be practically assessed at this point since information pertaining to the frequency of occurrence of either beneficial or detrimental food components has

not been established.

Interesting work in this respect has been initiated by Amonar (1969) and Reeves (1970) in bioassaying for toxic strains of California algae. However, detailed comparisons of the effects at various concentrations in terms of measurements on the mosquito's growth and development were not undertaken and only detrimental features were considered.

It is of interest to note with respect to mosquito control potential that the storage products of many algae are similar to that of some modern larvicides. Diatoms, for instance, characteristically store lipids (Fowden, 1954; Round, 1966; Jackson, 1968) which have similar chemical properties to some chemicals considered in mosquito control (Quraishi and Thorsteinson, 1967).

If such an investigation of the value and effects of components from larval diets were conducted, it would also be of considerable interest to find a species of micro-organism which might hold promise as a satisfactory single food source for larvae, which could be mass produced for standardizing uniform larval cultures so necessary for comparative physiology studies.

In the following thesis a technique for isolating, culturing and measuring the effect of single components of micro flora and detritus from a mosquito larva's natural diet is presented. Although the number of species investigated in this manner is small, further information is given on the role, relative food value and effect of these components. A more accurate assessment of the relative food value alone (apart from other effects) of larval diets could be realized by other methods, e.g. the actual measure of ingested and incorporated

carbon.

This type of study involving pure cultures of individual species of micro-organisms and measuring the response of larvae might be extended to other forms of aquatic invertebrates utilizing similar foods and could be used to develop a preliminary assessment of energy flow among various trophic levels in the aquatic ecosystem.

MATERIALS AND METHODS

Mosquito Rearing

Aedes atropalpus (Coquillett) (Belleville, Ontario population), which has not as yet been shown to be a native of Manitoba* was selected for study primarily because of its ease in routine laboratory culturing. The females are autogenous and do not require a blood meal for egg maturation. Its biology has been documented. *A. vexans* (Meign) has similar feeding habits and is anautogenous. It is a pestilent biter in North Central United States and most of Canada, especially the prairie provinces. Literature on its biology is considerable.

In the course of the study it was necessary to initiate new experiments at irregular intervals as time permitted, making it desirable to maintain a perpetual bank of eggs from which experimental larvae could be hatched as required. In consequence, laboratory culturing of *A. atropalpus* was undertaken and eggs were collected routinely by cycling this species in the laboratory. Eggs of *A. vexans* had to be obtained by washing sod samples extracted from likely

* *A. atropalpus* inhabits rock pools and is found in Eastern Canada as well as many regions of the U.S. Dr. S. M. Smith collected *A. atropalpus* in the spring of 1968, from a rock pool near Whitedog Falls, Ontario, very near Manitoba. It is reasonable to surmise that it probably exists in Eastern Manitoba.

oviposition sites in field habitats. *A. vexans* does not mate in confinement, and to date has not been colonized except by using the forced copulation procedure (Horsfall and Taylor, 1967; Fowler, 1972).

Culturing of *A. atropalpus* followed the procedure suggested by Gergerg (1970), modified in regard to oviposition and egg collection as suggested by Kalpagé (1970).

Eggs were set out to hatch in 1:1000 solution of nutrient broth* and tap water. After hatching they were removed and reared in shallow pans of distilled water (Fig. 1), the water being changed on alternate days. Laboratory cultures were fed 1 mg Tetramin** per larvae per day for the first two instars, and then 2 mg per larvae per day for the last two instars. Pupae were collected daily and placed in crystalizing dishes filled with distilled water which in turn were placed in 25 cm³ acrylic plastic cages (Fig. 2) where the adults emerged. The adults were fed on water-soaked raisins. When all emerged, the food was removed and the females deposited their eggs on the water surface (Kalpagé, 1970). Temperature fluctuated with that of the room, while the humidity of the cage was raised with rolled paper towel wicks in small beakers of water which were changed when fungi appeared. Eggs were collected daily from the water surface by means of a small eye dropper and placed on nylon mesh covering a layer of glass wool in petri dishes (Fig. 3). The eggs were allowed to embryonate for about one week at 20°C and then stored at

* Nutrient Broth--a product of Difco Laboratories, Michigan.

** Tetramin--commercial tropical fish food produced by TetraKraft-Werke, West Germany.

10°C. Non-diapause eggs were obtained by keeping the culture at L:D 15:9 or greater. Diapause results when the culture is kept at L:D 14:10 or shorter (Kalpagé, 1970). Although the life of non-diapause eggs wasn't as long as those in diapause, they provided readily hatchable eggs as desired. Some difficulty was experienced with eggs splitting and drying out when sprayed with the fungicide, parahydroxybenzoate. This treatment was devised for use with eggs on paper pads and was unnecessary with washed eggs placed on nylon pads over glass wool and was discontinued. Only the moisture content of the egg pads in storage was kept under occasional surveillance to prevent dehydration.

A. vexans eggs were identified after they had been washed from the sod and stored at room temperature until needed and hatched in the same way as those of *A. atropalpus*.

Preparation of Food for Larvae

Algal Cultures

The object of algal culturing was to supply an isolated component of the mosquito larva's natural diet and investigate the mosquito's growth, development and survival on this food. This endeavour presented the most diverse and time consuming part of the project and proved to be the limiting factor in the expansion of the primary objectives of the project.

Algal isolation was accomplished by placing about 10 mls of water from a mosquito larval habitat on solid Bristol's medium {as modified by Bold (1949) using Arnon's (1938) trace element solution} in petri dishes and incubating at 20°C with a light regimen of 16 hours light

and 8 hours dark until colonies appeared (Fig. 4). Using a small loop, the most isolated of the colonies were picked and added to about 10 mls of sterile distilled water and replated separately. As soon as new colonies appeared the process was repeated observing bacteriological technique and eventually unialgal cultures covering the plate were obtained (Fig. 5). Only those algae which grew appreciably under the culture regimen were used thereby avoiding the physical and chemical adjustments otherwise necessary for encouraging the growth of a selected species.

When good algal growth was obtained, a loop of the unialgal plate culture was introduced into liquid culture in test tubes and after satisfactory growth in this medium was evident, attempts were made to isolate the alga from accompanying bacteria.

Three basic methods of isolating algae from contaminating bacteria include serial dilution and plating (Pringsheim, 1967), mechanical separation (Chu, 1942; Droop, 1954; Pringsheim, 1967) and separation by bacteria antibiotics (Makismova and Vybornykh, 1967; Berland and Maestrini, 1969). Although it is more elaborate and required considerable practice, mechanical separation was chosen because it was direct.

The method of mechanical separation of algae from bacteria was similar to that used by Chu (1942), Pringsheim (1967) and others. Droop (1954) modified it to include separation of microflagellate algae. The technique utilizes a specially drawn micropipette just larger than the alga involved. By capillary action this pipette draws up selected individual algae out of a droplet of water on a well-slide under a binocular dissecting microscope housed in a

special chamber.

The chamber (Figs. 6 and 7) was made of acrylic plastic recessed to fit a dissecting microscope. The objective lens was directed above a flat transparent surface of the chamber directly above an elevated stage holding the well-slide. Access to the chamber was provided by two hand holes on either side of the microscope stage. The microscope light source was external, thereby reducing the heat incident on the slide. The light beam was easily focused from a source behind the chamber to the microscope base and reflected up through the bottom of the stage. The chamber was provided with a rack for instruments at the rear. Shallow pans were provided in the corners for holding ice packs necessary in maintaining a cool temperature. A monitoring thermometer was placed near the slide stage. The purpose of the chamber was to provide protection against contaminating, bacteria-ridden dust circulated by small air eddies. The entire chamber was disinfected before use by swabbing with a disinfectant.

A droplet of the liquid culture of the algae to be separated was placed in a well-slide on the stage inside the chamber. A small 20 microlitre capillary tube* was drawn down over a small alcohol flame to form a very fine micropipette and broken off so the diameter was just slightly larger than that of the alga. The flame ensures a sterile pipette. The short pipette was placed in a plastic holder provided with the capillary tubes and attached to a 25 cm rubber tube fitted with a check-valved squeeze bulb at the opposite end (Fig. 8). The micropipette and holder used were considerably shorter

* Cornin, Laboratory Products Department, Corning Glass Works, New York.

than suggested by Droop (1954) and Pringsheim (1967) and could be manipulated more steadily in algal selection. By means of this micropipette an alga in focus (80 to 160 power was commonly used) was picked out and taken up by capillary action. If just the correct pressure was kept on the squeeze bulb with the left hand, capillary pressure of the micropipette was balanced thereby preventing liquid from flowing up the tube until pressure at the bulb was reduced when required to extract selected algae.

A dozen or so algae were sucked up (depending on their size) and then transferred to a fresh droplet of sterile distilled water in another well-slide in a petri dish, the entire unit (petri dish, slide and droplet) having first been autoclaved and opened only inside the chamber. The algal cells had to be watched to avoid loss when they were expelled into the fresh droplet. They were usually found in a group and by means of a newly made pipette they were once more sucked up and expelled into a fresh droplet. This was performed serially five or six times (depending on the amount of original bacterial contamination) using a new slide and pipette each time until it was felt that the cells were clean of bacteria. Finally these cells were placed on sterile solid medium (in a few drops of sterile distilled water or sterile liquid culture medium). Usually the number of cells was reduced through serial washing and only a few were retrieved finally. It was usual to place these cells on sterile solid agar and culture them until they formed just noticeable colonies (Fig. 9) as a check for bacterial contamination. If free of contamination the colonies were transferred to slant cultures and grown as inoculum for larger liquid cultures.

Some of the isolated algae were placed on nutrient slants in screw-capped test tubes for reference and stored at 10°C in the dark (Fig. 10).

Only two species were successfully brought into continuous, bacteria-free culture, while several others were isolated and grown, but could not be kept axenic. One species of *Chlorella* and one of *Nitzschia* were primarily used in this work. *Chlorella* is a thick-walled, unicellular, non-motile alga (Fig. 11) belonging to the division Chlorophyta, order Chlorococcales. *Nitzschia* is a diatom belonging to the class Bacillariophyceae of the division Chrysophyta (Figs. 12 and 13). Both of these algae are commonly found in mosquito habitats and in the guts of mosquitoes collected in the field.

In culturing quantities of algae for larval food, a method similar to that used by Davis and Ukeles (1961) was adopted. The main culture flask consisted of a 1500 ml erlenmyer flask fitted as shown (Fig. 14) with a four hole stopper. The stopper admitted: (1) an air supply tube from a bacterial filter extending almost to the bottom of the flask, (2) a short exit vent bent downward at 45°, (3) a nutrient replenishing tube extending halfway to the bottom and sealed outwardly with a clamp on an attached hose, (4) a harvesting tube opening near the bottom of the flask. A short piece of rubber hose sealed with a clamp was attached above on the exterior of the harvesting tube. One litre of culture medium was placed in the flask, autoclaved and while still hot, the air supply was connected and turned on. When the medium cooled the stopper was carefully eased open with the air supply turned on to prevent dust-borne bacteria

from entering. Nutrients and vitamins were introduced via a micro-filter* attached to a syringe and a test tube of pure unialgal inoculum was flamed and added. The flask was then carefully sealed once more and incubated. Harvesting the cells was accomplished simply by stopping the vent exhaust and opening the harvesting tube, allowing the air pressure to displace as much of the culture as required.

After harvesting, the culture medium was replenished by connecting a second similar flask to the culture bottle. The replenishing flask contained freshly autoclaved medium and vitamins and was provided with a bacteria-filtering air inlet (a short tube opening just inside the flask) and an outlet tube from the bottom of the flask sealed outwardly by a clamp on a short piece of rubber hose. The outlet tube of the replenishing flask was connected to the culture flask by means of a small glass nipple. Before connecting, the nipple was autoclaved in metal foil and the ends of the culture flask replenishing inlet and the replenishing flask outlet hoses were soaked in 95% ethanol. The nipple was unwrapped and the connection made between the hoses of the two flasks over a small flame. The hose clamps were removed and the air pressure increased in the replenishing flask forcing the medium to flow across to the culture bottle. When full the clamps were replaced and the nipple removed.

Initially, several types of media were tried, including those of Arnon (1938); Chu (1942 and 1943), Bristol (Bold, 1949), Davis (1952), Eversole (1956), Jørgensen (1956), and Tamiya (1966). Media

* Gelman Instrument Co., U.S.A. Easy Pressure Filter Holder, 1 inch in diameter.

with organic constituents invited bacterial contamination while others containing soil extract were avoided in preference to a completely chemically defined medium. Bristol's medium was selected for ease in preparation and because it was a reliable and effective nutrient for those algae species of interest. Arnon's (1938) trace element solution was added to Bristol's stock solution. The complete medium was chemically defined.

The physical environment was provided by an incubator cabinet (Fig. 15) maintained at 20°C with a light regimen of 16 hours light and 8 hours dark. Illumination was provided by two 15 watt cool white florescent lamps above each shelf, providing an average light intensity of 100-250 foot-candles. The air supply was generated by an aquarium pump and filtered through cotton wool and silica gel before entering the culture flask through a bacterial filter. Initially an air supply enriched with 5% CO₂ was used, but since a rapidly growing culture was not essential, normal air was used.

A low level of bacterial contamination was a continual problem requiring considerable effort and caution to prevent. Population densities were estimated from the mean of three haemocytometer* counts.

Harvesting the cells was done by drawing off as much of the culture as needed after a certain density had been reached. An attempt was made to keep the culture in the exponential phase of growth by harvesting, then replenishing the medium. The harvested algae were used directly if the metabolites were to be included or centrifuged when it was desired to eliminate metabolites. A fine

* American Optical Co., U.S.A. Bright-line Haemocytometer.

centrifuge tube (0.05 ml divisions) was used for measuring wet cell volumes.

Bacterial Cultures

The culturing of bacteria (Breed, Murry, & Smith, 1957; Pelezar, 1957) was considerably easier than the culturing of algae due to the much faster growth rate.

The purpose of culturing bacteria in this study was to provide a food source for the larvae in useable quantity.

Initially a pure strain of *Escherichia coli* was obtained from the Microbiology Department to establish a pilot culture. The other species used were isolated from water taken from mosquito habitats. They were grown in pure culture in the laboratory from colonies that happened to grow on the medium when isolated. Identification was made by Dr. R. Z. Hawirko at the University of Manitoba Microbiology Department.

Four species of bacteria were isolated by means of standard microbiological dilution and plating technique as outlined in Pelczar (1957) and Frobisher (1958). These were *Irwinia* sp, *Pseudomonas* sp, *Coryneform* sp and *Achromobacter* sp. After the four species were growing vigorously in culture on solid medium in petri plates, screw-capped reference test tube slants were made. Several other plates and liquid test tube cultures were inoculated from the same colony.

These extra liquid and slant test tube cultures were coded and stored at 5°C to be used as starting inocula for larger liquid cultures. The freshly growing plate cultures were used for identification.

Bulk liquid cultures were made in large bottles (Fig. 16) fitted with two-holed rubber stoppers admitting a bacteriologically filtered air supply and an exit vent protruding downward at an angle of 45° . One litre of culture medium (8 g of nutrient broth per litre of distilled water) was added and the whole apparatus autoclaved. With the apparatus still hot from the autoclave, the air supply was connected and turned on. After the medium cooled the stopper was carefully eased out with the air supply turned up to prevent dust-borne bacterial contamination from entering and a test tube of inoculum was added over an open flame. The stopper was reseated and the entire flask cultured at room conditions which gave very good and fast growth for all bacterial species used, being ready for harvesting in three to four days. Because of the very rapid growth and large inoculum, as well as the precautions taken on inoculating, no contamination was detected.

The cells were harvested by centrifuging the culture at 10,000 r.p.m. for 25 minutes. The supernatant fluid was carefully poured off into pre-autoclaved receiving flasks and stored at 5°C so the accumulated metabolites in the cell-free media could be utilized for experiments. The centrifuged bacterial pellet was scraped off the centrifuge flask, wrapped in parafilm* and quickly frozen, then stored at -15°C .

Although the bacterial cells were frozen in storage prior to larval feeding, these cells were capable of generating viable cultures when reintroduced into liquid culture medium. This method of storage

* Parafilm, American Can Co., Wisconsin.

is practiced in some laboratories.

Larvae were tested on the cells and metabolites separately to ascertain the effect of each. Measured amounts of metabolites were added to the larval culture water by means of a pipette. The cells were fed by thawing a small fraction of the frozen pellet and weighing the required amount.

Pollen, Leaf Meals and Commercial Fish Food

Commercial tropical fish food ("Tetramin") was used as the standard laboratory reference food for assessing growth, development and survival of larvae fed on experimental diets. It was ground into a fine meal and food portions were measured by dry weight. It was preferred over other common larval foods such as dog food and liver powder since it caused little fouling of the larval rearing water.

Pollen was used as a food component because it is a known constituent of detritus consumed by larvae in the field. It is high in protein and forms the major food of various insects. The pollen used in this study was obtained from the Bee Biology section of the Entomology Department.

The leaf meals used were alfalfa (*Medicago sativa vernal*), wheat (*Triticum vulgare manitou*) and orchard grass (*Dactylis glomerata ottawa*). Although not normally available to mosquito larvae, they were included as diets in an attempt to simulate the macrophytic component of the larval diet. Considerable information is available on the chemical analysis of these grasses and their effect as food for some other insects is also known (Wilson, 1969). All three of these leaf meals contain the same amino acids as shown by Singh and Brown

(1957) and Akov (1962) to be necessary for development of *Aedes aegypti* and other insects (Rockstein, 1965), although the quantities vary.

Protozoa form a considerable part of the food of mosquito larvae and were originally considered for inclusion in this study but problems of pure-culturing protozoa which feed on bacteria presented a task better dealt with as a separate project. Utilizing fungi for larval food was not attempted for similar reasons.

Quantifying Food Portions

In order to compare the various experimental foods, commercial tropical fish food was taken as the basic control diet for reference. A standard ration (concentration) which produced optimum growth, development and survival was determined and equivalent portions by dry weight of the other foods were fed to the larvae.

Standard rations of algae were prepared by harvesting a fixed volume of culture, estimating the cell count and determining the dry weight. This was repeated for cultures of various concentrations and the dry weights per unit volume were plotted against a range of cell counts. For any given cell concentration the dry weight could then be estimated from the curve and an equivalent amount of live food obtained by diluting the harvested volume to the necessary concentration. Considerable variability was found in estimating algal food quantities.

Larvae were fed the harvested live food plus the culture medium if the algae and metabolites were to be tested or the cells were washed and separated by centrifuging to remove the metabolites and

culture medium if the cells were to be tested alone. By rearing larvae on standard rations of commercial fish food and adding various concentrations of algal metabolites to normal larval rearing water, metabolite effect was tested separately.

Attempts were made to harvest the algae in the exponential phase of growth to keep food populations at the same age and metabolite concentrations comparable since some algae become more toxic with age (Davis and Guillard, 1958).

Calculating the equivalent standard ration of bacteria in terms of dry weight was considerably easier since the entire culture was harvested at once by centrifuging and storing the cells in a frozen state while the metabolites and culture medium were kept refrigerated. The percent water in the pellet was determined so that the wet weight of the cells could be measured out directly to give the corresponding required dry weight equivalent.

The leaf meals were prepared by dehydration at 45°C for 48 hours in an oven and ground with a mortar and pestle. The required dry weight of leaf meal or pollen was measured out directly for each feeding.

Mosquito Cultures on Prepared Diets

General Procedure

Mosquito eggs were hatched in a 1:1000 solution of nutrient broth and distilled water and all larvae hatching within three hours were removed and washed by three serial transfers through sterile distilled water. Ten larvae and 50 mls of rearing medium were placed in each of three crystallizing dishes fitted with covers (Fig. 18). The standard

ration was 1 mg of commercial tropical fish food per larva per day in 5 mls of medium for instars I and II, and then 2 mg for instars III and IV. All larvae were incubated at $20^{\circ} \pm 2^{\circ}\text{C}$ and a light regimen of 16 hours light and 8 hours dark. The larvae were transferred to fresh rearing medium and fed after each observation. Daily records were kept of growth, development and survival and other information. The number of each instar was counted in each container and checked with the cast skins. All exuviae were collected and preserved for measuring in 75% ethanol beginning with the 4th instar. Males and females were recorded separately beginning with the pupal stage.

The normal rearing medium consisted of 25 mls of Bristol's medium as used for culturing algae added to 25 ml of distilled water in each container. Bristol's medium was found to contain all of the essential inorganic salts in the approximate concentration that Bates (1941) suggested as necessary for normal mosquito development.

Growth (i.e., weight, size and volume), development (stage reached) and survival (per stage) was measured in various ways for each of the mosquito stages.

Measuring Growth, Development and Survival

Larvae

An attempt was made to compare growth and development as early as the 4th instar because survival of larvae on some foods was so poor that they failed to develop further. Specimens in the larval stage were measured live rather than sacrifice a portion to conserve the number of reared larvae thus minimizing the need for excessive algal food production.

Conspicuous variations in thorax and abdomen sizes were observed (even when head capsule size was almost similar) suggesting that measurements of these parts would be useful in assessing growth.

A special measuring disk with retaining grooves was designed to facilitate measuring the thorax of live specimens (Fig. 18). Larvae to be measured were taken from the culture and individually placed in a water drop on the measuring stage. Excess water was drained off via a slender pipette until the larva was immobilized except for slight sporadic undulations. Two "V"-shaped grooves were cut into the disk, one being wide facilitating width measurements and the other narrow, forcing the larva onto its side and allowing thickness and length measurements to be taken more accurately. Several groups of larvae were measured in this manner and no mortality was encountered.

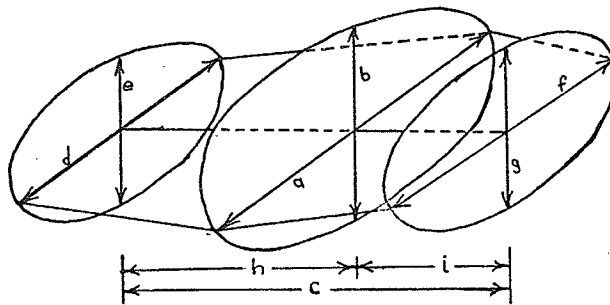
Overall length from the anterior edge of the head capsule to the siphon tip, width and thickness of the third abdominal segment and width, length, thickness and volume of the thorax were measured. The mean value $\pm$ the standard error in mm was recorded. These measurements were always made on larvae of comparable ages, and were always made as close as possible to 72 hours after half of the surviving larvae reached the 4th instar.

Development to the 4th instar was measured in days $\pm$ the standard error. Larval survival was recorded as a fraction of the initial number.

In calculating thorax volume it was found that although the size of the thorax differed between those larvae fed poor diets to those

fed good diet, the shape remained much the same (Fig. 19).

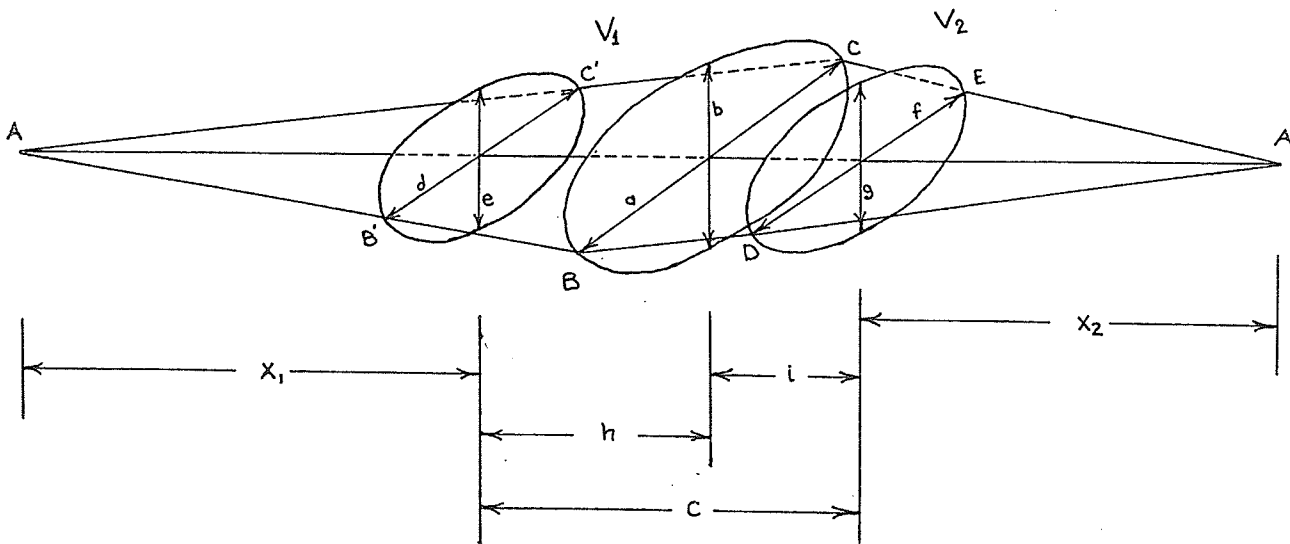
Groups of larvae fed poor diets and good diets were measured in detail and these measurements transferred to scale drawings. It was found that ratios of various dimensions of the two kinds of larvae were quite constant. It was postulated that by taking only three actual measurements and incorporating the proportions of other dimensions a formula for the volume could be devised. A flattened truncated cone was adopted as a model of the thorax.



General shape of larvae fed poor and control diets

Actual measurements and their proportions:

a = measured width
 b = measured thickness
 c = measured length
 $d = .50a$
 $e = .64b$
 $f = .65a$
 $g = .65b$
 $h = .55c$
 $i = .45c$



$$\frac{\frac{a}{2}}{\frac{d}{2}} = \frac{h + x_1}{x_1}$$

$$\frac{a}{d} = \frac{h + x_1}{x_1}$$

$$x_1 = \frac{hd}{a - d}$$

$$\text{volume of cone } ABC = \frac{\pi}{3} \left(\frac{a}{2} \cdot \frac{b}{2} \right) (h + x_1)$$

$$\text{volume of cone } ABC' = \frac{\pi}{3} \left(\frac{d}{2} \cdot \frac{e}{2} \right) (x_1)$$

required volume $V_1 = V_{ABC} - V_{AB'C'}$

$$= \frac{\pi}{12} ab(h + x_1) - \frac{\pi}{12} dex_1$$

$$= \frac{\pi}{12} \left[ab \left(\frac{a}{d} \right) \left(\frac{hd}{a - d} \right) - de \left(\frac{hd}{a - d} \right) \right]$$

$$= \frac{\pi}{12(a - d)} [a^2bh - d^2eh]$$

$$= \frac{\pi h}{12(a - d)} [a^2b - d^2e]$$

$$d = k_1a$$

$$e = m_1b$$

$$V_1 = \frac{\pi h}{12(a - k_1a)} [a^2b - k_1^2a^2m_1b]$$

$$= \frac{\pi ha^2b}{12a(1 - k_1)} [1 - k_1^2m_1]$$

$$= \frac{\pi hab}{12(1 - k_1)} [1 - k_1^2m_1]$$

$$k_1 = .50, m_1 = .64$$

$$(1 - k_1) = .50$$

$$1 - k_1^2m_1 = 1 - (.50)^2(.64) = 1 - .16 = .84$$

$$V_1 = \frac{\pi \cdot .84}{12 \cdot .05} hab$$

$$= .4398 hab$$

$$= .440 hab$$

$$V_2 = V_{A'BC} - V_{A'DE}$$

$$= \frac{\pi}{3} \left(\frac{ab}{4} \right) (i + x_2) - \frac{\pi}{3} \left(\frac{gf}{4} \right) x_2$$

$$\frac{a}{f} = \frac{i + x_2}{x_2} \quad x_2 = \frac{fi}{a - f}$$

$$\begin{aligned} V_2 &= \frac{\pi}{12} \left[ab \cdot \frac{a}{f} \cdot \frac{fi}{a - f} - gf \cdot \frac{fi}{a - f} \right] \\ &= \frac{\pi i}{12(a - f)} [a^2b - f^2g] \end{aligned}$$

$$f = k_2 a$$

$$g = m_2 b$$

$$k_2 = m_2 = .65$$

$$\begin{aligned} V_2 &= \frac{\pi i}{12(a - k_2 a)} [a^2b - k_2^2 a^2 m_2 b] \\ &= \frac{\pi i ab}{12(1 - k_2)} [1 - k_2^2 m_2] \\ &= \frac{\pi i ab}{12(.35)} [1 - (.65)^2 (.65)] \\ &= .543 iab \end{aligned}$$

$$\text{Total volume} = V_1 + V_2$$

$$= .440 hab + .543 iab$$

$$= ab(.440h + .543i)$$

$$= .486 abc$$

Females were aspirated into small cardboard cages (Fig. 21) on emergence and were kept inside a larger wick-humidified cage in the same environmental conditions as the larvae. These females were given only distilled water and kept forty hours to allow for ovarian development and then were killed by chloroform and frozen.

Ovarian development was assessed by thawing the frozen females and dissecting the ovaries to estimate the stage of development using Christopher's (1911) scale as modified by Macan (1950) and Clements (1963). A total follicle count was made of both ovaries. Development time to emergence was measured in days $\pm$ the standard error.

RESULTS AND DISCUSSION

A. Results

Since survival to pupal and adult stages was often low with some foods an attempt was made to assess the effect of the food as early as possible, beginning with the 4th instar. Various measurements were made in each stage to determine which best showed differences among the diets. Those found unsuitable are retained in the appendices.

The discussion primarily concerns *A. atropalpus*. The results for *A. vexans* on the diets tested indicate similar trends although its life cycle is more rapid and it appears to be less tolerant of poor diets (Table I).

B. Normal Rearing

In determining the optimal concentration, variations between 0.5 and 2 times a standard ration of Tetramin were tried. A standard ration consisting of 1 mg/larva/day for instars I and II and then 2 mg/larva/day for instars III and IV in 5 mls of culture water appeared to produce the best overall results. Although 0.5 standard ration gave development times significantly shorter in the larval and adult stages than those fed a standard ration ($P < 0.05$, Duncan's Multiple Range Test), the physical measurements of larvae were smaller; those fed double the standard ration showed a physical size increment but developed slower. In the pupa and adult stages the inverse occurred: larger physical sizes were realized with lower

TABLE I

Development time and survival of *A. vexans* reared on various foods

Food	Food Quantity*	Larvae		Pupae		Adults	
		Development Time to 4th Instar, Days	Mortality	Time to Pupation Mean±se	Mortality	Time to Emergence Mean±se	
		Mean±se		♂	♀	♂	♀
CONTROL ("Tetramin")	1	5.32±.17(25)	4/30	7.80±.18(15)	7.80±.83(11)	1/15 0/11	10.4±.25(14) 10.9±.40(11)
<i>Chlorella</i> sp	1	-	30/30	-	-	-	-
<i>Nitzschia</i> sp	1	6.46±.40(22)	15/30	16.5±1.5(8)	17.9±2.0(7)	0/8 0/7	19.4±1.5(8) 19.9±1.8(7)
<i>E. coli</i> + metab-olites	1	-	30/30	-	-	-	-
<i>E. coli</i> + silica sand, 1:4	1	-	30/30	-	-	-	-
<i>E. coli</i> + <i>Chlorella</i> sp	1	-	30/30	-	-	-	-

* Standard ration = 1:1 mg dry wt/larva/day in 5 ml of culture through instars I & II, then 2 mg for III & IV, in 1:1 vols. distilled water and Bristol's medium without vitamins.

Note: sample sizes in parentheses.

concentrations of the control diet although differences between the physical measures were not statistically significant in either stage ($P>0.05$). Mortality was least with a standard ration. Ovarian development and the number of follicles matured did not differ significantly in the female adults ($P>0.05$) reared on food concentrations through one half to double one standard ration.

Larvae reared on standard rations of Tetramin (fish food) required 6.71 days to reach the 4th instar, 10.67 and 10.78 days respectively for male and female pupation and 13.91 and 14.80 days for male and female adults to emerge (Tables II to V). These times are similar to those reported in the literature (Trembly, 1945; Shaw and Maisey, 1961).

Mortality was low (7/30 or 23%) in the larval stage and nil in the pupal stage for both sexes. Adult females dissected 40 hours after emergence gave a mean follicle count of 133 ± 27 and most follicles were developed to the IVa stage. Forty hours was allowed for development since first oviposition usually occurs 48 hours after emergence at 80°F (Gerberg, 1970). These normal values compare favourably with those found by Trembly (1945) and Kalpagé (1970).

Physical measurements are shown for the larvae in Table II and Appendix A. Larval thorax volume was chosen as an estimation of growth because differences were apparent in the thorax size among larvae reared on various diets. Thorax size was considered a good indicator of food utilization since this body region contains developing flight muscles, the size of which should reflect food quality. Good flight muscle development can be considered of vital significance to the success of the adults. Thorax volumes were $1.25 \pm 0.07 \text{ mm}^3$

TABLE II

Development time, survival and size of 4th instar larvae of *Aedes atropalpus* fed on various foods

Food and Larval Rearing Water Composition ¹	Food Quantity ²	Development Time to 4th Instar, Days Mean±se	Total Larval Mortality	Abdominal Segment III Width, mm Mean±se	Thorax 3 Volume mm ³ Mean±se
control food nw	.5	5.88±.08(25)	5/30	1.02±.03(25)	1.25±.07(25)
CONTROL ("Tetramin") nw	1	6.71±.10(24)	9/30	1.05±.03(23)	1.33±.09(22)
control food nw	2	6.82±.15(17)	13/30	1.12±.03(17)	1.46±.12(17)
control food dw	1	6.27±.08(29)	2/30	0.86±.05(9)	-
control food Bm	1	6.35±.12(17)	13/30	1.05±.04(17)	1.13±.09(17)
control food Bm:3dw	1	6.59±.11(29)	1/30	-	-
control food Bmv:dw	1	7.70±.26(10)	1/10	0.96±.04(9)	0.99±.10(9)
<i>Nitzschia</i> sp nw	1	8.52±.34(29)	4/30	-	-
<i>Nitzschia</i> sp for 3rd and 4th instars nw	1	7.31±1.7(29)	12/30	-	-

¹ Bm = Bristol's medium, without vitamins, Bmv = Bm + vitamins, dw = distilled water, nw = normal larval rearing water--1:1 volumes Bm:dw.

² Standard ration = 1 = 1 mg dry wt./larva/day in 5 mls of culture medium through instars I and II, then 2 mg for III and IV.

Note: Sample size in parentheses. Volume calculation method given in text.

TABLE II (continued)

Development time, survival and size of 4th instar larvae of *Aedes atropalpus* fed on various foods

Food and Larval Rearing Water Composition		Food Quantity	Development Time to 4th Instar, Days Mean±se	Total Larval Mortality	Abdominal Segment III Width, mm Mean±se	Thorax ³ Volume mm ³ Mean±se
<i>Chlorella</i> sp	nw	91	23.8±1.1(12)	26/30	-	-
<i>Chlorella</i> sp	nw	41	24.0±1.6(6)	27/30	-	-
<i>Chlorella</i> sp for 3rd and 4th instars	nw	1	7.81±.77(16)	28/30	-	-
<i>Chlorella</i> sp ground with silica sand	nw	1	7.20±.13(10)	1/10	0.81±.02(10)	0.58±.03(10)
control food in nw + 25% <i>Chlorella</i> metabolites		1	6.63±.18(8)	2/10	1.06±.05(8)	1.19±.12(8)
pollen	nw	1	7.72±.30(18)	23/30	0.69±.03(14)	-
pollen	nw	.5	6.48±.19(23)	12/30	0.91±.02(20)	0.81±.04(20)
alfalfa	dw	1	8.30±.25(23)	7/30	1.16± (1)	-
alfalfa	nw	1	6.70±.12(30)	1/30	1.02±.02(27)	-
wheat	dw	1	9.14±.26(22)	23/30	0.79±.06(7)	-
wheat	nw	1	6.25±.11(28)	5/30	0.95±.02(28)	-

TABLE II (continued)

Development time, survival and size of 4th instar larvae
of *Aedes atropalpus* fed on various foods

Food and Larval Rear- ing Water Composition	Food Quan- tity	Development Time to 4th Instar, Days Mean±se	Total Larval Mort- ality	Abdominal Segment III Width, mm Mean±se	Thorax 3 Volume mm ³ Mean±se
orchard grass dw	1	8.59±.34 (29)	2/30	0.84±.05 (14)	-
orchard grass nw	1	6.15±.21 (27)	5/30	0.84±.02 (25)	0.69±.03 (23)
<i>E. coli</i> + metabolites nw	1	8.78±.48 (23)	18/30	-	-
<i>E. coli</i> nw	1	9.83±1.0 (7)	23/30	0.92±.10 (3)	0.67±.16 (3)
<i>E. coli</i> nw	.5	17.0±- (3)	28/30	-	-
control food in nw + 25% <i>E. coli</i> metabolites	1	-	30/30	-	-
control food in nw + 5% <i>E. coli</i> metabolites	1	-	30/30	-	-
control food in nw + 40% nutrient broth sol'n	1	-	30/30	-	-
control food in nw + 10% nutrient broth sol'n	1	6.00±- (1)	29/30	-	-
control food in nw + 5% nutrient broth sol'n	1	6.90±.15 (19)	12/30	1.01±.03 (18)	1.23±.08 (18)

TABLE 11 (continued)

Development time, survival and size of 4th instar larvae
of *Aedes atropalpus* fed on various foods

Food and Larval Rear- ing Water Composition	Food Quan- tity	Development Time to 4th Instar, Days Mean±se	Total Larval Mort- ality	Abdominal Segment III width, mm Mean±se	Thorax 3 Volume mm ³ Mean±se
<i>Irwinia</i> sp	1	-	30/30	-	-
<i>Irwinia</i> sp	.25	15.5±4.5(2)	28/30	-	-
control food in nw + 5% <i>Irwinia</i> metabolites	1	7.17±.14(6)	24/30	-	-
<i>Pseudomonas</i> sp	1	12.6±.48(15)	20/30	0.89±.03(10)	0.74±.06(10)
<i>Pseudomonas</i> sp	.5	15.3±.45(14)	22/30	0.78±.04(10)	0.55±.05(10)
<i>Pseudomonas</i> sp	.25	13.8±.58(21)	15/30	0.73±.02(10)	0.43±.05(10)
control food in nw + 5% <i>Pseudomonas</i> metabolites	1	6.95±.11(20)	0/30	1.11±.04(15)	1.44±.12(14)
<i>Coryneform</i> sp	1	12.7±.54(20)	13/30	0.75±.02(10)	0.39±.02(10)
<i>Coryneform</i> sp	.5	14.4±.72(24)	8/30	0.66±.02(10)	0.33±.03(10)
<i>Coryneform</i> sp	.25	16.9±.63(28)	14/30	0.60±.02(10)	0.26±.02(9)
control food in nw + 5% <i>Coryneform</i> metabolites	1	7.00±.08(25)	5/30	1.06±.03(25)	1.23±.06(25)

TABLE II (continued)

Development time, survival and size of 4th instar larvae
of *Aedes atropalpus* fed on various foods

Food and Larval Rear- ing Water Composition		Food Quan- tity	Development Time to 4th Instar, Days Mean±se	Total Larval Mort- ality	Abdominal Segment III Width, mm Mean±se	Thorax 3 Volume mm Mean±se
<i>Coryneform</i> sp & silica sand	nw	1	9.33±1.6(6)	4/10	0.88±.04(5)	0.69±.05(5)
<i>E. coli</i> & silica sand	nw	1	8.25±.75(4)	27/30	-	-
<i>E. coli</i> + <i>Chlorella</i> sp	nw	1	-	30/30	-	-

TABLE III

Development time, survival and size of pupae of *Aedes atropalpus*
reared on various foods

Food and Larval Rearing Water Composition ¹	Food Quantity ²	Time to Pupation, Days		Mortality		Length to Apex of Wing Case	
		♂	♀	♂	♀	♂	♀
control food	nw	9.83±.12(18)	10.1±.26(7)	1/18	1/7	2.03±.02(10)	2.54±.02(6)
CONTROL ("Tetramin")	nw	10.7±.16(12)	10.8±.23(9)	0/11	0/10	2.02±.01(11)	2.53±.01(10)
control food	nw	11.4±.30(7)	11.8±.22(9)	1/7	1/10	2.01±.02(6)	2.48±.02(9)
control food	dw	9.80±.20(15)	10.54±.18(13)	1/15	0/13	2.03±.01(7)	2.53±.01(10)
control food	Bm	10.3±.15(10)	10.3±.18(7)	2/10	0/7	2.04±.02(8)	2.49±.03(6)
control food	Bm:3dw	10.6±.20(16)	11.2±.22(13)	0/16	0/13	-	-
control food	Bmv:dw	11.6±.40(5)	12.0±.41(4)	1/5	0/4	2.02±.02(3)	2.51±.02(4)
<i>Nitzschia</i> sp	nw	17.1±.66(16)	20.4±.88(10)	3/16	1/10	1.78±.04(13)	2.12±.03(10)
<i>Nitzschia</i> sp for 3rd and 4th instars	nw	24.8±1.0(13)	29.0±1.2(5)	3/13	0/5	-	-

¹ Bm = Bristol's medium without vitamins, Bmv = Bm + vitamins, dw = distilled water, nw = normal larval rearing water--1:1 volumes Bm:dw.

² Standard ration = 1 = 1 mg dry wt/larva/day in 5 mls of culture medium through instars I and II, then 2 mg for III and IV.

Note: Sample size in parentheses. Wing case length measured from base of left respiratory trumpet.

TABLE III (continued)

Development time, survival and size of pupae of *Aedes atropalpus*
reared on various foods

Food and Larval Rearing Water Composition	Food Quantity	Time to Pupation, Days		Mortality		Length to Apex of Wing Case	
		♂	♀	♂	♀	Mean±se in mm	♀
<i>Chlorella</i> sp	≥1	30.0±4.0 (2)	30.0±3.0 (2)	1/2	0/2	-	-
<i>Chlorella</i> sp	≤1	38.0±- (1)	45.0±1.7 (2)	1/1	0/2	-	-
<i>Chlorella</i> sp for 3rd and 4th instars	1	31.5±1.0 (2)	-	2/2	-	-	-
<i>Chlorella</i> sp ground with silica sand	1	13.0±.55 (5)	15.0±.41 (4)	2/5	1/4	1.98±.04 (3)	2.47±.03 (3)
control food in nw + 25% <i>Chlorella</i> metabolites	1	10.8±.49 (5)	11.0±.58 (3)	1/5	0/3	1.98±.01 (3)	2.49±.02 (4)
pollen	1	13.5±1.3 (4)	18.3±1.5 (3)	2/4	2/3	1.78±.01 (2)	2.14±- (1)
pollen	.5	12.7±.34 (10)	15.5±.95 (8)	3/10	3/8	1.74±.04 (7)	2.09±.06 (5)
alfalfa	1	13.9±.61 (9)	15.7±.46 (14)	2/9	0/14	2.00±.02 (7)	2.50±.02 (14)
alfalfa	1	10.9±.21 (11)	12.6±.25 (18)	1/11	2/18	1.98±.01 (10)	2.39±.02 (16)
wheat	1	14.7±.67 (3)	19.0±.41 (4)	3/3	3/4	-	2.22±- (1)
wheat	1	12.2±.59 (12)	14.2±.44 (13)	5/12	2/13	1.86±.03 (5)	2.16±.06 (3)

TABLE III (continued)

Development time, survival and size of pupae of *Aedes atropalpus*
reared on various foods

Food and Larval Rearing Water Composition	Food Quantity	Time to Pupation, Days		Mortality		Length to Apex of Wing Case	
		♂	♀	♂	♀	♂	♀
orchard grass dw	1	21.5±.78(16)	25.2±.55(10)	0/18	0/10	-	-
orchard grass nw	1	14.5±.58(14)	16.6±.59(11)	0/14	1/11	1.80±.02(13)	2.04±.04(10)
<i>E. coli</i> + metabolites nw	1	17.9±1.3(9)	22.0±3.5(3)	8/9	1/3	1.81±- (1)	2.07±- (1)
<i>E. coli</i> nw	1	18.5±1.7(4)	20.0±3.8(3)	1/4	1/3	1.76±.02(4)	2.13±.03(8)
<i>E. coli</i> nw	.5	25.0±- (1)	26.0±- (1)	0/1	0/1	1.89±- (1)	2.22±- (1)
control food in nw + 25% <i>E. coli</i> metabolites	1	-	-	-	-	-	-
control food in nw + 5% <i>E. coli</i> metabolites	1	-	-	-	-	-	-
control food in nw + 40% nutrient broth sol'n	1	-	-	-	-	-	-
control food in nw + 10% nutrient broth sol'n	1	11.0±- (1)	-	1/1	-	1.87±- (1)	-
control food in nw + 5% nutrient broth sol'n	1	10.7±.23(12)	11.2±.48(6)	0/12	1/6	2.06±.01(11)	2.51±.02(5)

TABLE III (continued)

Development time, survival and size of pupae of *Aedes atropalpus*
reared on various foods

Food and Larval Rear- ing Water Composition	Food Quan- tity	Time to Pupation, Days		Mortality		Length to Apex of Wing Case	
		♂	♀	♂	♀	Mean±se in mm	♀
<i>Irwinia</i> sp	1	-	-	-	-	-	-
<i>Irwinia</i> sp	.25	-	18.5±4.5(2)	-	2/2	-	-
control food in nw + 5% <i>Irwinia</i> metabolites	1	11.3±.33(3)	12.0±1.0(3)	0/3	0/3	2.06±.02(3)	2.60±.02(3)
<i>Pseudomonas</i> sp	1	21.3±.75(7)	23.3±1.2(3)	4/7	1/3	1.85±.02(4)	2.28±.09(2)
<i>Pseudomonas</i> sp	.5	23.7±.88(6)	27.5±1.5(2)	4/6	1/3	1.85±.02(4)	2.28±.09(2)
<i>Pseudomonas</i> sp	.25	27.7±1.8(10)	30.8±1.1(5)	7/10	3/5	1.76±.06(3)	1.93±.21(2)
control food in nw + 5% <i>Pseudomonas</i> metabolites	1	10.8±.18(11)	11.6±.24(9)	1/11	0/9	2.03±.01(9)	2.50±.01(9)
<i>Coryneform</i> sp	1	24.6±.43(11)	29.2±.70(6)	2/11	0/6	1.82±.04(9)	2.08±.07(6)
<i>Coryneform</i> sp	.5	29.5±1.0(12)	33.9±1.3(10)	7/12	3/10	1.67±.05(4)	1.98±.08(7)
<i>Coryneform</i> sp	.25	34.0±1.8(7)	38.9±.63(9)	3/7	1/9	1.73±.04(4)	2.03±.04(6)
control food in nw + 5% <i>Coryneform</i> metabolites	1	10.8±.11(15)	11.4±.16(10)	0/5	1/10	2.04±.01(14)	2.48±.02(9)

TABLE III (continued)

Development time, survival and size of pupae of *Aedes atropalpus*
reared on various foods

Food and Larval Rear- ing Water Composition	Food Quan- tity	Time to Pupation, Days		Mortality		Length to Apex of Wing Case	
		♂	♀	♂	♀	♂	♀
<i>Coryneform</i> sp & silica sand	1	17.5±2.5(2)	22.5±2.7(4)	1/2	3/4	1.81±- (1)	2.04±- (1)
<i>E. coli</i> & silica sand	1	14.0±1.0(2)	15.0±- (1)	0/2	0/1	-	-
<i>E. coli</i> + <i>Chlorella</i> sp	1	-	-	-	-	-	-

TABLE IV

Development time, weight and size of adult male *Aedes atropalpus*
reared on various foods

Food and Larval Rear- ing Water Composition ¹	Food Quan- tity ²	Time to Emergence Days, Mean±se	Dry Weight in mg Mean±se	Length of Left Hind Femur in mm Mean±se	
control food	nw	.5	12.8±.43(13)	.561±.01(13)	2.06±.01(14)
CONTROL ("Tetramin")	nw	1	13.9±.21(11)	.533±.01(10)	2.05±.03(9)
control food	nw	2	14.0±.26(6)	.521±.02(6)	2.01±.04(6)
control food	dw	1	13.2±.11(14)	-	-
control food	Bm	1	13.4±.18(8)	.570±.03(6)	2.05±.01(7)
control food	Bm:3dw	1	14.2±.10(16)	-	-
control food	Bmv:dw	1	15.8±.48(4)	.507±.02(3)	1.96±.03(3)
<i>Nitasschia</i> sp	nw	1	20.2±1.2(13)	-	-
<i>Nitasschia</i> sp for 3rd and 4th instars	nw	1	28.1±.73(13)	-	-

¹ Bm = Bristol's medium without vitamins, Bmv = Bm + vitamins, dw = distilled water, nw = normal larval rearing water--1:1 volumes Bm:dw.

² Standard ration = 1 = 1 mg dry wt/larva/day in 5 mls of culture medium through instars I and II, then 2 mg for III and IV.

Note: Sample size in parentheses.

TABLE IV (continued)

Development time, weight and size of adult male *Aedes atropalpus*
reared on various foods

Food and Larval Rear- ing Water Composition		Food Quan- tity	Time to Emergence Days, Mean±se	Dry Weight in mg Mean±se	Length of Left Hind Femur in mm Mean±se
<i>Chlorella</i> sp	nw	≥1	29.0±- (1)	-	-
<i>Chlorella</i> sp	nw	≤1	-	-	-
<i>Chlorella</i> sp for 3rd and 4th instars	nw	1	-	-	-
<i>Chlorella</i> sp ground with silica sand	nw	1	16.7±.33(3)	.470±.06(3)	1.84±.04(3)
control food in nw + 25% <i>Chlorella</i> metabolites		1	14.5±.48(4)	.490±.02(3)	2.02±.02(3)
pollen	nw	1	15.5±1.5(2)	.305± - (1)	1.87±.05(2)
pollen	nw	.5	16.0±.38(6)	.358±.02(7)	1.74±.04(7)
alfalfa	dw	1	17.6±.69(7)	.495±.01(7)	2.07±.04(7)
alfalfa	nw	1	14.8±.33(10)	.496±.02(9)	2.04±.03(8)
wheat	dw	1	20.3±.54(10)	-	-
wheat	nw	1	14.9±.68(7)	.407±.02(5)	1.78±.09(4)

TABLE IV (continued)

Development time, weight and size of adult male *Aedes atropalpus*
reared on various foods

Food and Larval Rearing Water Composition	Food Quantity	Time to Emergence Days, Mean±se	Dry Weight in mg Mean±se	Length of Left Hind Femur in mm Mean±se
orchard grass	dw	25.4±.68(17)	-	-
orchard grass	nw	18.2±.65(14)	.317±.01(13)	1.80±.02(13)
<i>E. coli</i> + metabolites	nw	18.0±- (1)	-	-
<i>E. coli</i>	nw	21.7±2.0(3)	.275±- (1)	1.74±.23(2)
<i>E. coli</i>	nw	28.0±- (1)	.460±- (1)	1.87±- (1)
control food in nw + 25% <i>E. coli</i> metabolites	1	-	-	-
control food in nw + 5% <i>E. coli</i> metabolites	1	-	-	-
control food in nw + 40% nutrient broth sol'n	1	-	-	-
control food in nw + 10% nutrient broth sol'n	1	-	-	-
control food in nw + 5% nutrient broth sol'n	1	14.1±.19(12)	.549±.01(10)	2.04±.02(10)

TABLE IV (continued)

Development time, weight and size of adult male *Aedes atropalpus*
reared on various foods

Food and Larval Rear- ing Water Composition	Food Quan- tity	Time to Emergence Days, Mean±se	Dry Weight in mg Mean±se	Length of Left Hind Femur in mm Mean±se
<i>Irwinia</i> sp	1	-	-	-
<i>Irwinia</i> sp	.25	-	-	-
control food in nw + 5% <i>Irwinia</i> metabolites	1	14.7±.33(3)	.593±.03(3)	2.07±0(3)
<i>Pseudomonas</i> sp	1	24.0±.58(3)	.385±.04(4)	1.75±.04(4)
<i>Pseudomonas</i> sp	.5	29.0±0(2)	.340±- (2)	1.82±.06(2)
<i>Pseudomonas</i> sp	.25	28.0±1.5(3)	.325±.11(2)	1.60±.19(2)
control food in nw + 5% <i>Pseudomonas</i> metabolites	1	14.5±.22(10)	.542±.01(9)	2.04±.02(9)
<i>Coryneform</i> sp	1	28.4±.56(9)	.317±.02(5)	1.71±.03(6)
<i>Coryneform</i> sp	.5	31.4±1.1(5)	.275±.04(5)	1.66±.08(4)
<i>Coryneform</i> sp	.25	36.8±3.5(4)	.315±.05(4)	1.69±.05(4)
control food in nw + 5% <i>Coryneform</i> metabolites	1	14.1±.09(15)	.532±.01(12)	2.05±.02(12)

TABLE IV (continued)

Development time, weight and size of adult male *Aedes atropalpus*
reared on various foods

Food and Larval Rear- ing Water Composition	Food Quan- tity	Time to Emergence Days, Mean±se	Dry Weight in mg Mean±se	Length of Left Hind Femur in mm Mean±se
<i>Coryneform</i> sp & silica sand	1	19.0±- (1)	.350±- (1)	1.69±- (1)
<i>E. coli</i> & silica sand	1	17.0±- (2)	-	-
<i>E. coli</i> + <i>Chlorella</i> sp	1	-	-	-

TABLE V

Development time, size and ovarian development of adult females
of *Aedes atropalpus* reared on various foods

Food and Larval Rear- ing Water Composition ¹	Food Quan- tity ²	Time to Emergence Days, Mean±se	Length of Left Hind Femur in mm Mean±se	Number of Follicles Mean±se	Mean Stage of Follicle Development
control food	nw	13.7±.21(6)	2.37±.04(6)	151±7.7(6)	IIIb
CONTROL ("Tetramin")	nw	14.8±.20(10)	2.41±.01(10)	134±9.1(9)	IVa
control food	nw	14.0±.22(5)	2.34±.02(8)	130±7.5(8)	IIIb
control food	dw	14.1±.14(13)	-	-	-
control food	Bm	13.9±.26(7)	2.37±.02(6)	135±14(6)	IIIb
control food	Bm:3dw	14.4±.18(13)	-	-	-
control food	Bmv:dw	15.8±.25(4)	2.36±.03(3)	141±7.1(3)	IVa
<i>Nitzschia</i> sp	nw	24.6±.92(9)	-	-	-
<i>Nitzschia</i> sp for 3rd and 4th instars	nw	32.2±.86(5)	-	-	-

¹ Bm = Bristol's medium without vitamins, Bmv = Bm + vitamins, dw = distilled water, nw = normal larval rearing water--1:1 volumes Bm:dw.

² Standard ration = 1 = 1 mg dry wt/larva/day in 5 mls of culture medium through instars I and II, then 2 mg for III and IV.

Note: Sample size in parentheses.

TABLE V (continued)

Development time, size and ovarian development of adult females of *Aedes atropalpus* reared on various foods

Food and Larval Rearing Water Composition	Food Quantity	Time to Emergence Days, Mean±se	Length of Left Hind Femur in mm Mean±se	Number of Follicles Mean±se	Mean Stage of Follicle Development
<i>Chlorella</i> sp	≥1	33.5±2.5(2)	-	-	-
<i>Chlorella</i> sp	≤1	46.5±16(2)	-	-	-
<i>Chlorella</i> sp for 3rd and 4th instars	1	-	-	-	-
<i>Chlorella</i> sp ground with silica sand	1	19.0±0(3)	2.18±.02(3)	133±2.1(3)	IVa
control food in nw + 25% <i>Chlorella</i> metabolites	1	15.7±.33(3)	2.41±.03(4)	140±4.5(4)	IVa
pollen	1	20.0±- (1)	1.97±- (1)	82±- (1)	IIIB
pollen	.5	18.4±.51(5)	1.99±.08(4)	69±9.4(5)	IIIB
alfalfa	1	19.0±.45(14)	2.38±.02(11)	81±8.2(9)	IIIB
alfalfa	1	16.1±.30(16)	2.34±.02(12)	77±8.8(9)	IIIB
wheat	1	23.0±- (1)	-	-	-
wheat	1	17.7±.41(11)	2.04±.05(7)	69±7.2(4)	IIIIa

TABLE V (continued)

Development time, size and ovarian development of adult females of *Aedes atropalpus* reared on various foods

Food and Larval Rearing Water Composition	Food Quantity	Time to Emergence Days, Mean±se	Length of Left Hind Femur in mm Mean±se	Number of Follicles Mean±se	Mean Stage of Follicle Development
orchard grass dw	1	28.2±.52(11)	-	-	-
orchard grass nw	1	20.6±.65(10)	1.94±.03(10)	56±2.6(6)	IIIa-b
<i>E. coli</i> + metabolites nw	1	23.0±1.0(2)	-	-	-
<i>E. coli</i> nw	1	24.5±6.5(2)	1.97±- (1)	101±- (1)	IIIa-b
<i>E. coli</i> nw	.5	30.0±- (1)	2.05±.01(2)	85±10(2)	IIIb
control food in nw + 25% <i>E. coli</i> metabolites	1	-	-	-	-
control food in nw + 5% <i>E. coli</i> metabolites	1	-	-	-	-
control food in nw + 40% nutrient broth sol'n	1	-	-	-	-
control food in nw + 10% nutrient broth sol'n	1	-	-	-	-
control food in nw + 5% nutrient broth sol'n	1	14.4±.51(5)	2.34±.04(5)	138±11(5)	IVa

TABLE V (continued)

Development time, size and ovarian development of adult females of *Aedes atropalpus* reared on various foods

Food and Larval Rearing Water Composition	Food Quantity	Time to Emergence Days, Mean±se	Length of Left Hind Femur in mm Mean±se	Number of Follicles Mean±se	Mean Stage of Follicle Development
<i>Irwinia</i> sp nw	1	-	-	-	-
<i>Irwinia</i> sp nw	.25	-	-	-	-
control food in nw + 5% <i>Irwinia</i> metabolites	1	16.0±1(3)	2.44±.02(3)	155±5.0(3)	IIb-IVa
<i>Pseudomonas</i> sp nw	1	27.0±1(3)	2.14±.13(2)	123±13(2)	IIa-b
<i>Pseudomonas</i> sp nw	.5	-	-	-	-
<i>Pseudomonas</i> sp nw	.25	31.5±.50(2)	2.07±- (1)	90±- (1)	IIa
control food in nw + 5% <i>Pseudomonas</i> metabolites	1	14.9±.32(9)	2.39±.03(6)	169±12(5)	IVa
<i>Coryneform</i> sp nw	1	32.3±.80(6)	1.91±.10(5)	78±8.4(5)	IIb
<i>Coryneform</i> sp nw	.5	36.6±1.1(7)	1.82±.08(7)	51±4.4(7)	IIb
<i>Coryneform</i> sp nw	.25	42.5±.87(8)	1.92±.06(6)	61±6.9(6)	IIa
control food in nw + 5% <i>Coryneform</i> metabolites	1	14.8±.22(9)	2.32±.02(9)	148±4.6(7)	IVa

TABLE V (continued)

Development time, size and ovarian development of adult females
of *Aedes atropalpus* reared on various foods

Food and Larval Rear- ing Water Composition	Food Quan- tity	Time to Emergence Days, Mean±se	Length of Left Hind Femur in mm Mean±se	Number of Follicles Mean±se	Mean Stage of Follicle Development
<i>Coryneform</i> sp & silica sand	nw 1	22.0±- (1)	-	-	-
<i>E. coli</i> & silica sand	nw 1	19.0±- (1)	-	-	-
<i>E. coli</i> + <i>Chlorella</i> sp	nw 1	-	-	-	-

for those fed on 0.5 standard ration of fish food and $1.22 \pm .09 \text{ mm}^3$ and $1.46 \pm .12 \text{ mm}^3$ for those fed on one and two times a standard ration but the differences do not pass the 5% test in an analysis of variance.

Physical measurements for the pupae reared on control diets are presented in Table III and Appendix B, for each sex. In estimating pupal size in this group of experiments, it was found that measurements of wing lobe size (Table III) gave more consistent comparisons when correlated to development times than total length measurements (Appendix B). Although genitallia width is sometimes used as a criterion in size comparisons because of its sclerotization, the wing lobe measurements were adopted because they were easier to make.

Wing lobe measurements varied from $2.03 \pm .01 \text{ mm}$ to $2.02 \pm .01 \text{ mm}$ and $2.01 \pm .02 \text{ mm}$ for male pupae fed 0.5, one and two times standard rations respectively. This decrease in size with increasing food concentration was also expressed in the other physical measures made on pupae (Appendix B). Females also displayed a decrease in wing lobe size with increasing food concentrations: $2.54 \pm .02 \text{ mm}$, $2.53 \pm .01 \text{ mm}$ and $2.48 \pm .02 \text{ mm}$ for pupae reared on 0.5, one and two times standard rations. These differences do not pass the conventional 5% level of statistical significance but are suggestive enough to warrant further investigation.

In comparing the various food concentrations for effect on adult size, several physical measurements were taken. Adult males were weighed dry (Table IV). Weights ranged from $.561 \pm .01 \text{ mg}$ to $.533 \pm .01 \text{ mg}$ and $.521 \pm .02 \text{ mg}$ for adults reared on 0.5, one and two times standard rations. The mean differences fall short of the 5%

level of statistical significance but the order concordance suggests that there may be a real effect. The sample weights are similar to those found by Kalpagé (1970). Front and hind femur lengths correlated roughly with the weights. Only hind femur lengths were tested statistically (Tables IV and V). Front femur lengths are given in Appendix C. Hind femur lengths ranged from $2.06 \pm .01$ mm to $2.05 \pm .03$ mm and $2.01 \pm .04$ mm for males and $2.37 \pm .04$ mm to $2.41 \pm .01$ mm and $2.34 \pm .01$ mm for females raised on 0.5, one and two times standard rations. These do not differ significantly ($P > 0.05$). Again, males exhibited a size range inverse to the food concentration. This same trend does not occur for female femur lengths but is displayed in the number of follicles which were most numerous for those fed on the 0.5 standard ration of the control diet.

Although varying the food concentrations from 0.5 to one and two times standard rations of the control diet did not yield statistically significant differences in most measures, suggestive that fairly large alterations must occur in the diet before growth, development or survival is significantly affected, the orders of measurement means, however, conforms to a consistent pattern that should encourage further study of the influence of food concentration.

Larvae were raised on standard rations of control diets in different compositions of rearing water. Concentrations of 100% Bristol's medium, 50% Bristol's medium with and without vitamins, and 25% Bristol's medium in distilled water and distilled water only were tested (Tables I to V and Appendices A to C). Insects reared in distilled water had significantly shorter development times than control insects ($P < 0.05$) in the larval and male pupal stages only (6.27 and

9.80 days compared with 6.71 and 10.67 days respectively). The physical measures did not differ significantly ($P>0.05$) from the control.

Larvae reared in 50% Bristol's medium with the addition of vitamins thiamine, biotin and vitamin B₁₂ (algal culture medium) showed retarded development times and reduced larval physical sizes which could possibly be due to the abnormal concentrations of the vitamins which were available to the larvae. A developmental time lag was found beginning in the larval stage which persisted throughout the later stages. These vitamins would not be present in as high a concentration when this medium was introduced with the addition of algae to the rearing water for two reasons: firstly, only small amounts of the algal culture medium-food were added, ensuring dilution, and secondly, the growing algae would very quickly utilize the vitamins from their culture medium reducing the free vitamin concentration in the water before the larvae were placed in it.

Mosquitoes reared in water composed of a 5% addition of nutrient broth solution as used in bacteria culturing did not differ significantly ($P>0.05$) from the control group in any measure.

C. Algae

Of the two algae tested, the diatom *Nitzschia* sp was considerably better than the green alga *Chlorella* sp, but still a significantly poorer food by comparison to commercial fish food ($P<0.05$).

Larvae given *Nitzschia* sp required a longer time for developing through each stage, taking 20.2 days for male adults to emerge and 24.6 days for females compared to about 13.9 and 14.8 days for those

reared on commercial fish food (Tables IV and V). The mortality was lower than normal in the larval and similar in the succeeding stages. From the physical measurements available, however, pupae reared on *Nitzschia* sp had considerably smaller wing lobes than normal, $1.78 \pm .04$ mm for males and $2.12 \pm .03$ mm for females as compared to $2.06 \pm .01$ mm and $2.07 \pm .04$ mm. Physical measures are not available for the adult stage.

Chlorella sp provided an extremely poor diet (Tables II to V). Mortality was very high in the larval stage (90%) and very few (one male and two females) reached the adult stage. Development time was very extended, taking 29.0 days for males to emerge and 46.5 days for females. Increasing the amount of food ration did not improve results much (females development time was reduced to 33.5 days). Larvae reared on commercial fish food for the first two instars and then on *Chlorella* sp for the remaining instars showed slight improvement. Larval development times shortened from 23.8 to 7.81 days and pupal development times shortened from 38 to 31.5 days for males. Mortality was again high (93%) in the larval stage and none progressed beyond the pupal stage. It appears that *Chlorella* sp could not be utilized even by the later instars.

In both trials, a great many viable *Chlorella* sp cells were observed in the posterior portion of the larval gut and also in the feces. These cells were cultured without difficulty. However, the larvae grew normally when reared on fish food in water composed of a 25% solution of cell-free *Chlorella* sp metabolites and development times and other measures were close to the control values ($P > 0.05$) indicating that the metabolites of *Chlorella* sp are not toxic.

Chlorella sp is known to have a relatively high protein content by comparison to other algae (Fowden, 1954). When *Chlorella* sp cells were freeze-dried, ground with sterile silica sand to crush the cells and fed to larvae, the insects developed as well as on the control diet. Development times were slightly longer than normal, and were significantly so only for female pupae and adults ($P < 0.05$).

Size was significantly smaller in the larval stage ($P < 0.05$). Ovarian development and follicle numbers were the same as for insects on the control diet.

Chlorella sp is an algae with a thick cell wall (Round, 1966) and it seems likely that this cellulose wall hinders the larvae's ability to assimilate the cell contents. Live cells of other algae have been reported in the rear portions of the alimentary tract and expelled in feces (Hinman, 1930). It appears probable that other algae high in nutrients are also resistant to digestion (Fowden, 1954). Davis and Guillard (1958) observed the same phenomenon with clam and oyster larvae feeding on *Carteria* sp, a species of green alga.

Although diatoms have cell walls composed of silica it might be thought that this would prevent or reduce their utilization by the larvae. However, motile diatoms possess a longitudinal groove called a raphe which exposes the inner part of the cell. As well, all cell walls of diatoms and other silicious algae are perforated with many small openings (Patrick and Reimer, 1966) which could allow digestive enzymes to come into contact with the inner parts of the cell. Senior-White (1928) and Romney and Neilson (1968) have shown that diatoms constitute a large portion of gut contents.

Time of passage through the gut is a factor in the utilization of foods and has been shown to be quite rapid for all instars of some species. In "fourth instar *A. aegypti* and *Culex pipiens*, food reached the hind end of the midgut 20 to 30 min. after ingestion at 27°C and 50 to 60 min. after ingestion at 20°C, whereas in 2nd and 3rd instars only 15 to 20 min. were required at 27°C. Fourth instar larvae of *Anopheles maculipennis* and *A. superpictus* required about 60 min. at 27°C." (Clements, 1963). Dadd (1968, 1970a and b) has also shown that gut passage times are short. This suggests that micro-organisms with more resistant cell walls probably contribute less to the nutrition of the larvae during their short stay in the gut. This in turn affirms the importance of more delicate micro-organisms or those species more likely to be digested.

D. Detritus

The value of organic detritus has always been considered significant in the nutrition of mosquito larvae "Although non-living organic matter may also be important, the extent to which it can supplement or replace micro-organisms is not known." (Clements, 1963).

In the present study two types of organic detritus were considered: pollen, a common constituent of larval gut contents; and plant fragments. These are sometimes referred to as "organic detritus" or "amorphous material" in gut content analysis (Senior-White, 1928; Romney and Lewis, 1968).

When standard rations of pollen were fed, larval development to the fourth instar required 7.72 days, time required to reach pupal stage was 13.5 days for males and 18.3 for females and time required to reach adult stage was 15.5 and 20.0 days for males and females,

significantly longer than normal development times ($P < 0.05$). Mortality was much higher than for the control. Physical measures were also significantly smaller ($P < 0.05$) for each stage. Ovarian development and total follicle number were considerably less than the values found for normally reared specimens (Tables II to V).

When the pollen diet was reduced to 0.5 standard ration all measures improved although these were still significantly poorer than for insects fed similar concentrations of the control diet ($P < 0.05$). Larvae required 6.48 days to reach the 4th instar, 12.7 and 15.5 days for males and females to reach pupal stage and 16.0 and 18.4 days for male and female adults to emerge. Mortality was similar to that of normally reared specimens. Physical measures were greater than for insects fed a standard ration of pollen in all but pupal wing lobe size (male and female) and adult male hind femur length. Ovarian development and total follicle count did not increase.

This suggests the presence of a toxic factor in the pollen. Another hypothesis is that the shape of pollen grains might cause mechanical interference with the mouthparts in the filtering process, but observations have not confirmed this.

Although alfalfa, wheat and orchard grass are not normally available to mosquito larvae they were included as diets in an attempt to simulate macrophytic detritus ingested by mosquito larvae in the natural habitat. Results of previous nutritive studies using these three diets were available for another insect (grasshopper) affording an indication of utilization (Wilson, 1969).

For the mosquito larvae, none of the three provided as good a diet as commercial fish food (Tables II to V). Alfalfa was the best

of these but development times and physical measures were mostly somewhat inferior to those of the control. Only female pupae wing lobe size (2.39 ± 0.02 mm compared with 2.53 ± 0.01 mm) and ovarian development and follicle number (77 ± 8.8 at stage IIIb versus 134 ± 9.1 at stage IVa) differed significantly ($P < 0.05$). Mean development time (6.7 days) for the larval stage was the same as for the control larvae (6.71 days) but development was slower in the following stages with male and female pupae requiring 10.9 days and 12.6 days respectively compared with 10.7 and 10.8 days for the control. Male and female adults required 14.8 and 16.1 days compared with 13.9 and 14.8 days respectively for those reared on the control diet. Mortality in the larval stage was very low (1/30 compared with 9/30 for the control).

Wheat leaf meal was generally poorer than alfalfa but better than orchard grass. All physical measures of larvae, male pupal wing lobe size, female pupal development time, male adult weight and hind femur length and in all measures of the female adult were significantly smaller for insects reared on wheat meal than on the control. Most measures were also significantly inferior ($P < 0.05$) than those obtained with alfalfa, primarily in adult development times and physical measures of both sexes.

Orchard grass was a significantly poorer diet ($P < 0.05$) than the control group in all measures except larval development time (6.15 days) and mortality (5/30). Orchard grass was also significantly poorer than alfalfa in all measures excepting larval development time and poorer than wheat in most measures of pupae and adults.

Wilson (1969) tested grasshoppers (*Melanopus sanguinipes* (Fabricus)) on alfalfa, wheat and orchard grass leaf meals and found

orchard grass to give the lowest growth index of all diets tested, i.e., the grasshoppers had to consume more orchard grass diet than alfalfa or wheat per unit adult weight.

When all three of these diets were fed to the mosquito larvae in distilled water (without the addition of Bristol's medium), the development times were longer, probably due to a diminished supply of mineral salts necessary for normal ecdysis (Clements, 1963). Bristol's medium is a solution containing salts that Bates (1941) found necessary for normal development. In natural pools this factor would not influence the development since all natural waters contain these minerals in adequate concentrations (Hutchinson, 1957).

From the results of the leaf meal diets it appears that macrophytic detritus of diverse origins is not of equal nutritional potential.

E. Bacteria

Of the four species of bacteria used, *Pseudomonas* sp and *Coryneform* sp could be considered of slight nutritive value and *Erwinia* sp and *E. coli* were very poor.

Larvae fed *Pseudomonas* sp or *Coryneform* sp required almost twice as long to reach the 4th instar than larvae fed on the control diet (Table II and Appendix A). Mortality was greater with *Pseudomonas* sp (20/30) and *Coryneform* (13/30) than with the control (9/30). Measures for the width of segment III were considerably smaller than normal on specimens reared on *Pseudomonas* sp and *Coryneform* sp on diets compared to the control. The thorax volumes were only $0.74 \pm .06 \text{ mm}^3$ and $0.39 \pm .02 \text{ mm}^3$ compared with $1.33 \pm .09 \text{ mm}^3$ for the control.

Pseudomonas sp appeared to be slightly more favourable for larval development than *Coryneform* sp.

A reduction in food concentration to 0.5 and 0.25 standard rations further reduced all measures.

Males and females reared on *Pseudomonas* sp pupated in 21.3 and 23.3 days, not significantly shorter than *Coryneform* sp, requiring 24.6 and 29.2 days respectively. However, these were significantly longer than the control development times of 10.7 days for males and 10.8 days for females ($P < 0.05$). Pupal mortality was generally slightly greater than for the control for both sexes. Physical measures, e.g., wing lobe lengths, were smaller than those of the control ($P < 0.05$). The difference was greater for larvae fed low concentrations of the diet.

Males and females reared on *Pseudomonas* sp and on *Coryneform* sp required twice as long to reach the adult stage as compared with the control times of 13.9 and 14.8 days for males and females. Dry weights of the males were $.385 \pm .04$ mg and $.317 \pm .02$ mg for those specimens reared on *Pseudomonas* sp and *Coryneform* sp respectively, compared with $.533 \pm .01$ mg of the control. Femur lengths of both sexes were less than those of the control specimens. On dissecting the ovaries, *Pseudomonas* sp reared females produced 123 ± 13 follicles and *Coryneform* sp. only 78 ± 8 , significantly less than 134 ± 9 observed in the control insects ($P < 0.05$). Follicles of adult females reared on both bacterial diets were developed to about the IIIb stage while those from females reared on the control diets usually reached the IVa stage of development. Development was reduced on lower concentrations of these diets.

When *A. atropalpus* was reared on Tetramin in water to which *Pseudomonas* sp and *Coryneform* sp metabolites had been added (3 ml or 5% by volume) no significant difference was observed in any of the growth, development or survival measures compared to those of the control specimens, in any stage of either sex ($P>0.05$). These results suggest that metabolites of the two bacteria cannot be considered detrimental when present in this concentration. Since larvae never encountered metabolite concentrations higher than 5% the poor performance of *A. atropalpus* reared on the bacteria cell diets must then be attributed largely to the low nutritional value of the intact bacteria.

When standard rations of *Coryneform* sp cells were combined with sterile silica sand in 1:4 mixtures, development times, mortality and physical measures for all stages improved significantly. Both males and females required 19.0 days to emerge compared with 28.4 and 32.3 days for diets consisting of cells only ($P<0.05$). Survival showed little improvement. Larval thorax volume increased almost twice to $0.69 \pm 0.05 \text{ mm}^3$ but remained much smaller than normal ($1.33 \pm 0.09 \text{ mm}^3$). Pupal and adult physical measures also increased.

It would appear that the addition of mechanical bulk to supplement a poor food improved the diet. Under natural conditions a larva would normally consume various quantities of inorganic detritus which might contribute to the utilization of other foods present. Nayar (1966) has reported the presence of mechanical bulk as necessary for proper alimentation of *A. taeiorhynchus*. In the case of *A. atropalpus*, the addition of silica sand as mechanical bulk produced a decidedly beneficial effect.

Irwinia sp and *E. coli* were found to be poor foods and metabolites from these two bacteria were decidedly detrimental (Tables II to V). Mortality of *A. atropalpus* on a standard ration of *Irwinia* sp was total (30/30). Reducing the ration to 0.5 produced almost as complete (28/30) and the few surviving larvae succumbed early in the pupal stage. On testing the metabolites from *Irwinia* sp, they did not prove to be quite as toxic as the cells (24/30). The survivors did not differ significantly from the control specimens in any stage ($P>0.05$). It appears that metabolites of *Irwinia* sp were toxic only during food ingestion and did not interfere with the later stages of development.

E. coli, like *Erwinia* sp gave poor results, but conversely, *E. coli* metabolites were highly toxic. When small amounts (3 ml or 5% by volume) of *E. coli* metabolites were added to rearing water all larvae died. The cells, however, were not as toxic although growth, development and survival remained significantly inferior to the control ($P<0.05$). A few adults did emerge, requiring on average 21.7 days for the three males and 24.5 days for the two females. Reducing the ration to 0.5 further depressed development. Combining the cells with sterile silica sand as was done for *Coryneform* sp effected only a slight improvement.

The toxic metabolite effect of some bacteria has been reported by others (Hinman, 1933). A similar effect was found with a few species of algae, although not with the species used in this study. Amonkar (1969) and Reeves (1970) isolated strains of blue-green algae having metabolites detrimental to larvae of four species of *Culex* and *Aedes* mosquitoes.

In the bacteria as with the algae, a wide variation in nutritional value was found between members of the same food taxa. Not only can the cells or metabolites be detrimental or toxic as with *Irwinia* sp and *E. coli*, but the cells per se can be very poor in food value as exemplified by *Coryneform* sp. In such a vast taxonomic group as the algae or the bacteria it would seem likely that more nutritious as well as poor or harmful species should exist.

F. General Discussion

A controlled diet comprising a mixed complex of organisms would presumably provide a better diet than any one component alone since those nutritional factors incomplete in one species would likely be supplemented by others. This mixed food composition was not tested on the mosquitoes in this study due to the problems of maintaining controlled proportions of each constituent of the live food. In nature, however, it is from such a varied selection that the mosquito larva derives its nutrition. All micro-organisms and detritus contribute and while some constituents are detrimental or toxic, others are probably of more positive value and the resulting component usually favours adequate growth, development and survival. It seems unreasonable considering the limited results of this study, to stress any one taxa of food flora as most important. Each group probably includes beneficial and detrimental components. The effect of low nutritive, detrimental or toxic cells or metabolites are probably so diluted as to be innocuous. Perhaps the most beneficial or detrimental species are, in fact, few. A preponderance of a single component as could occur during an algal bloom, bacterial infestation or accumulations of decaying aquatic plant material would probably increase the

likelihood of ingestion by the larva's indiscriminate feeding process. This concentration or abundance would become more acute in smaller pools where floral species diversity is less and which are subjected to more violent fluctuations in floral succession throughout the seasons (Tribbey, 1965). Studies of temporary water habitats characteristic of Western Canada have been insufficiently treated. The regional characterization of these pools limnologically and ecologically (especially the micro-fauna, flora and the bacteria) through the seasons would provide a valuable basis for similar programs.

The approach and methods worked out in this study for isolating and culturing constituents of mosquito larval diets are practical, but to obtain pure culture diets of larger assortments of micro-organisms, in particular the algae and protozoa, would present a sizable technical undertaking. The culturing of mosquitoes on pure diets or mixtures of micro-organisms would be useful for further studies on the value of natural constituents in larval diets as a controlled diet for comparative mosquito physiology or for considering biological control agents.

SUMMARY

1. Fairly large alterations in the diet of *A. atropalpus* larvae must occur before measures of growth, development or survival are significantly affected. Varying the concentration of a standard ration of a basic control food from 0.5 to two times did not effect significant changes. Mineral composition of the larval rearing water influences *A. atropalpus* development and growth.

2. Of the two algae tested, *Nitzschia* sp was considerably more nutritious for *A. atropalpus* larvae than *Chlorella* sp although significantly poorer than the control food. Increasing the ration did not compensate.

3. It was found that the thickness of the algal cell wall hampered digestion by the larva, even by older instars. Gut passage time may be a factor affecting assimilation. By crushing the cells to facilitate digestion a significant improvement in growth, development and survival occurred.

4. Organic detritus of macrophytes and pollen can be utilized by mosquito larvae of *A. atropalpus* but do not provide satisfactory foods by themselves. Although alfalfa was more favourable than wheat and orchard grass it was significantly less nutritious than the control diet. The pollen provided a better diet when 0.5 standard ration was used. The inferior results may have resulted from a toxic factor or from mechanical interference of the higher concentration of

pollen particles with the mouthparts.

5. The bacteria provided a sub-optimal diet, although some growth and development to maturity was obtained on *Pseudomonas* sp, *Coryneform* sp and *E. coli* in that order. The addition of mechanical bulk (silica sand) improved the diet. Reducing the concentration of these micro-organic diets caused a further deterioration in all measures.

6. Cells of some bacteria were toxic while the metabolites were not and vice versa. *Erwinia* sp cells were very toxic to the larvae and caused high mortality. The metabolites were less toxic but the few survivors were not significantly different from normally reared specimens. *E. coli* cells, however, were less toxic than their metabolites.

7. A diet comprising a mixed complex of food organisms would allow the various deficiencies of individual components to be supplemented by the contributions of other species. This depicts the natural situation.

8. Some micro-organic constituents are toxic while others are benign. It seems that, in nature, mixtures likely favour adequate growth, development and survival.

9. A great discrepancy was found among the various biological taxa in their food contribution and each probably includes favourable and unfavourable components of larval food. The preponderance of a single component as could occur during an algal bloom would probably increase the likelihood of ingestion by the indiscriminant filter feeding of mosquito larvae.

APPENDICES

APPENDIX A

Measurements of overall length, abdominal segment III and thorax length, width and depth of live 4th instar *Aedes atropalpus* fed various foods (mm, mean±se, n bracketed)

Food and Larval Rearing Water Composition ¹	Food Quantity ²	Overall length	Abdominal Segment III Depth	Thorax Length	Thorax Width	Thorax Depth
control food	nw .5	7.60±.16(25)	0.76±.02(25)	1.35±.03(25)	1.67±.04(25)	0.97±.02(25)
CONTROL ("Tetramin")	nw 1	7.46±.18(25)	0.80±.03(23)	1.38±.03(23)	1.70±.05(24)	0.97±.02(24)
control food	nw 2	7.76±.23(17)	0.82±.03(17)	1.36±.05(17)	1.76±.05(17)	1.04±.03(17)
control food	dw 1	6.72±.29(9)	0.86±.05(9)	1.04±.07(9)	1.40±.08(9)	-
control food	Bm 1	7.39±.20(17)	0.76±.03(17)	1.28±.03(17)	1.65±.05(17)	0.92±.02(17)
control food	Bm:3dw 1	-	-	-	-	-
control food	Bmv:dw 1	7.44±.25(9)	0.72±.04(9)	1.27±.05(9)	1.53±.06(9)	0.88±.03(9)
<i>Nitzschia</i> sp	nw 1	-	-	-	-	-
<i>Nitzschia</i> sp for 3rd and 4th instars	nw 1	-	-	-	-	-

¹ Bm = Bristol's medium without vitamins, Bmv = Bm + vitamins, dw = distilled water, nw = normal larval rearing water--1:1 volumes Bm:dw.

² Standard ration = 1 = 1 mg dry wt/larva/day in 5 mls of culture medium through instars I and II, then 2 mg for III and IV.

APPENDIX A (continued)

Measurements of overall length, abdominal segment III and thorax length, width and depth of live 4th instar *Aedes atropalpus* fed various foods (mm, mean±se, n bracketed)

Food and Larval Rearing Water Composition	Food Quantity	Overall Length	Abdominal Segment III Depth	Thorax Length	Thorax Width	Thorax Depth
<i>Chlorella</i> sp nw	≤1	-	-	-	-	-
<i>Chlorella</i> sp nw	≥1	-	-	-	-	-
<i>Chlorella</i> sp for 3rd and 4th instars nw	1	-	-	-	-	-
<i>Chlorella</i> sp ground with silica sand nw	1	6.71±.14(10)	0.61±.02(10)	1.06±.02(10)	1.32±.03(10)	0.74±.02(10)
control food in nw + 25% <i>Chlorella</i> metabolites	1	7.80±.30(8)	0.77±.04(8)	1.32±.05(8)	1.63±.07(8)	0.96±.03(8)
pollen nw	1	6.08±.23(14)	-	0.92±.04(14)	1.17±.05(14)	-
pollen nw	.5	7.24±.16(20)	-	1.12±.02(20)	1.43±.03(20)	0.89±.02(20)
alfalfa dw	1	9.35±- (1)	-	-	1.94±- (1)	-
alfalfa nw	1	8.21±.11(28)	-	1.28±.02(27)	1.62±.03(27)	-
wheat dw	1	6.12±.30(7)	-	-	1.31±.10(7)	-
wheat nw	1	7.25±.11(28)	-	1.16±.02(28)	1.49±.03(28)	-

APPENDIX A (continued)

Measurements of overall length, abdominal segment III and thorax length, width and depth of live 4th instar *Aedes atropalpus* fed various foods (mm, mean±se, n bracketed)

Food and Larval Rearing Water Composition	Food Quantity	Overall Length	Abdominal Segment III Depth	Thorax Length	Thorax Width	Thorax Depth
orchard grass dw	1	6.35±.24(14)	-	-	1.39±.07(14)	-
orchard grass nw	1	7.30±.11(25)	0.63±.01(25)	1.07±.02(25)	1.36±.02(25)	0.81±.02(25)
<i>E. coli</i> + metabolites nw	1	-	-	-	-	-
<i>E. coli</i> nw	1	6.79±.64(3)	-	1.01±.08(3)	1.40±.13(3)	0.81±.05(3)
<i>E. coli</i> nw	.5	-	-	-	-	-
control food in nw + 25% <i>E. coli</i> metabolites	1	-	-	-	-	-
control food in nw + 5% <i>E. coli</i> metabolites	1	-	-	-	-	-
control food in nw + 40% nutrient broth sol'n	1	-	-	-	-	-
control food in nw + 10% nutrient broth sol'n	1	-	-	-	-	-
control food in nw + 5% nutrient broth sol'n	1	7.27±.16(18)	0.77±.02(18)	1.31±.03(18)	1.67±.03(18)	0.98±.03(18)

APPENDIX A (continued)

Measurements of overall length, abdominal segment III and thorax length, width and depth of live 4th instar *Aedes atropalpus* fed various foods (mm, mean±se, n bracketed)

Food and Larval Rearing Water Composition	Food Quantity	Overall Length	Abdominal Segment III Depth	Thorax Length	Thorax Width	Thorax Depth
<i>Irwina</i> sp	1	-	-	-	-	-
<i>Irwina</i> sp	.5	-	-	-	-	-
control food in nw + 5% <i>Irwina</i> metabolites	1	-	-	-	-	-
<i>Pseudomonas</i> sp	1	6.56±.22(10)	0.65±.02(10)	1.07±.03(10)	1.44±.04(10)	0.84±.02(10)
<i>Pseudomonas</i> sp	.5	6.03±.25(10)	0.59±.10(10)	0.96±.05(10)	1.31±.05(10)	0.76±.03(10)
<i>Pseudomonas</i> sp	.25	5.92±.22(10)	0.55±.02(10)	0.92±.05(10)	1.18±.04(10)	0.68±.03(10)
control food in nw + 5% <i>Pseudomonas</i> metabolites	1	7.92±.22(15)	0.82±.03(15)	1.38±.04(15)	1.76±.05(15)	1.00±.03(15)
<i>Coryneform</i> sp	1	5.84±.16(10)	0.53±.01(10)	0.89±.02(10)	1.17±.03(10)	0.66±.02(10)
<i>Coryneform</i> sp	.5	5.61±.16(10)	0.47±.02(10)	0.82±.03(10)	1.09±.03(10)	0.65±.03(10)
<i>Coryneform</i> sp	.25	5.20±.14(10)	0.46±.01(10)	0.75±.03(10)	1.01±.02(10)	0.59±.01(10)
control food in nw + 5% <i>Coryneform</i> metabolites	1	7.64±.14(25)	0.78±.02(25)	1.32±.03(25)	1.70±.03(25)	0.96±.02(25)

APPENDIX A (continued)

Measurements of overall length, abdominal segment III and thorax length, width and depth of live 4th instar *Aedes atropalpus* fed various foods (mm, mean±se, n bracketed)

Food and Larval Rearing Water Composition		Food Quantity	Overall Length	Abdominal Segment III depth	Thorax Length	Thorax Width	Thorax Depth
<i>Coryneform</i> sp & silica sand	nw	1	6.95±.11(5)	0.65±.03(5)	1.09±.03(5)	1.40±.03(5)	0.81±.02(5)
<i>E. coli</i> & silica sand	nw	1	-	-	-	-	-
<i>E. coli</i> + <i>Chlorella</i> sp	nw	1	-	-	-	-	-

APPENDIX B

Measurements of length from left respiratory trumpet to paddle tip and width of genitalia of *Aedes atropalpus* pupae reared on various foods (mm, mean±se, n bracketed)

Food and Larval Rearing Water Composition ¹	Food Quantity ²	Length to Paddle Tip		Width of Genitalia	
		♂	♀	♂	♀
control food	nw	5.42±.04(10)	6.90±.05(6)	0.51±.003(10)	0.22±.005(6)
CONTROL ("Tetramin")	nw	5.35±.04(11)	6.71±.09(10)	0.50±.004(11)	0.23±.004(9)
control food	nw	5.33±.04(6)	6.56±.10(9)	0.52±.005(6)	0.22±.005(9)
control food	dw	5.52±.12(7)	6.86±.06(10)	0.51±.002(7)	0.23±.002(11)
control food	Bm	5.52±.07(8)	6.76±.07(6)	0.51±.005(8)	0.22±.005(6)
control food	Bm:3dw	-	-	-	-
control food	Bmv:dw	5.18±.08(3)	6.52±.07(4)	0.51±.011(3)	0.22±.006(4)
<i>Nitazschia</i> sp	nw	4.70±.08(13)	5.51±.10(10)	0.46±.006(12)	0.21±.005(12)
<i>Nitazschia</i> sp for 3rd and 4th instars	nw	-	-	-	-

¹ Bm = Bristol's medium without vitamins, Bmv = Bm + vitamins, dw = distilled water, nw = normal larval rearing water--1:1 volumes Bm:dw.

² Standard ration = 1 = 1 mg dry wt/larva/day in 5 mls of culture medium through instars I and II, then 2 mg for III and IV.

APPENDIX B (continued)

Measurements of length from left respiratory trumpet to paddle tip and width of genitalia of *Aedes atropalpus* pupae reared on various foods (mm, mean±se, n bracketed)

Food and Larval Rear- ing Water Composition		Food Quan- tity	Length to Paddle Tip ♂	Length to Paddle Tip ♀	Width of Genitalia ♂	Width of Genitalia ♀
<i>Chlorella</i> sp	nw	41	-	-	-	-
<i>Chlorella</i> sp	nw	51	-	-	-	-
<i>Chlorella</i> sp for 3rd and 4th instars	nw	1	-	-	-	-
<i>Chlorella</i> sp ground with silica sand	nw	1	5.31±.16(3)	6.28±.12(3)	0.50±.015(3)	0.21±.008(3)
control food in nw + 25% <i>Chlorella</i> metabolites		1	5.25±.24(3)	6.60±.09(4)	0.50±.004(3)	0.23±0(4)
pollen	nw	1	4.58±0(2)	4.97±-(1)	0.44±.006(2)	0.18±-(1)
pollen	nw	.5	4.70±.10(7)	5.52±.15(5)	0.46±.007(7)	0.20±.004(5)
alfalfa	dw	1	5.31±.05(7)	6.78±.06(14)	0.49±.004(7)	0.23±.002(14)
alfalfa	nw	1	5.24±.04(10)	6.32±.04(16)	0.50±.003(10)	0.23±.002(15)
wheat	dw	1	-	5.42±- (1)	-	0.19±- (1)
wheat	nw	1	5.51±.07(4)	5.83±.13(3)	0.48±.008(5)	0.20±- (2)

APPENDIX B (continued)

Measurements of length from left respiratory trumpet to paddle tip and width of genitalia of *Aedes atropalpus* pupae reared on various foods (mm, mean±se, n bracketed)

Food and Larval Rear- ing Water Composition	Food Quan- tity	Length to Paddle Tip ♂	Length to Paddle Tip ♀	Width of Genitalia ♂	Width of Genitalia ♀
orchard grass	dw	-	-	-	-
orchard grass	nw	4.79±.04(13)	5.41±.11(10)	0.46±.003(13)	0.19±.004(10)
<i>E. coli</i> + metabolites	nw	4.84±- (1)	5.61±- (1)	0.48±- (1)	0.18±- (1)
<i>E. coli</i>	nw	4.42±.12(4)	5.56±.07(8)	0.44±.006(4)	0.20±.006(9)
<i>E. coli</i>	nw	5.03±- (1)	5.61±- (1)	0.49±- (1)	0.19±- (1)
control food in nw + 25% <i>E. coli</i> metabolites	1	-	-	-	-
control food in nw + 5% <i>E. coli</i> metabolites	1	-	-	-	-
control food in nw + 40% nutrient broth sol'n	1	-	-	-	-
control food in nw + 10% nutrient broth sol'n	1	4.97±- (1)	-	0.50±- (1)	-
control food in nw + 5% nutrient broth sol'n	1	5.51±.03(11)	6.61±.05(5)	0.51±.02(10)	0.23±.004(5)

APPENDIX B (continued)

Measurements of length from left respiratory trumpet to paddle tip and width of genitalia of *Aedes atropalpus* pupae reared on various foods (mm, mean±se, n bracketed)

Food and Larval Rearing Water Composition	Food Quantity	Length to Paddle Tip ♂	Length to Paddle Tip ♀	Width of Genitalia ♂	Width of Genitalia ♀
<i>Irwinia</i> sp nw	1	-	-	-	-
<i>Irwinia</i> sp nw	.5	-	-	-	-
control food in nw + 5% <i>Irwinia</i> metabolites	1	5.42±.04(3)	6.88±.11(3)	0.20±.004(3)	0.21±.008(3)
<i>Pseudomonas</i> sp nw	1	4.79±.04(4)	6.29±.29(2)	0.47±.007(4)	0.22±.019(2)
<i>Pseudomonas</i> sp nw	.5	4.71±- (2)	5.23±- (1)	0.47±.006(2)	0.19±- (1)
<i>Pseudomonas</i> sp nw	.25	4.55±.36(2)	5.16±.71(2)	0.44±.007(3)	0.17±.006(2)
control food in nw + 5% <i>Pseudomonas</i> metabolites	1	5.48±.05(9)	6.76±.07(9)	0.50±.006(9)	0.22±.003(9)
<i>Coryneform</i> sp nw	1	4.71±.07(9)	5.39±.16(6)	0.46±.005(9)	0.19±.008(6)
<i>Coryneform</i> sp nw	.5	4.69±.12(4)	5.21±.16(7)	0.44±.013(4)	0.18±.009(7)
<i>Coryneform</i> sp nw	.25	4.56±.09(4)	5.30±.12(6)	0.44±.014(4)	0.18±.004(6)
control food in nw + 5% <i>Coryneform</i> metabolites	1	5.51±.04(14)	6.57±.07(9)	0.52±.004(14)	0.23±.001(9)

APPENDIX B (continued)

Measurements of length from left respiratory trumpet to paddle tip and width of genitalia of *Aedes atropatus* pupae reared on various foods (mm, mean±se, n bracketed)

Food and Larval Rearing Water Composition	nw	Food Quantity	Length to Paddle Tip		Width of Genitalia	
			♂	♀	♂	♀
<i>Coryneform</i> sp & silica sand	nw	1	4.77±- (1)	5.48±- (1)	0.45±- (1)	0.18±- (1)
<i>E. coli</i> & silica sand	nw	1	-	-	-	-
<i>E. coli</i> + <i>Chlorella</i> sp	nw	1	-	-	-	-

APPENDIX C

Measurements of left front tarsi lengths of adult *Aedes atropalpus* reared on various foods (mm, mean±se, sample size parentheses)

Food and Larval Rearing Water Composition ¹	Food Quantity ²	Length of Front Femur ♂	Length of Front Femur ♀
control food	nw .5	1.93±.01 (14)	2.02±.12 (6)
CONTROL ("Tetramin")	nw 1	1.88±.02 (8)	2.19±.02 (10)
control food	nw 2	1.85±.01 (6)	2.15±.02 (8)
control food	dw 1	-	-
control food	Bm 1	1.94±.02 (7)	2.12±.02 (6)
control food	Bm:3dw 1	-	-
control food	Bmv:dw 1	1.86±.03 (3)	2.13±.01 (3)
<i>Nitsschia</i> sp	nw 1	-	-
<i>Nitsschia</i> sp for 3rd and 4th instars	nw 1	-	-

¹ Bm = Bristol's medium without vitamins, Bmv = Bm + vitamins, dw = distilled water, nw = normal larval rearing water--1:1 volumes Bm:dw.

² Standard ration = 1 = 1 mg dry wt/larva/day in 5 mls of culture medium through instars I and II, then 2 mg for III and IV.

APPENDIX C (continued)

Measurements of left front tarsi lengths of adult *Aedes atropalpus* reared on various foods (mm, mean±se, sample size parentheses)

Food and Larval Rearing Water Composition	Food Quantity	Length of Front Femur ♂	Length of Front Femur ♀
<i>Chlorella</i> sp	nw ≤1	-	-
<i>Chlorella</i>	nw ≥1	-	-
<i>Chlorella</i> sp for 3rd and 4th instars	nw 1	-	-
<i>Chlorella</i> sp ground with silica sand	nw 1	1.84±.04(3)	2.06±.02(3)
control food in nw + 25% <i>Chlorella</i> metabolites	1	1.88±.02(3)	2.12±.04(4)
pollen	nw 1	1.66±.23(2)	1.74± (1)
pollen	nw .5	1.66±.04(7)	1.82±.05(5)
alfalfa	dw 1	1.95±.04(4)	2.15±.02(12)
alfalfa	nw 1	1.87±.03(8)	2.12±.02(11)
wheat	dw 1	-	-
wheat	nw 1	1.64±.12(4)	1.84±.03(9)

APPENDIX C (continued)

Measurements of left front tarsi lengths of adult *Aedes atropalpus* reared on various foods (mm, mean±se, sample size parentheses)

Food and Larval Rearing Water Composition		Food Quantity	Length of Front Femur ♂	Length of Front Femur ♀
orchard grass	dw	1	-	-
orchard grass	nw	1	1.71±.02 (13)	1.76±.04 (10)
<i>E. coli</i> + metabolites	nw	1	-	-
<i>E. coli</i>	nw	1	1.56±- (2)	1.84±- (1)
<i>E. coli</i>	nw	.5	1.76±- (1)	1.88±.04 (2)
control food in nw + 25% <i>E. coli</i> metabolites		1	-	-
control food in nw + 5% <i>E. coli</i> metabolites		1	-	-
control food in nw + 40% nutrient broth sol'n		1	-	-
control food in nw + 10% nutrient broth sol'n		1	1.74±- (1)	-
control food in nw + 5% nutrient broth sol'n		1	1.91±.03 (10)	2.19±.04 (5)

APPENDIX C (continued)

Measurements of left front tarsi lengths of adult *Aedes atropalpus* reared on various foods (mm, mean±se, sample size parentheses)

Food and Larval Rearing Water Composition	Food Quantity	Length of Front Femur ♂	Length of Front Femur ♀
<i>Irwinia</i> sp	1	-	-
<i>Irwinia</i> sp	.5	-	-
control food in nw + 5% <i>Irwinia</i> metabolites	1	1.92±.06 (3)	2.27±.04 (3)
<i>Pseudomonas</i> sp	1	1.73±.04 (3)	1.97±.13 (2)
<i>Pseudomonas</i> sp	.5	1.71±0 (2)	-
<i>Pseudomonas</i> sp	.25	1.49±.25 (2)	1.84±- (1)
control food in nw + 5% <i>Pseudomonas</i> metabolites	1	1.89±.01 (9)	2.19±.04 (6)
<i>Coryneform</i> sp	1	1.61±.03 (5)	1.79±.09 (5)
<i>Coryneform</i> sp	.5	1.49±.07 (5)	1.77±.06 (6)
<i>Coryneform</i> sp	.25	1.61±.05 (4)	1.77±.06 (6)
control food in nw + 5% <i>Coryneform</i> metabolites	1	1.93±.02 (12)	2.09±.02 (9)

APPENDIX C (continued)

Measurements of left front tarsi lengths of adult *Aedes atropalpus* reared on various foods (mm, mean±se, sample size parentheses)

Food and Larval Rear- ing Water Composition	Food Quan- tity	Length of Front Femur σ^7	Length of Front Femur σ^7
<i>Coryneform</i> sp & silica sand	1	1.59±- (1)	1.74±- (1)
<i>E. coli</i> & silica sand	1	-	-
<i>E. coli</i> + <i>Chlorella</i> sp	1	-	-

Figure 1. Larval rearing pan with approximately
2-3 cm water, and 200 larvae.

Figure 2. Adult cage showing crystallizing dish with
pupae, moist raisins, and rolled wet paper
towel for humidifier. Access sock can be
seen on the left side.

FIGURES



Figure 1

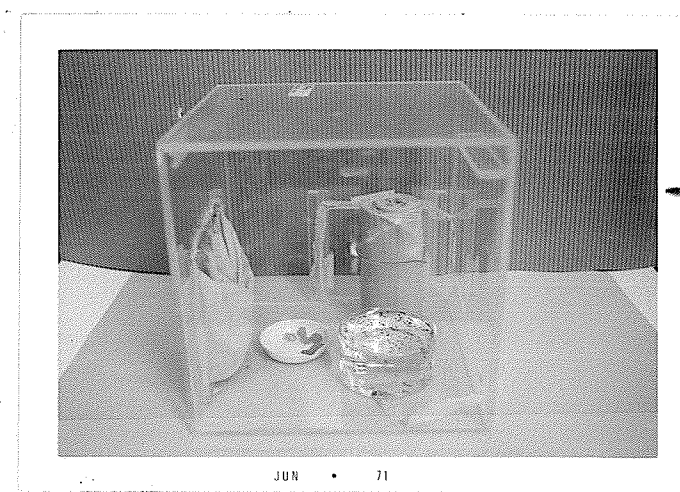


Figure 2

Figure 3. Egg bank storage containers: large container holds smaller petri dishes with eggs visible on moistened nylon-covered glass wool pads.

Figure 4. Mixed colonies of algae on plate culture grown from 10 mls of habitat water.

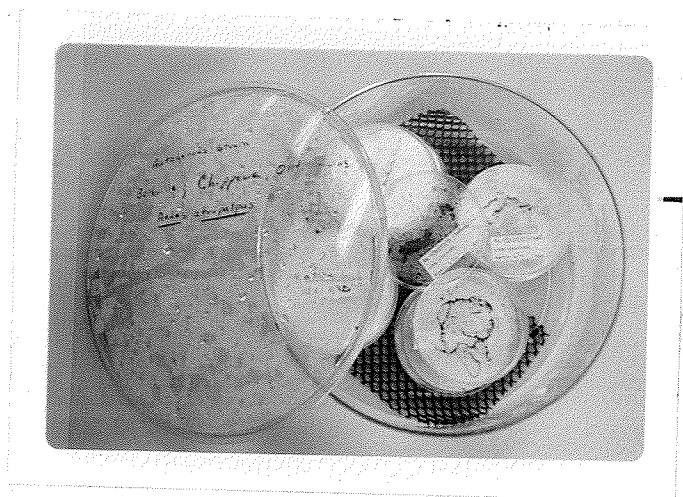


Figure 3



Figure 4

Figure 5. Unialgal growth of *Nitzschia* sp. Culture now ready for mechanical separation from bacteria by micropipetting.

Figure 6. Isolation chamber shown with microscope fitted into "V"-shaped recess. Operating platform and access holes for hands can be seen.



Figure 5

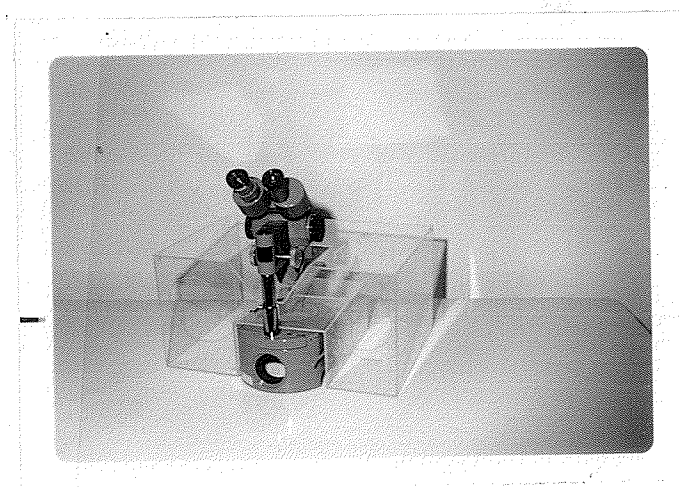


Figure 6

Figure 7. Isolating chamber in operational set-up for mechanically isolating algae from bacteria by micropipette. Note ice packs in corner and on top, covered by towel to help offset heat liberated from hands. Supply of sterile petri plates and slides can be seen in left front corner.

Figure 8. Micropipettes, pipette holder, hose (with in-line cotton filter), and squeeze bulb with check valve, as used in the mechanical separation process.



Figure 7

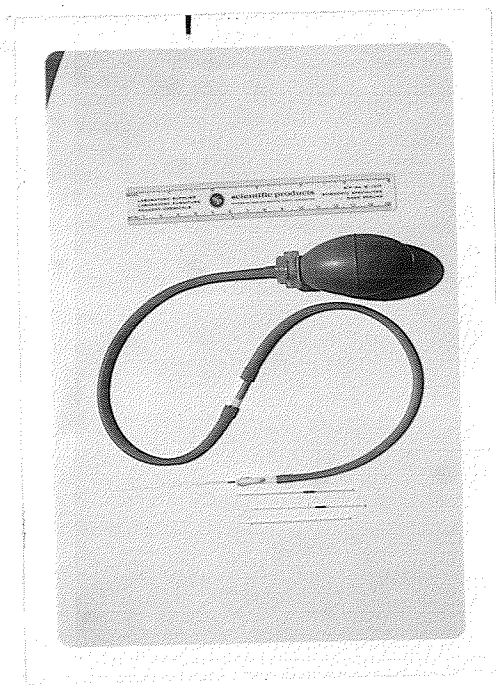


Figure 8

Figure 9. Pure algal colonies each arising from single cells isolated by manual micropipette technique using chamber and apparatus shown in Figures 7 and 8.

Figure 10. Screw-capped test tube slant cultures as used for storing reference cultures and inoculum cultures.

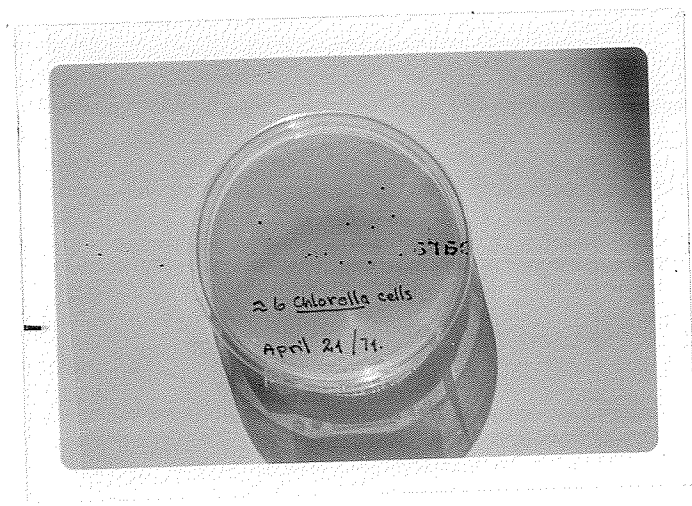


Figure 9

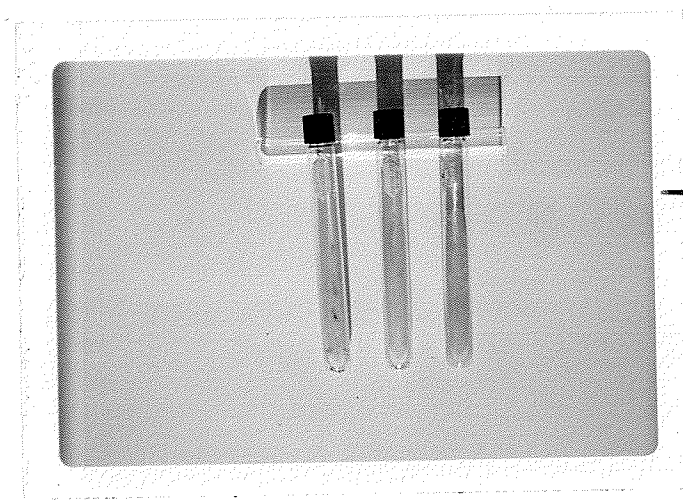


Figure 10

Figure 11. Pictorial representation of *Chlorella* sp cells
showing their characteristic round appearance.
(From "Standard Methods", Orland, 1965)

Figure 12. Pictorial representation of *Nitzschia* sp cells.
Perforations in the silica frustule can be seen.
(From "Standard Methods", Orland, 1965)

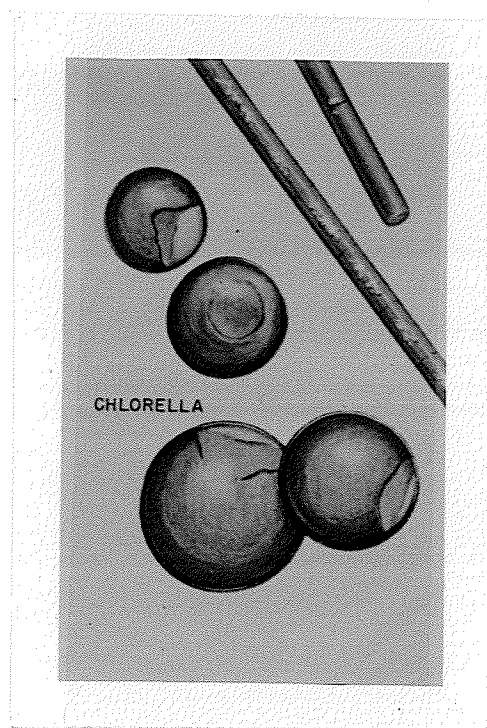


Figure 11

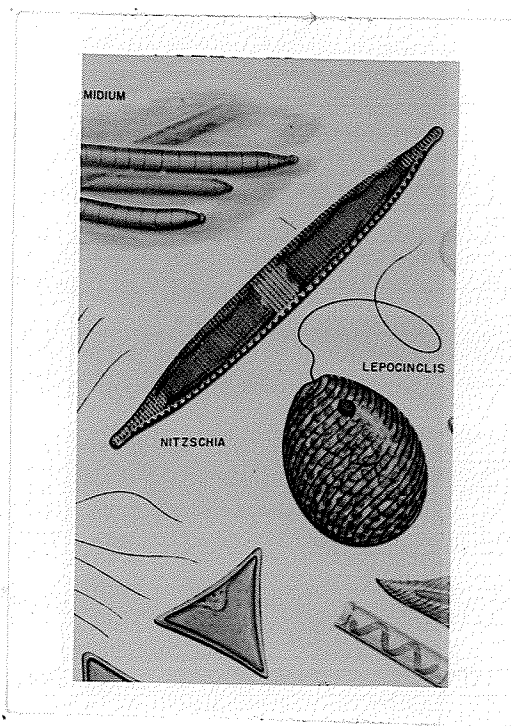


Figure 12

Figure 13. Photomicrograph of *Nitzschia* sp. 1:250.

Figure 14. Culture flask. Air inlet with bacteria filter on left upper, replenishing tube at left lower, vent exhaust on right and harvesting tube at centre of flask mouth.

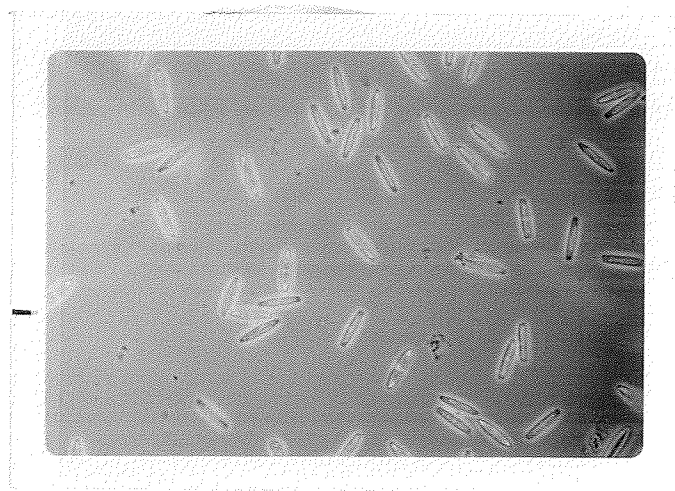


Figure 13

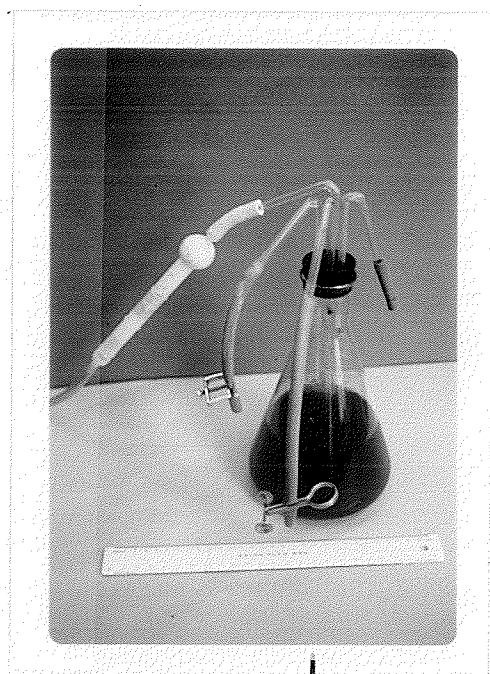


Figure 14

Figure 15. Interior of incubator cabinet showing lighting and culture arrangement.

Figure 16. Bacterial culture flask showing filtered air inlet provided with bacterial filter and exhaust.

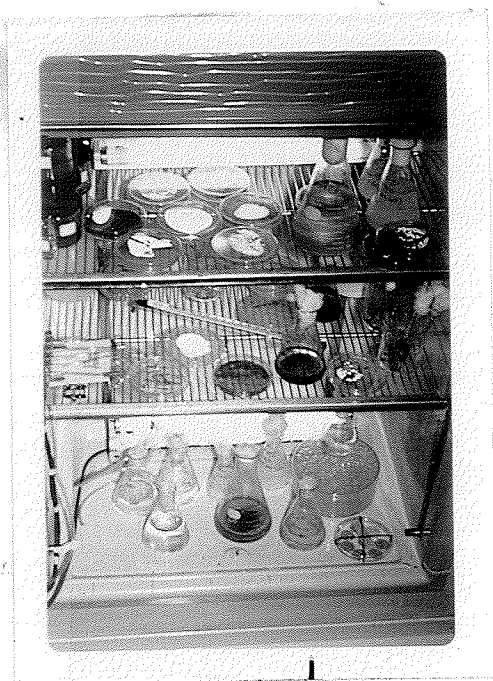


Figure 15

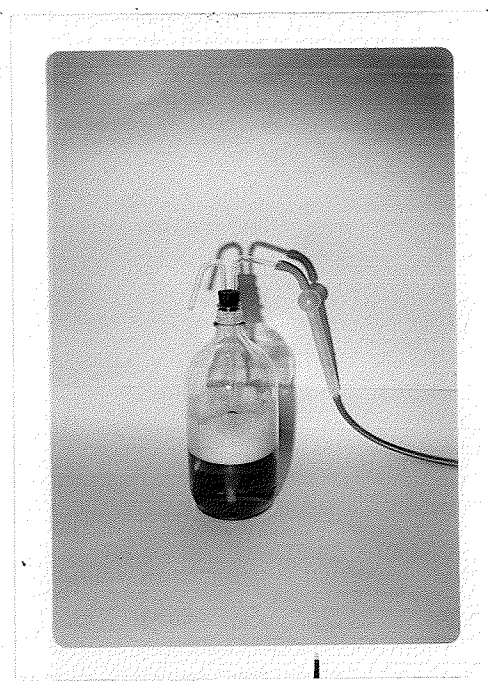


Figure 16

Figure 17. Experimental containers with lids. Each diet tested in triplicate, with 10 larvae in each crystallizing dish.

Figure 18. Measuring disk used to measure 4th instar larvae. Outer ring has wide sloping "V" for measuring widths and lengths of larvae, while inner "V" ring is much steeper forcing larvae in sideways, enabling measurements of the thickness of the thorax and abdomen.

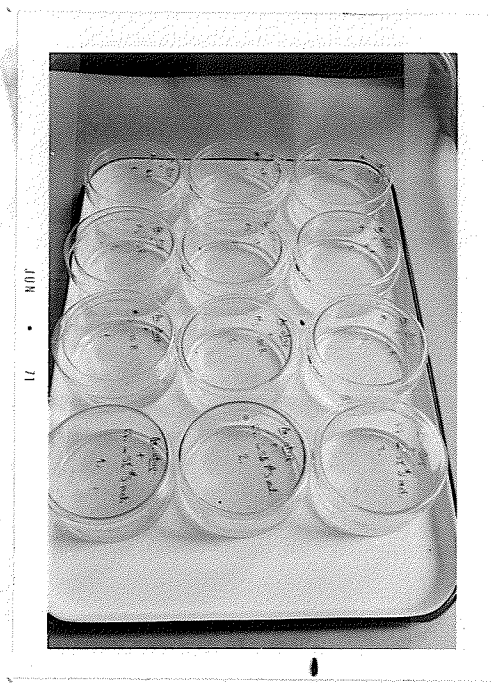


Figure 17

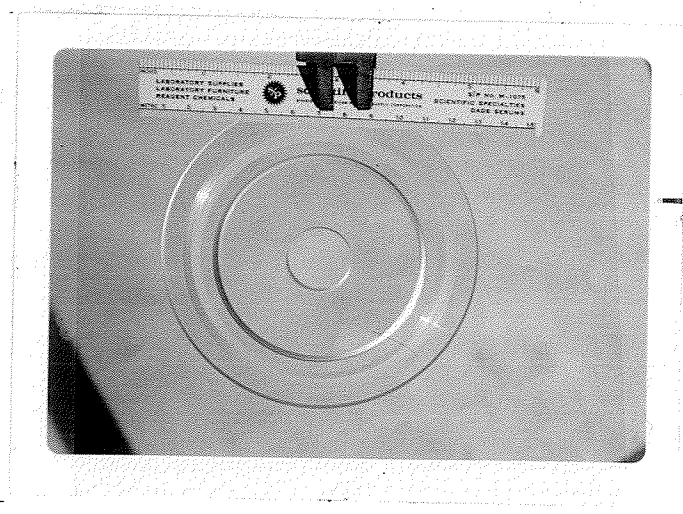


Figure 18

Figure 19. Sketches illustrating *A. atropalpus* fed poor diets (.5 standard ration *Pseudomonas* sp) left, and those fed good diets (standard ration of control food "Tetramin").

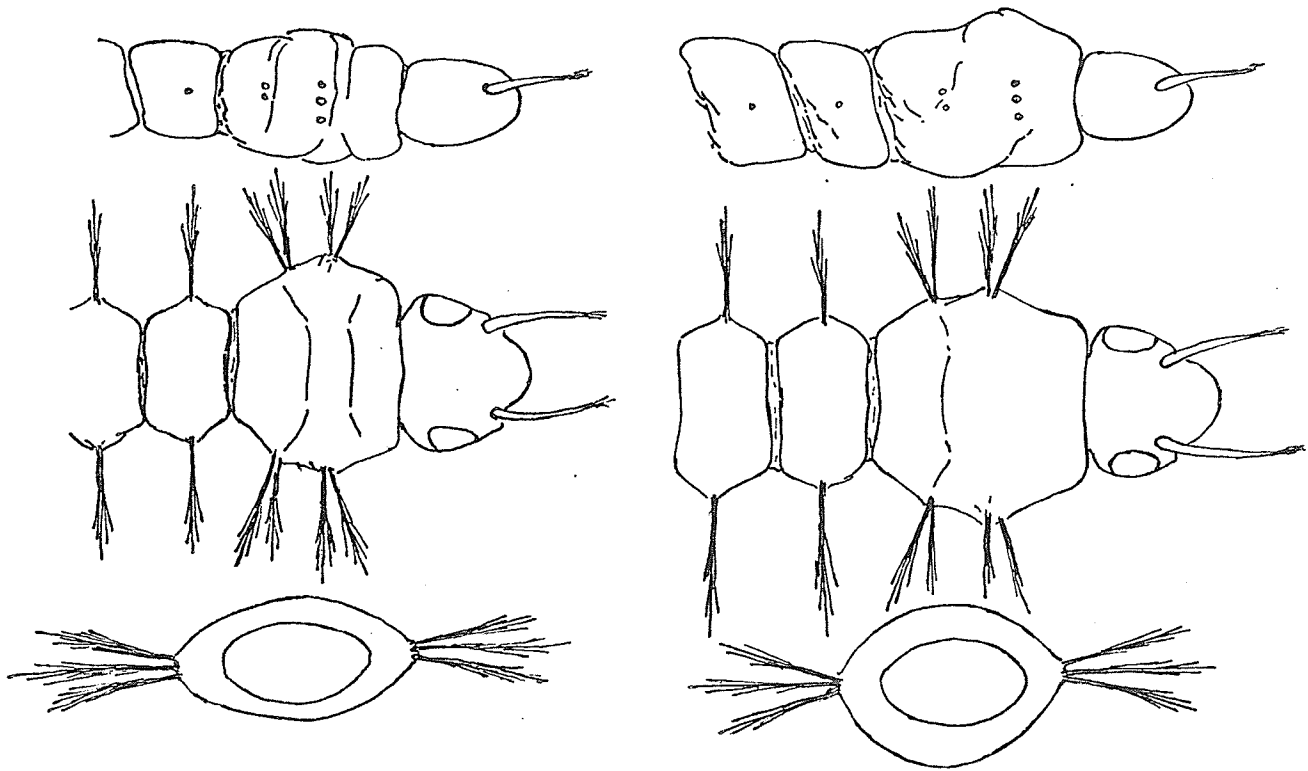


Figure 19

Figure 20. Various apparatus used: right, spatula and dispensing measure for powdered foods; chloroform killing jar; aspirator with short plastic mouth-piece and longer intake pipe, screened at inside end: wide and narrow mouthed eye droppers; and forceps.

Figure 21. Individual cardboard adult cages used for holding females for 40 hours after emergence. Note screen top with moistened cloth partially covering.

- * Split rubber flap on right covers access hole.

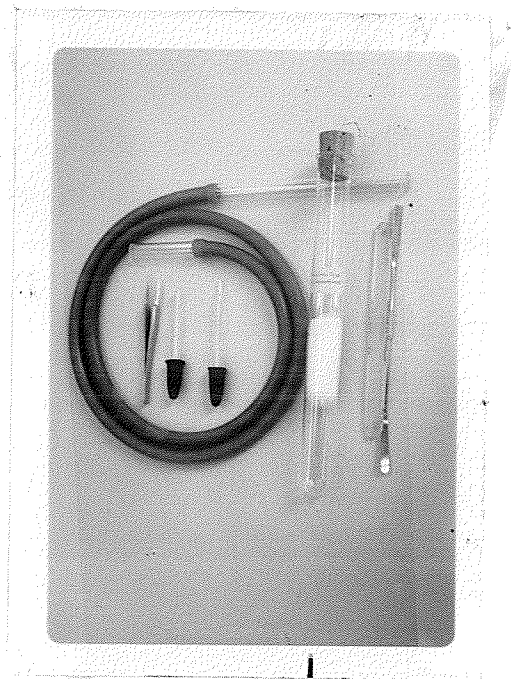


Figure 20



Figure 21

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