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Pedro Barbosa
University of Massachusetts Amherst

SOME EFFECTS OF OVERCROWDING ON THE RESPIRATION OF LARVAL AEDES AEGYPTI (L.)

A Dissertation Presented

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Pedro Barbosa

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Approved as to style and content by:

Donald W. Hall

Donald W. Hall, Dissertation Committee

Gary L. Jensen

Gary L. Jensen, Dissertation Committee

T. Michael Peters

T. Michael Peters, Chairman of
Dissertation Committee
Head of Department

TABLE OF CONTENTS

CHAPTER I

INTRODUCTION

The various facets of overcrowding are a major aspect of insect population regulation. The control and suppression of insects can be more effectively achieved through an increase in research data on the mechanisms involved in regulating population abundance. Experimentation into the nature and manifestations of overcrowding in mosquito populations is of significance due to the impact of Aedes aegypti and other species as vectors of deseases.

The early work on overcrowding in mosquitoes was primarily of a general nature. These studies illustrated the occurence of basic changes in larval and adult biology induced by the stress effects of overcrowding. These changes included density-dependent modifications such as size, survival, larval growth rate, and larval-pupal ecdysis. Aspects of adult changes have been investigated but these have been neither extensive nor conclusive.

Another approach to the study of overcrowding in mosquitoes has been exemplified by experimentation into the origins and direct causes of changes in overcrowded mosquitoes. This approach emphasizing cause and effect, has resulted in several studies depicting the qualitative nature of interactions in overcrowded mosquito populations. The study by Peters et al. (1969a) on the competitive interactions between Culex pipiens and Aedes aegypti was among the first to direct

greater emphasis on the possible role of biochemically active intrinsic factors occuring in the rearing media of mosquitoes. These density-induced compensatory responses of larval populations are now under closer scrutiny by several researchers.

A causal agent produced in overcrowded conditions has been reported in mosquitoes. It has been demonstrated in both A. aegypti (Moore and Fisher, 1969) and Culex quinquefasciatus (Ikeshoji and Mulla, 1970a, 1970b). It has been most frequently referred to as the growth retardant factor (GRF) (Moore and Fisher, 1969). The qualitative and quantitative identification of the growth retardant factors still has not been achieved. It has been illustrated that for Culex quinquefasciatus the agent produced is composed of many complex factors each causing a particular type of physiological effect. The prime modes of action of this complex substance can be of two general types. It can have a direct toxic effect or it can act as a bactericidal agent (Ikeshoji and Mulla, 1970a, 1970b). The bactericidal effect may cause the death of bacteria or the inhibition of their growth in the rearing media and thus deny larvae a direct food supply or a source of essential dietary requirements. In summary, there is strong evidence for the existence of biochemically active causal agents in C. quinquefasciatus and A. aegypti. In C. quinquefasciatus and perhaps in A. aegypti the impact of dietary deficiencies under overcrowded conditions may be mediated or influenced by these GRF. In addition,

there appear to be indications, at least in C. quinquefasciatus, suggesting that dietary effects are contingent on the degree of overcrowding imposed.

Past experimentation has indicated that the effects of stress due to overcrowding are manifested as changes in size, larval growth rate, mortality, and larval-pupal ecdysis. These density-related physiological changes suggest the occurance of some major and basic metabolic alteration in overcrowded individuals. In mosquitoes, as in most animals, metabolic activities are a function of the food or energy input they acquire. One can generalize and state that the available (food) energy input is utilized in three ways. A portion is utilized in growth or is stored as reserves (e.g. fat, glycogen, etc.). A second drain of food (input) energy is the energy loss in fecal materials or other excretory products. A third area of metabolic energy consumption is emphasized in this study. That is, energy utilized in maintenance metabolism; which assures the contiguity of essential body functions.

One of the metabolic sinks for food input energy has been indirectly investigated in past experiments. These studies have demonstrated that very little energy (food) is utilized for growth (the addition of new tissue) by overcrowded mosquitoes. The size of overcrowded individuals is reduced with increasing density. Rearing in overcrowded conditions significantly lowers pupal weights (Barbosa et al., in lit.). Similarly, adults resulting from overcrowded larvae

have also been reported to be smaller than adults reared under less stressed conditions (Krishnan et al., 1959).

One of the goals of this investigation is to ascertain whether the energy allotted to maintenance is affected by the stress imposed by overcrowding. It would seem logical to investigate the phenomenon of overcrowding in mosquitoes in comparison to two other types of populations, a starved and a standard. Such a comparative investigation of changes in larval populations has been undertaken in this study. The comparison of overcrowded, starved, and standard populations was accomplished by using differences in oxygen uptake as a criterion of metabolic shifts.

In the past, no experimentation has been conducted on the relative respiration rates of standard, overcrowded, and starved larval mosquito populations. Thus, experimentation covering all aspects of larval mosquito respirometry is presented.

CHAPTER II

LITERATURE REVIEW

Respiration is the process by which energy is provided for the continuation of life. It involves the biochemical processes of oxidative and nonoxidative enzymatic reactions. The importance of respiration lies in the fundamental necessity of these reactions for biological activity. The emphasis in this section will be primarily ecological in that the role of respiration in community interactions among organisms will be discussed.

Respiratory uptake can be considered to be related to that part of the energy input (assimilated food) which is directed toward the maintenance of a functional homeostasis in individuals. Functional homeostasis is defined as a state allowing for the integrity and survival of an organism (Engelmann, 1966). Respiration is a good criterion for measuring how a system operates and changes, whether this system is an individual, a population, or a community. Changes in oxygen uptake can indicate shifts in metabolism. These changes in oxygen uptake may serve as a sensitive index of environmental influences.

Estimates of the amount of energy loss through respiration vary from researcher to researcher and with each species investigated. Engelmann (1966) estimated that most animals utilized around 70 per cent of the assimilated energy in respiration. In specific examples the values calculated may

be even higher. Such an example is a study of the respiration of oribatid mites in a sample one meter square by 12.5 cm. deep. It was reported that of the assimilated energy (25 per cent), 95 per cent was dissipated in respiration (Engelmann, 1968). Similarly, the herbivores of a monophytic community were found to dissipate about 80 per cent of assimilated energy in respiration (Menhinick, 1967).

Factors Modifying Respiration

Surface Law

Certain relationships exist between respiration and factors such as weight and surface area and these relationships bear on external factors of the environment. The Surface Law, developed in the early nineteenth century, states that "the metabolic rate per unit weight decreases with increasing size, but is constant per unit surface." This concept was first developed for homeothermic animals and was based on heat production data. Most of the continuing work on this topic has emphasized homeothermic groups, primarily mammals (Kleiber, 1947). Exceptions to the surface law have been reported and thus the law is not universally accepted. The surface law seems to hold for poikilothermic vertebrates and some invertebrates, however, there are many exceptions (Bertalanffy, 1951). Edwards (1953b) has drawn several wide generalizations pertaining to the applicability of the surface law. He indicated that the law should apply when the weight range is large and when there is a similarity in the

structures of the organisms.

Bertalanffy (1951) suggested that the relationship between metabolic rate and body size be divided into three categories. The first type is one in which metabolic rate is proportional to surface area or its equivalent; weight to the two-third power. This surface proportionality relationship is reported to exist in some mammals, fish, and invertebrates; mainly crustaceans such as isopods and mussels. The relationship in this category is manifested by a decreasing oxygen consumption (per unit weight) with increasing body weight; but a constant uptake when expressed per unit surface area. Among the specific examples of this inverse O_2 consumption-weight relationship are those reported by Sayle (1928), Edwards (1958), Drummond and Chamberlain (1961), Weigert (1964), Knight and Gaufin (1966), Czerpak and Czeczuga (1969), and Querra and Cochran (1970). The weight-growth curve in this type of relationship is characteristically sigmoidal. In the second category the metabolic rate is directly proportional to the body weight. Metabolic rates per unit weight are constant with increasing body size. Examples of organisms in this group include insects. Some annelids and snails also belong in this group. Weight-growth curves in this group are generally exponential. The third relationship is intermediate between weight and surface proportionality. Thus, metabolic rates (expressed in terms of oxygen consumption) decrease with weight but tend to increase with surface area. Some pond snails and planarians

fall into this group. The weight growth curve is similar to that in type one, in that it is sigmoidal (Bertalanffy, 1951).

The choice of units used in expressing respiration has long been a controversial issue due in part to the unsure relevance of the surface law. The debate continues but many important associated concepts have developed from this controversy. The relationships between body size and metabolism have been expressed generally by the exponential equation $Y=aX^b$, where Y is metabolism (expressed for example, as oxygen consumption), and X is body size (expressed as weight or body nitrogen). This formula (for a parabola) is usually transformed to a straight-line log form (Zeuthen, 1953; Edwards, 1958; Richman, 1958; Engelmann, 1966). In the log formula, a and b are constants representing the Y-intercept and the slope of a plot of log oxygen consumption, respectively. The value of b is of significance since it can give an indication of weight or surface proportionality in the relationship between metabolism and body size. For example, in Bertalanffy's (1951) Type I, the weight (X) would be raised to a power of two-thirds or 0.66 (the value of b) indicating a surface proportionality. Similarly, in the second type b would have a value of 1.0 or very close to this value (e.g. 0.95). This would indicate a weight-proportional relationship. Finally, in the third intermediate type, b would have a value between 0.66 and 1.0.

Experimentally the values of b vary extensively from 0.67 to 1.0 (Edwards, 1953b). Edwards (1953b) indicated that

while most holometabolous insects would have weight-proportional relationships (b=1.0), hemimetabolous insects would have surface proportional relationships indicated by a value of b=0.75.

Temperature Changes

The interaction between respiratory rates and temperature changes has been confirmed experimentally in many insects. Essentially it is comprised of an increase in respiratory rates with increasing temperatures (Keister and Buck, 1964). Experimental evidence of the above relationship is discussed in reviews by Edwards (1946, 1953b), Keister and Buck, (1964). A typical example, among experimental reports is that of Edwards (1958). Investigating the oxygen consumption of Chironomus riparius Meigen, as a function of size and temperature, he reached the basic conclusion that the rate of oxygen consumption at a higher temperature (20°C) was 2.6 times greater than at a lower temperature (10°C).

Specific temperature-respiration curves not only differ among insect species, but are affected by a variety of other factors. Nevertheless, a generalized temperature-respiration curve can be described for insects. Such a theoretical curve exhibits a rising but low respiratory rate at low temperatures. A plateau is reached around the mid-range of temperatures, approximately 15° - 20°C. Finally, an inflection in the curve is noted when lethal temperatures are approached (Keister and Buck, 1964). These lethal temperatures are commonly known as thermal death points but vary with each particular species.

Although temperature-oxygen consumption curves may be similar in general shape they differ in relative position on the temperature axis due to the species' differential physiologically-manifested genetic adaptions (Edwards, 1946).

Changes in respiration rates due to changes in temperature may be indirectly influenced by ancillary factors. The effect of temperature on respiration can be mediated by preconditioning, such as the temperature of the rearing substratum previous to measurement of respiration (Sayle, 1928; Keister and Buck, 1964). The ability and degree to which an insect can acclimate may also affect the interpretation of respiration-temperature relationships. For example, temperature compensating insects may overshoot and then settle down to a new respiratory level. Thus, in the analysis and interpretation of respiratory rates, note must be made of regulating mechanisms. These may include activity, orientation to the sun, clustering, aggregation, shelter, and water utilization (Keister and Buck, 1964).

Starvation

The influence of starvation on insect respiratory rates is not completely understood, although, certain trends are evident. Information on this topic is scanty. Some of the available data are obtained in conjunction with the study of some other ecological factor such as circadian rhythm. Richards (1969), for example, reported that in cockroaches under conditions of complete inanition, two major phenomena

can be observed. First, the peak activity in oxygen consumption corresponds to the general circadian activity rhythm. Secondly, over a period of eighteen days the overall oxygen consumption dropped. Considering the close correlation between activity rhythms and oxygen uptake rhythms, lower activity was postulated as the cause of lower oxygen consumption under starvation. Richards also stated that a sudden burst of respiratory activity occuring during the final hour preceding the death of individual cockroaches was probably due to the breakdown of body tissues.

Sayle (1928) investigated the effects of starvation on the carbon dioxide output of dragonfly nymphs. She reported the existence of fluctuating patterns in CO_2 output. Initially (during the first week) the CO_2 output decreased but increased during the second week. In the third and fourth weeks the CO_2 output dropped again. The increase during the second week was attributed to the utilization of reserves or autolysis of body tissues. A similar fluctuating pattern was found in a different animal group, the planaria (Hyman, 1919). She reported that the oxygen consumption dropped continually; more severely as time proceeded. The level of oxygen consumption then reached a minimum level. After about three weeks of starvation, the rate of oxygen consumption began to rise and continued to do so for as long as five to eight weeks. From these data she concluded that under starvation conditions the rate of oxygen consumption in planaria rises. In addition, she concluded that this increased consumption made them

metabolically similar to young forms when compared to old fed individuals. Hyman also found that feeding consistently had a stimulatory effect on respiratory activity. She did not attribute this increased activity to assimilation or any other allied digestive process. A similarly induced rise in rate of metabolism was reported in starved grasshoppers upon the ingestion of food (Bodine, 1921). In a study on the respiratory energy loss of a spider, Lycosa pseudoannulata, Ito (1964), found a similar trend in the effects of starvation. Taking CO_2 output measurements before feeding and after several days of starvation, the per cent decrease in respiration due to starvation averaged 76.68 per cent $\pm$ 18.5 per cent.

A general trend towards a decrease in respiratory activity due to the effects of starvation is evident in several studies. In a comparative study of wild and vestigial Drosophila melanogaster, Orr (1937), reported a rapid starvation-induced decline in respiration for both females and males. In a study of the relationship between starvation and various humidities on the Japanese beetle similar results were also found (Bellucci, 1939). After falling throughout the initial two days of starvation, the metabolic rate of the beetle larvae was maintained, fluctuating around a low level until death insued. This shift was suggested as the compensating adjustment allowing for larval survival for about a month at high humidity (Bellucci, 1939). Ludwig (1937) also found that in the grasshopper Chortophaga viridifasciata an increasingly drastic drop in oxygen consumption (54.1 per cent,

61.5 per cent and 67.8 per cent of initial values) with increasing humidity (5 per cent, 82 per cent and 96 per cent respectively). Unfortunately, high variability with each test sequence caused these results to be statistically insignificant.

The increase in respiratory rate brought about by feeding is also exhibited in blood feeding adults (Mercado et al., 1956; Rajagopal and Bursell, 1966). Mercado et al. studied Aedes aegypti, A. atropalpus, Anopheles albimanus, A. quadrimaculatus As freeborni and Culex pipiens while Rajagopal and Bursell studied the tsetse fly. Rajagopal and Bursell suggested that the increased metabolic activity was associated with digestion, deamination and detoxication. This conclusion was at odds with the conclusions reported by Hyman (1919).

Genetics

Investigations have been made on the differences in respiratory activity between closely related animals. Petersen (1960) investigated the differences in rates of oxygen consumption among the sibling species Drosophila willistoni, D. paulistorum, and D. equinoxialis. No significant differences were found in oxygen consumption among these species. In a non-insect group, the cladocera, the genetic condition of the population was found to affect respiration. One normal clone and two mutant clones derived parthogenetically from "a single common mother" were used in experimentation (Obreshkove and Banta, 1930). They reported that the normal clone showed a much greater rate of oxygen consumption than the reproductively

weaker mutant clones. Orr (1937) studying Droso phila melanogaster compared the respiratory rates of wild, vestigial and hybrid flies. Under nearly identical conditions the rate of oxygen consumption was higher for vestigial flies than for the wild type and both were higher than the hybrid respiratory activity. Although, the vestigials were consistently larger, the respiratory differences were not merely size-dependent differences since higher rates existed when expressed either per fly or per unit of body weight (Orr, 1937).

Life Cycle and Age

The life stage of an insect can also have an influence upon the magnitude and pattern of respiratory activity. Even the overall type of metamorphosis occuring in different insects is sometimes correlated with characteristic respiratory patterns (cf., Surface Law section). Various aspects of the insect's life cycle may also change the magnitude of respiratory activity. For example, diapause is reported to cause lower respiratory values in many insect groups (Keister and Buck, 1964).

Golley and Gentry (1964) stated that differences occur in respiratory rates with changes in life stages. They reported that the oxygen consumption per unit weight of soldier ants was much lower than that of worker ants. The degree of difference was indicated in the 40.8 ul O_2 mg.$^{-1}$ hr.$^{-1}$ for the workers as compared to 3.8 ul O_2 mg.$^{-1}$ hr.$^{-1}$ for the

soldiers. Differences such as these are indirectly correlated to other influencing factors. For example, the soldiers are much larger individuals and would be expected to respire less oxygen. Melampy and Willis (1939) offer still another example when they reported that the queens of colonies of Italian bees had higher respiratory rates than the other colony forms. This higher metabolic rate was concluded to be associated with the fast growth rate of the reproductive caste. Allen's (1959) analysis of the patterns of respiratory rates in honeybees as a function of age and temperature, went a step further than the above study. He concluded that older bees were better able to regulate their temperature than younger worker bees. In other situations temperature, activity, numbers or other factors may influence age-induced shifts in respiratory rates.

Relative metabolic rates differ depending upon the insect, its environment, and its adaptations to its surroundings. Examples of these effects can be found in studies discussed by Roeder (1953) and Rockstein (1964). A specific example of these effects is illustrated in the investigation by Petitpren and Knight (1970) on the oxygen consumption of the dragonfly. They reported that adult respiration was approximately three times the respiratory rate of larvae when analysed at comparable weights and temperatures. Not only were there differences between young and adults, but, as in other insects, there were differences in oxygen consumption within the immature period. As previously mentioned, there can be an additional factor which indirectly may cause differences

in respiration. Pititpren and Knight (1970) suggested that in this situation the greater activity and amount of active tissues (i.e. gonads and flight muscles) of the adult may cause the three-fold difference in respiration.

Similarly, size related differences in oxygen consumption between young and old nymphs were reported in stoneflies (Knight and Gaufin, 1966). Generally, the oxygen consumption per unit weight was lower for older animals than for younger individuals. The per cent decrease in oxygen consumption per unit weight from one age grouping to the next ranged from 22 per cent to 47 per cent, depending on the species.

Habitat and Trophic Levels

Habitat can play a major part in influencing shifts of metabolic activity, particularly, if the features of that habitat require adaptations affecting metabolic rates (Fox and Simmonds, 1932). Such a habitat-dependent shift occurs in two species of stoneflies. Significant respiratory differences between Acroneuri pacifica Banks and Pteronarcys californica Newport were reported by Knight and Gaufin (1966). They concluded that these metabolic differences might be correlated to the insect's habitat in the wild. A. pacifica was very active, usually inhabiting small spring-fed streams, while P. californica was usually found in large slower-moving waters and the stonefly itself was more sedentary. Laboratory experimentation indicated a higher rate of oxygen consumption for larval A. pacifica than for P. californica within comparable conditions. Thus, the rates seem to correlate well with

activity and habitat differences.

The metabolic or respiratory rates of organisms may also be viewed as a function of the relative trophic level in which they are found (trophic level in the sense of Lindeman, 1942). Lindeman (1942) suggested a generalization assuming a greater activity of organisms at higher trophic levels. The generalization established a relationship between respiratory losses and trophic levels. This trophic principle states that "the percentage loss of energy due to respiration is progressively greater for higher levels in the food cycle." Thus, the per cent respiration relative to growth would be expected to increase from primary consumer to secondary consumer, etc. This relationship has been repeatedly illustrated in field studies. For example, Golley (1960) reported the respiratory losses of major organisms of an old-field community as follows: Vegetation, 15 per cent; Mice, 68.2 per cent and Least Weasels, 93.3 per cent.

Cuticular Respiration

The possible utilization of cuticular respiration under stress necessitates a discussion of the role of this type of respiration. All insect eggs depend upon cuticular gaseous exchange as a means of respiration. In addition some primitive forms such as the family Poduridae (Order Collembola) also use cutaneous diffusion as a means of respiration (Ebeling, 1964; Edwards, 1953a). Many aquatic nymphs and terrestial immatures utilize cutaneous respiration. It occurs in all

insects during the molting process (Ebeling, 1964). Edwards (1953a) stated that cutaneous respiration may play a major role in the survival of common aquatic nymphs such as dragonflies and mayflies. In other insects such as Aphelocheirus sp adults, respiration occurs through plastron breathing, but the immature fifth instar respires completely through the integument. Similarly, Edwards stated that all larval and pupal marine chironomids as well as some adults respire cutaneously.

In terrestial forms partial cutaneous respiration may occur. For example, inligaturing experiments on Phormia regina larvae Buck and Keister (1956) reported that the amount of oxygen entering through the cuticle was less than 2.5 per cent. If all the spiracles are experimentally plugged the percentage oxygen diffusing through the cuticle increased to 10 per cent (Buck and Keister, 1956). In experimentation using Calliphora erythrocehpala, Fraenkel and Herford (1938) calculated the normal cuticular respiration to be about one tenth (10 per cent) of the total respiration. Fraenkel and Herford (1938) also indicated that for submerged larval Culex sp. the shift to cuticular respiration also occured. For these forcibly submerged larvae the amount of oxygen entering cuticularly was about one half that amount normally consumed.

Mosquito Respiration

The quantitative and qualitative aspects of mosquito

respiration are poorly known. Richards (1964) investigated trends in developmental rates and oxygen consumption as affected by temperature. Eggs of six species of insects including Culex pipiens were used ir the study. Data on larval respiration is also not abundant. Most of the early work on larval respiration revolved around the effectiveness of the technique of "oiling" as a larvicidal procedure. The evaluation of these techniques necessitated data on cuticular and tracheal respiration.

The majority of mosquitoes take in atmospheric gas directly while at the water's surface. The gaseous exchange is generally accomplished tracheally through a pair of spiracles located on the eighth abdominal segment (Krogh, 1941; Goma, 1966). Nevertheless, there are a few exceptions to this general respiratory pattern. Aedes argenteopunctatus uses modified papillae to respire cutaneously. Two pair of these large, densely tracheated and thin-walled papillae occur in the head area and a similar anal papilla also serves as a respiratory surface. Water currents produced during feeding are passed over the papillae thus improving respiratory exchange. Other species of mosquitoes utilizing similar structures include Culex poicilipes, Culex sinaiticus, Culex morsitans and Culiseta morsitans (Clements, 1963). Among other areas modified for cutaneous respiration and usually extensively tracheated are the ventral margin, the antennae, and evaginations of the head cuticle. Once it was thought that the anal gills were actively involved in the respiration of all larval mosquitoes, however this is not now considered

correct (Wang, 1938).

As previously mentioned, the emphasis in the early research was on evaluation of larval respiration while they were forcibly submerged. Among the research highlighting this approach to the study of larval mosquito respiration was that of Sen (1914), Macfie (1917), Hacker, (1925), Ginsburg, (1929), and Richards, (1941). Richards (1941) investigating the toxic and suffocating effects of petroleum oils on Culex pipiens, made some basic conclusions on cuticular respiration. He stated that cuticular respiration and similarly survival upon submergence, depended on oxygen and carbon dioxide concentration in the water, temperature, activity, species-specific cuticle permeability and size of the individual.

Recent research on larval mosquito respiration still revolves around the relationship between the mode of action of oils and surfactants and respiration. Hagstrum (1970) concluded that Fraenkel and Herford's (1938) estimation of submerged mosquito cuticular respiration of 50 per cent was too high and actually nearer 5 to 20 per cent of aerial (tracheal) respiration. In addition, Hagstrum (1970) reported that the respiratory values among three species of mosquitoes under either control or treated (oiled) conditions showed little variation. These three species of mosquitoes were Aedes aegypti, Anopheles albimanus and Culex pipiens quinquefasciatus. The larvae (fourth instar) of A. aegypti and Culex pipiens (third and fourth instars) showed no major increase in

cuticular respiration after oiling, but the fourth instar A. albimanus and C. pipiens pupae, showed a three to four-fold increase in cuticular respiration. The seeming importance of this fluctuation is tempered by a fluctuation of similar magnitude between two replications of normal (untreated) cuticular respiration of Aedes fourth instars and Culex third instars. Finally, Hagstrum (1970), stated that in all cases tracheal respiration drops by a magnitude of two to four times after oil application. Piper and Maxwell (1971) reported that greater concentrations of surfactants (agents with the ability to reduce surface tension) were required to produce LC_{50} and LC_{90} values in larvae than in pupae of Culex pipiens quinquefasciatus. This greater tolerance exhibited by larvae was attributed to the enhanced capacity of larvae to utilize cuticular respiration. Hedeen (1953) studied the process of cuticular respiration in greater detail. He investigated the differential responses of different instars to forced submergence. The fourth instars of Aedes atropalpus had a greater capacity for cuticular respiration than younger instars.

The above information representing data from various sources, represents a major part of the work reported concerning the respiration of mosquitoes. Various other aspects of the respiratory phenomenon have also been investigated. For example, the capacity for thermal homeostasis was investigated in Culex pipiens pipiens by Buffington (1969). He concluded that thermal adjustment was accomplished by any

of three mechanisms; acclimation, temperature compensation, or adaptive responses to selective pressures. Oxygen consumption was used as a measure of temperature acclimation and was graphically manifested in weight-oxygen consumption curves.

Circadian patterns in the respiration of larval Culex pipiens were studied by Buffington (1968). He found a close correlation between metabolism and fluctuations in environmental temperatures. In addition, it was concluded that light was the entraining factor in this rhythm. The respiratory trends in second, third, and fourth instars of C. pipiens were reported to be essentially the same. Direct evidence of periodicity in larval respiration of Anopheles maculipennis was earlier demonstrated by Olifan (1947). He reported that drops in respiratory activity coincided with molting periods. The highest periods of oxygen consumption were noted during the middle portion of each stadium.

Kolesov (1956) measured the effect of 5.5 per cent DDT dust on the respiration of larval Aedes caspius dorsalis. He reported that larval contact with DDT dust for five minutes reduced the time spent at the water's surface from 43 minutes 29 seconds to 14 minutes 12 seconds. A 20 minute exposure reduced surface time to 6 minutes and 17 seconds. The closure of the spiracles was suggested to be a defensive reaction against DDT poisoning.

Some research has also been conducted on the respiratory biology of adult mosquitoes. No significant differences were

reported in respiratory values between males and females of Aedes aegypti, A. atropalpus, Anopheles albimanus, Anopheles quadrimaculatus, Anopheles freeborni, and Culex pipiens (Mercado et al., 1956). Feeding was found to stimulate respiration in both sexes. The initial oxygen consumption rate at feeding was high but fell after several days, finally reaching a level lower than the preceeding level. Adult A. aegypti fed on sucrose water had a much higher oxygen consumption rate than those fed on blood (Mercado et al., 1956). Bat-Miriam and Galun (1962) also studied oxygen consumption of adult mosquitoes, but as a function of sex and age. Older A. aegypti adults were reported to have a higher oxygen consumption than young adults at all temperatures tested. A definite trend began with a high oxygen uptake during the first two days of the adult life of males and females. On the third and fourth days consumption rate dropped in both sexes. After this decrease in oxygen consumption rate stayed constant and was independent of age. On the basis of oxygen uptake (per weight) males showed a higher rate than females at experimental temperatures (Bat-Miriam and Galun, 1962).

Galun (1960) also studied the respiration of decapitated Aedes aegypti adults. She reported a slight increase in oxygen consumption rate after decapitation, however the values were close to those of the intact adults. Although "haltereless" individuals were reported to be more active, the uptake was much lower than in either intact or decapitated individuals. Galun (1960) suggested that regulation of oxygen

consumption was accomplished by the action of the spiracles. The suggested modes of action for increasing spiracular activity were; to remain open longer, enlarge the opening, or increase the frequency of opening activity. Krafsur et al. (1970) and Krafsur and Graham (1970), studied these possible spiracular mechanisms in more detail. Krafsur et al. (1970) found that spiracular activity was less vigorous in decapitated individuals. They suggested that this discrepancy between their work and Galun's (1960) might be due to the hypoxic (CO_2) conditions of their own experiments.

Animal Assemblages and Oxygen Consumption

Most studies on the relationships between numbers of animals and oxygen consumption have revolved around the investigation of the influences of "group effect" (Allee, 1931). Group effect can be defined as "effects produced by proximity upon the individuals in populations of low density" generally compared with an isolated individual (Chauvin, 1967).

No single major trend is evident in the relationship between oxygen consumption and grouping of individuals. The experimental indications of group effect vary depending upon the species studied and the differing features of its ecology. Thus Allee (1934) concluded that grouping causes one of two opposite types of effects upon the rate of respiration of animals. In the more widespread category the grouping of individuals causes a depression in the rate of oxygen uptake. In the second category grouping acts to induce higher oxygen

consumption rates. In studies using the aquatic isopod Asellus communis, four species of land isopods, Daphnia pulex (a cladoceran) and Ophioderma brevispina (an ophiurid), the grouping of individuals caused a decrease in the rate of respiration. These differences were not caused by lowered oxygen tension around the grouped individuals as compared to that of isolated individuals (Allee, 1934). Similarly, groups of four Trichodactylus petropolitanus crabs were reported to exhibit a significantly lower rate of oxygen consumption than isolated individuals (Valente, 1943). In addition, the absence of light did not diminish the group effect on respiratory rates. The decrease in respiration was also exhibited by isolated individuals kept in a mirrored aquarium, thus indicating the visual mediation of the group effect.

Fish exhibit effects which are very similar. An early conflict over the relationship between group effect and respiration (Schuett, 1933, 1934) was resolved in later work by Shlaifer (1938, 1939). Shlaifer (1939) reported that a decreased rate of oxygen consumption occured in grouped Carassius auratus (goldfish) as compared to isolated individuals. This group effect was lost in complete darkness or when individuals were blinded. Isolated individuals could also have their oxygen uptake depressed by confinement in mirrored aquariums (Shlaifer, 1939).

The second type of group effect yields opposite results to those indicated in the above studies. Grouped bullheads (Ameiurus melas) were reported to consume a greater amount of

oxygen per individual than isolated individuals (Allee, 1934). Similarly the rise in respiratory rate in confined Daphnia was represented by a two and one half increase in oxygen consumption of confined as compared to unconfined individuals (Zeiss, 1963).

Some animals are intermediate between these extremes in their response to grouping. This response may be a function of ecological factors. Grouping of the brittle starfish (Ophioderma sp.) during the breeding season induced higher respiratory rates. Conversely, at the end of the breeding season, grouping depressed respiratory values (Allee, 1934).

In insects affected by grouping, the changes in respiratory rates due to the number of individuals may be generally represented by differences in gregarious, versus solitary phases. Butler and Innes (1936) working with Locusta migratoria, found that both sexes of all instars in migratory phases showed a higher rate of respiration than individuals of the solitary phase. Gardiner (1958) investigated the relationship between respiration of nymphs as affected by the previous density conditioning of the parent population. Young nymphs (three-day old) reared from gregarious parent populations were reported to exhibit increased oxygen consumption rates as compared to those reared from solitary forms. The effect of nymphal rearing density (in the same study) depended upon their phase parentage. Nymphs of gregarious parents were unaffected by rearing density until about seven days. Conversely, hoppers of solitary parentage

manifest a definite respiratory effect resulting from various rearing densities. Although Blackith and Howden (1961) were unable to report any significant phase-related respiratory differences, due to interstock variation, they did indicate an effect caused by grouping. The grouping of three Locusta hatchlings resulted in a consistent decrease in respiration of 30 per cent.

The basic relationship of higher respiratory rates in gregarious forms has been periodically reaffirmed such as in Pener (1964). Similarly, the allied relationships between oxygen consumption and age or weight have also been investigated (Clarke, 1957). Just as the phase theory holds for other insects so the relationship between respiration and phase status also holds true. Shibazaki and Ito (1969) indicated that in Leucania seperata, the armyworm, the CO_2 output of the crowded (gregarious) black larvae was higher than that of the isolated (solitary) green larvae.

There is no single generalized relationship between the numbers of individuals and respiratory activity. Therefore the total ecological picture must be considered in the evaluation of the data on these interrelationships. Not only must one be aware of the influencing role of physical factors such as temperature, humidity and light but other parameters such as activity, sex, age, habitat, etc. The importance of these considerations are illustrated by the differing results of Shlaifer (1939) and Valente (1943). Shlaifer used Carassius auratus, the goldfish and Valente used Trichodactylus

petropolitanus, a freshwater crab. While both studies reported that confinement of isolated individuals in mirrored aquariums induced results similar to those in grouped individuals (decreased respiration) conditions of darkness did not result in similar effects. In the goldfish, darkness eliminated the differences between isolated and grouped individuals. In the sandcrab the respiratory differences were not lost. It was suggested that the crab's dark river habitat, under stones and aquatic plants, made it less susceptible to influences of darkness (Valente, 1943). Similarly, the shift in respiratory activity may be an adaptive metabolic feature of a species. Thus, Weigert and Coleman (1970) suggested that Nasutitermes costalis shifted to a low fasting metabolic rate as an adaptation to a crowded environment. These are only two possible justifications, of which there are many, depending upon the many species-specific limiting factors of the environment.

Overcrowding In Mosquitoes

Larvae

Most apparent among all the changes occuring in overcrowded mosquitoes are the increases in larval developmental time with increasing density. Culex pipiens pallens was the subject of a study on the effect of population density on larval development conducted by Ishii (1963). In a series of experiments he determined that increased densities resulted in extended development. Densities ranged from one to eighty

larvae per 20cc of medium. Among the different types of experiments he conducted were those in which the medium was not changed. In others, the rearing medium was changed daily. In a third experiment the larvae were grown together for six days and then subdivided into various density levels. In all cases development and emergence were delayed in overcrowded conditions. Ingram (1954) working with Aedes polynesiensis found that larval development took two days longer in overcrowded conditions as compared to individuals under low density conditions. The overcrowded conditions consisted of 375 larvae in a pint-sized container while non-overcrowded conditions consisted of 52 larvae in the same size container. "Excess food" was provided in both situations. Prolongation of developmental time with increasing numbers of larvae was also reported by Surtees (1959). In addition, increase mortality with increasing density was demonstrated.

Higher mortality is another major change evident in overcrowded populations of mosquitoes. Shannon and Putnam (1934) studied Aedes aegypti at densities of 100, 400, 1000, and 4000 eggs per lot. They found that higher densities were correlated with increased developmental period and higher mortality. Although the onset of pupation was identical in the first two density levels, the per cent mortality of eggs and larvae was three times greater at the 400 than at the 100 density level. Shannon and Putnam did not believe that toxic materials were the cause of developmental retardation. They placed 25 larvae in water previously conditioned with over-

crowded individuals and reported normal development. They suggested mechanical agitation as a possible cause of decreased feeding and thus changes in development. Kurihara (1963) also worked on the effects of density on larval growth, mortality, and size using Culex pipiens s.l. He found that using identical vials with 50ml of water and densities ranging from 25 to 150 larvae the mortality increased with rising density.

Not all studies indicate agreement as to the effects of overcrowding. Krishnan et al. (1959) for example, studied the effects of overcrowding on Culex fatigans. "Overpopulated" larvae had 0.2 square inches of surface area available per larva, while "underpopulated" larvae had 2.3 square inches available. Larvae were placed in identical volumes of water and fed equal amounts of yeast. They concluded that there was no significant difference in developmental time between underpopulated and overpopulated larvae.

Barbosa et al. (in lit.) described several changes that occur in overcrowded larval A. aegypti. They reported the delayed onset of pupation with increasing density. As the combined density-starvation stress increased (40 to 1280 larvae per 80ml of rearing medium) the survival of both sexes dropped significantly. Among the other changes reported were shifts towards a one-to-one sex ratio and smaller pupal weights with increasing density. Bar-Zeev (1957) studied the effects of density on larval Aedes aegypti using densities ranging from 30 to 1000 larvae per 100ml of water. She concluded that lack

of food was the cause of retardation of development. After conducting experiments similar to those of Shannon and Putnam she concluded that retardation was not due to metabolites.

Jones (1960) reaffirmed Bar-Zeev's contention that the effects exhibited in overcrowded conditions were proportional to the amount of food available. Wada (1965a) working on A. aegypti differed by concluding that increasing density increase the duration of the larval period when food was constant. Larval mortality also increased with rising density. Wada reported that "undercrowding" also seemed to be suboptimal. Lack of food was indicated to be another cause of retardation of development.

A different approach to the study of mosquito overcrowding consists of the investigation of the chemical mediation of stress effects of overcrowding. The chemical mode of action was suggested by Peters et al. (1969a). In this experiment the competitive interactions between Culex pipiens and A. aegypti were studied. Various populations of 100 larvae with differing ratios of Culex pipiens and A. aegypti were used (i.e. 100:1, 75:25, 50:50, etc.). It was reported that A. aegypti inhibited the pupation of C. pipiens. There existed a reciprocal effect by C. pipiens but only a very slight one. In discussing the results achieved it was concluded that the active agents might be chemical factors (either secretory or excretory), in the water. Moore and Fisher (1969) duplicated the above work using A. aegypti and A. albopictus. Various ratios of the two species were

evaluated as well as two density levels. The experimental results illustrated that A. aegypti had inhibitory effects on A. albopictus. Moore and Fisher extracted what they called GRF or growth retardant factors.

The most recent study of the chemical factors has been performed by Ikeshoji and Mulla (1970a, 1970b). They were able to illustrate its affect by extracting the GRF and bioassaying it, primarily on Culex quinquefasciatus. The death of 50 per cent of the population occured quicker with increasing concentrations of the GRF. The variable lethality of the subfactors comprising the overcrowding factors were demonstrated by extraction and separation of components by silica gel chromatography. These factors were also found to be bactericidal. Considering the need for bacteria by larvae as a nutrient source, Ikeshoji and Mulla suggested that the GRF caused starvation by their effect on bacteria.

Pupae

In addition to reported effects on larvae, Krishnan et al. (1959) also reported differential pupation due to overcrowding. A reported 82 per cent of the larvae pupated in the "underpopulated" containers as compared to 65.6 per cent in "overpopulated" containers. Terzian and Stahler (1955) did research on population density effects on Anopheles quadrimaculatus Say. Higher densities (150/pan) had 2.3 square inches per larvae. All larvae were fed maximally by adding food when the water surface was clean of food. Terzian and Stahler concluded that overcrowded conditions affected both

the onset and the duration of pupation. "Underpopulated" larval populations began pupation on the eighth day and completed pupation on the tenth. Overpopulated larvae began pupating on the tenth day and did not terminate pupation until the twentieth or twenty-first day. Pupae were found to be significantly larger and weighed more when reared in undercrowded pans. Pupal mortality was found to increase with increasing density.

Studying the effects of photoperiod, salinity, and food on Culex nigripalpus Nayar (1968) also investigated the effects of density. He found that different quantities of food and high larval density did not affect the initiation of pupation. It was, however, affected by the amount of food available to each larvae and by temperature. Nayar stated that the primary reason for the extention or shortening of the pupal period was the quantity of food. This can be illustrated by data indicating that on a basic ration the duration of the pupal ecdysial period was 90 hours at a density of 75 larvae and 113 hours at a density of 200 larvae. At two times the basic ration and a density of 75 larvae the period lasted 47 hours as compared to 75 hours at the 200 larvae density.

Wada (1965a) concluded that no distinct difference existed in pupal period with changing density. He stated that pupal period was a function of temperature, as compared to larval period which was a function of temperature, density, and quantity and quality of food.

The relationship between pupal development and increasing density has been investigated by Nayar and Sauerman (1968, 1970a, 1970c). Nayar and Sauerman (1968) studied the characteristics and effects of larval aggregation formation in Aedes taeniorhynchus. They concluded that these aggregations aided in the synchronization of larval-pupal ecdysis and were induced by large numbers of individuals. In more extensive studies of up to 15 species of mosquitoes Nayar and Sauerman (1970a,c) made similar conclusions. Manipulation of rearing conditions including quantity of food, density, and salinity were found to control both diurnal rhythm and synchronization of larval-pupal ecdysis. Species differ as to the combination of rearing conditions needed to produce the changes in pupal patterns. In four species synchronization could be increased by increasing the amount of food and by lowering the density and salinity. In the other six species a larger amount of food but at higher density improved synchronization (Nayar and Sauerman, 1970a). Similarly, temporary overcrowding as well as changes in food quantity in nine of ten species studied enhanced synchronization of the number of pupae in pupation peaks.

Adults

The study of the effects of larval overcrowding on resultant adults has received less emphasis. Krishnan et al. (1959) in a study of the effects of high density on Culex fatigans reported that "underpopulated" females weighed more and had a higher biting rate than "overpopulated" females,

i.e. 51.4 per cent as compared to 31.2 per cent. Similarly, Terzian and Stahler (1955) working with Anopheles quadrimaculatus reported that females emerging from overcrowded pans had lower biting rates than underpopulated females. Their survival time was in an inverse relationship to the length of the larval development.

Wada (1965a) reported that wing and thorax length in adult Aedes aegypti were decreased with increasing density. Females seemed more sensitive to stress. Nayar and Sauerman (1970b) studied changes in morphological characteristics such as wing length in eleven species of mosquitoes. Other rearing conditions studied included food, density and salinity. In all species tested they found that minimal expression of adult morphological traits were exhibited by rearing larvae in "crowded" conditions, at low salinities and with small quantities of food. Identical results were reported in similar work on Aedes taeniorhynchus (Nayar, 1969).

Nayar and Sauerman (1969) reported that Aedes taeniorhynchus when reared in crowded conditions manifested migratory flight activity not apparent in non-crowded individuals. Crowded individuals exhibited an early main flight peak on the day of emergence. This peak was not demonstrated in non-crowded individuals. Interestingly, an earlier report by Wada (1965b) while not providing any experimental evidence suggested that overcrowded Culex pipiens may exhibit dispersal tendencies.

In some studies stress due to larval overcrowding have

been reported to have no effect on resulting adults. Wallis (1954) concluded that increasing density had very little effect on ovipositing female Aedes aegypti. On a much wider scale Jones (1960) concluded that if an adequate supply of food is available to larval Aedes aegypti, under aseptic conditions, crowding has no effect on survival, weight, sex ratio, hatchability, longevity, or fecundity of adults.

The above sections have highlighted experimental information on factors which can influence respiration. With this information as a background, a more productive evaluation and interpretation of experimental conclusions can be achieved.

CHAPTER III

MATERIALS AND METHODS

General Procedures

Rearing

All eggs utilized in experimentation were obtained from cultures of the Rockefeller strain of *Aedes aegypti* (L.). This strain was originally obtained from Dr. George Craig of Notre Dame University and has been maintained since 1969. Adults of the stock culture were kept in a cage 32" x 18" x 23" enclosed by glass on all sides with the exception of its plywood bottom and cloth-sleeved entrance. The culture cages were kept in a controlled-environment walk in room. A thermoregulated Electromode space heater provided a room temperature of 27°C ± 2.0°C. An automatic humidifier kept the humidity at an average of 60 per cent relative humidity. A Tork single-pole, single-throw timing motor maintained the daily light-dark regime set at 10 hours light, 14 hours dark.

Vacuum hatching was used in obtaining larvae for both the stock culture and experimentation. In order to replenish adults of the stock cultures, eggs were hatched under a vacuum pressure of 25 psi for 30 minutes using a Gast air and vacuum pump (Barbosa and Peters, 1969). Approximately 400 larvae were placed in 9" x 12" x 2¼" enameled pans, each containing two liters of modified Trager's solution. The larvae were fed dry brewer's yeast, and kept in the environmental walk-in

room. Adults were fed on a ten per cent sucrose solution, presented on soaked cotton wicks. Females were blood fed on a rabbit and oviposition occurred on dated filter paper cones. The egg cones were stored in a dessicator kept at 75 per cent relative humidity (maintained by a saturated solution of NaCl).

Larvae for all experiments were obtained by placing eggs, in Trager's, under a vacuum pressure of 25 psi for 30 minutes. In all situations requiring liquid media, modified Trager's salt solution was used (Trager, 1935). The experimental rearing universes were 100 x 20mm petri dishes. Each dish contained 80ml of the modified Trager's solution and 40 larvae. The larvae were transferred daily to fresh solutions throughout the whole of the rearing period. Larvae were fed on suspensions of Brewer's yeast in modified Trager's solution in accordance with a previously reported standard rearing technique (Peters, et al., 1969b). In experiments using larvae reared in accordance to this procedure, the larval population will be referred to as "standard larvae." Overcrowded experimental populations were reared and fed identically to standard populations but differ in the number of individuals per petri dish. Starved populations were reared identically and had the same number of individuals per dish as standard populations but were fed lesser amounts depending on the specific experimental design. All larval populations were reared in an environmental chamber set at a temperature range of 76°F (night temperature) to 84°F (day

temperature), a relative humidity of 90 per cent, and a light-dark regime of 12 hours light, 12 hours dark.

Respiratory Measurements

The Gilson GR-20 refrigerated differential respirometer was used in all measurements of oxygen consumption (Gilson, 1963). In all respirometric tests 15ml reaction flasks were used. Ten millimeters of modified Trager's solution or treated media were used as the larval media in the reaction vessels. A 20 per cent solution of KOH was used for absorption of evolved CO_2. One milliliter of the 20 per cent KOH solution was placed in the side arm of each vessel. The KOH was absorbed onto a strip of fluted No. 40 Whatman filter paper (Dixon, 1951). The strip of filter paper was placed in the side arms of the reaction vessels with an end of the strip extending out into the main center area of the vessels. The side arm was used for the KOH because use of the center well would have caused KOH contamination of the liquid medium. Ten milliliters were used in each vessel so that the ratio of media per larva would be as close as possible to the media per larva ratio of the standard rearing technique. The water bath temperature was set at 80°F. This temperature was selected because it was the mean temperature at which all larvae were reared.

Comparative measurements of larval respiration were performed on fourth instars. Using the standard technique, fourth instars could be consistently obtained within approximately 96 hours. Overcrowded and starved larvae were

differentiated as fourth instars by morphological differences of the respiratory siphon. The larvae used in the respiration tests were washed twice with Trager's solution before placement into the reaction flasks. The number of larvae per vessel varied with the particular experiment. The reaction vessels were placed on the manometers of the respirometer and the larvae (or pupae) equilibrated in the water bath for approximately one hour, with all valves open. At the end of this period of equilibration, all valves were closed and from that point readings were taken at fifteen minute intervals (in some experiments the intervals were longer). Readings were continued for three to four hours. At the end of the period of measurement the larvae were recounted and placed in marked 5ml beakers for drying and weighing. The larvae from each vessel (replication) were oven dried for an hour at 100°C. After drying the beakers were placed in a dessicator containing KOH tablets to maintain specimens moisture free. The beakers were left in the dessicator overnight. Larvae were then weighed on a Mettler balance.

Using data on dry weights of larvae and total hourly oxygen uptake the oxygen consumption in ul/mg. dry wt./hr. was calculated. A split plot in time analysis of variance design was used to analyze differences in variation in treatments effects, hourly effects and in treatment by hour interaction. Duncan's new multiple range test and single-degree of freedom orthogonal comparisons were used to test mean differences.

Experimental Procedures

Experiment I

This experiment was designed to ascertain the minimum number of *A. aegypti* larvae per vessel which would be most suitable for the measurement of oxygen consumption. This was necessary because of preliminary indications that the oxygen uptake of very low numbers of larvae per vessel was not observable in the manometers. Additionally, a close concurrence was desired between the media per larvae ratio in the standard technique and the ratio in the reaction vessels. Standard larvae were used in all treatments. No calculations were made to convert respiratory values to ul/mg. dry wt./hr. since only the comparative absolute values, representing respiration, were of interest.

To accomplish the desired goal, treatments were designed with 5, 7, 10, 15, and 20 larvae per reaction vessel. Each treatment consisted of two replications (i.e. two vessels). The standard larvae used were all fourth instars. All other procedures used, either preparatory or experimental were identical to those discussed in General Procedures (Materials and Methods).

Experiment II

This experiment was designed to measure the effect of an overcrowding stress during respirometric testing, but not imposed during the rearing procedures. This overcrowding stress was induced by establishing a series of treatments

with increasing larval numbers per reaction vessel. This was the opposite approach to the measurement of the effects of overcrowding stress imposed during the rearing process. The effect of overcrowding during rearing was evaluated in other experiments.

Fourth instar standard larvae were used in this experiment. Three treatments were designed with four replications (i.e. reaction vessels) in each. The treatments consisted of 5 (T_1), 35 (T_2), and 65 (T_3) larvae in each vessel. All other procedures used in this experiment are described under the section General Procedures. No calculations were made to convert to ul O_2/mg. dry wt./hr.

Experiment III

The purpose of this experiment was to test the precision and uniformity of oxygen uptake measurements. Precision may be defined as the degree of reproducibility among several independent measurements. This series of experiments was performed with standard larvae since these were logistically the best choice due to their rapid development and uniformity. The oxygen consumption observations were made on larval populations of identical and dissimilar ages. The larvae used in experiments were obtained from eggs laid on different days. Rearing of the larvae was initiated on different days and thus their oxygen uptake was measured with the Gilson respirometer on different days.

All tests in this series of experiments were conducted with fourth instar standard larvae. All treatments consisted

of four replications with 15 larvae in each vessel. Measurements of oxygen consumption were taken, for each treatment, on different days. Treatment 1 consisted of larvae from eggs that had been laid on September 5, 1970 and were hatched on October 9, 1970. Treatment 2 used larvae from eggs laid on September 20, 1970 and were hatched on October 10, 1970. Treatment 3 used larvae from eggs laid on September 20, 1970 and hatched on October 14, 1970. Treatment 4 utilized eggs that were laid on October 14, 1970 and hatched on October 17, 1970. All other operational procedures for both rearing and measurement of respiration were identical to those described in General Procedures. Oxygen consumption values were converted to ul/mg/hr. and were statistically analysed.

This experiment was an attempt to justify the design and procedure of the major experiments of this study. Since the overall length of the developmental period varied for each of the three major experimental populations, standard, overcrowded, and starved, respirometric measurements would have to be made on different days within any single experiment. Even if differential timing of the rearing of all three populations could be made to coincide, measurements could not be made on the same day. The physical limitations of the Gilson would not allow the running of replicated treatments of all three populations simultaneously. Therefore, the experimental justification of alternate day testing had to be considered.

Experiment IV

The purpose of this experiment was to evaluate the

relative respiratory rates of standard, overcrowded, and starved larval populations of Aedes aegypti. The three populations were reared under conditions described in General Procedures. A density of 640 larvae per 80ml. of media per petri dish was used as the overcrowded population. The starved larval population consisted of 40 larvae per 80ml. of medium per petri dish. The standard larval populations (40 larvae/80ml.) and the overcrowded populations (640 larvae/80ml.) were fed equal quantities of food according to techniques previously described. An attempt was made to feed individuals of both starved and overcrowded populations substantially the same average quantities. Each petri dish (the experimental container) of the starved populations contained a sixteenth of the number of larvae present in dishes of overcrowded populations. Therefore, in all daily feedings, standard and overcrowded populations would receive the full aliquot of food while the starved populations would receive a sixteenth of that amount. In reality, overcrowded populations were also partially starved since they received the same amount of food per diem as standard populations (40 larvae/80ml.). Thus, each individual of the overcrowded populations also received an average of one sixteenth of that available to individuals of the standard populations. Consequently the way to isolate the starvation effects was to design a third population with a standard number of larvae, 40, but fed the same amount per larvae as overcrowded individuals. In all the treatments six replications

(reaction vessels) were used and each vessel contained 15 larvae.

Experiment V

In this experiment the effects on respiration of overcrowding fourth instar standard larvae within reaction vessels was investigated. These larvae were reared without any previous overcrowding stress during rearing. Four replications of each of three treatments were used. The three treatments consisted of 15 larvae per vessel (T_1), 25 larvae per vessel (T_2) and 35 larvae per vessel (T_3). This experiment represents a repetition of Experiment II and is based on the knowledge of the limitation of its design. All procedures for rearing, pre-experimental preparation and respiratory techniques are previously described.

Experiment VI

The purpose of this experiment was to ascertain the effects of growth retardant factors (GRF), extracted from overcrowded populations on standard larval populations. The density of the overcrowded populations from which the GRF were extracted was 640 larvae per 80ml. The extracted material was tested on standard fourth instar larvae. Five replications (15 larvae/reaction vessel) were designed for each treatment and each reaction vessel contained 15 larvae. The vessels of the control were filled with plain Trager's salt solution while those of the second treatment contained the GRF extract dissolved in Trager's solution.

The extraction procedures were those reported by Ikeshoji and Mulla (1970a). Essentially, this procedure calls for the washing of the rearing media with three or four 100ml. portions of ether. The resultant ether fractions were vacuum evaporated. The residue was then suspended first in 1 ml. of ethanol (95 per cent) and then with Trager's solution. The Trager-extract solution was heated at 60°C for a few hours to evaporate any dissolved ether. After heating, the liquid was brought back up to its initial volume. This step was performed using Trager's solution. Ten milliliters of solution, whether plain Trager's or Trager-extract solution were pipetted into each of five reaction vessels. Respiratory values were then recorded.

Experiment VII

This experiment was conducted to determine if the respiratory rates of larvae reared in unchanged medium differed from those of individuals reared in medium that was changed daily. The experiment was conducted with overcrowded larval populations of 640 per 80ml. Both treatments were designed with five replications. The daily changing of rearing medium involved the transfer of larvae into fresh Trager's suspension containing Brewer's yeast. The daily amounts fed to larval are reported in an earlier work (see General Procedures). The Trager's solution of the "unchanged" treatment was filtered daily with a Buchner funnel. Due to evaporation, the volume of medium usually dropped from day to day. The total volume of solution was brought up to its initial

volume by adding additional Trager's solution. The appropriate amount of yeast was then added. Respiratory measurements were taken for three hours.

Experiment VIII

The purpose of this experiment was to investigate the effects on respiration of overcrowding fourth instar larvae within each reaction vessel. These larvae had been reared in overcrowded conditions. The experiment was designed as a comparison to Experiment V in which fourth instar standard larvae were overcrowded in the reaction vessels. The treatment densities were the same in both experiments, i.e. 15 (T_1), 25 (T_2), and 35 (T_3) larvae per vessel. In this experiment replications varied from five (T_2 and T_3) to six (T_1). The rearing density of all larvae used was 640 larvae per 80ml. Observations on oxygen consumption were taken for three hours.

Experiment IX

This experiment was designed to determine the effects on standard larvae of the growth retardant factors (GRF) extracted from overcrowded larval populations. The experiment was similar to Experiment VI but with two concentrations of GRF among the four treatments. Growth retardant factors were extracted from overcrowded populations comprised of 640 larvae per dish. The GRF were tested on fourth instar standard larvae. The extraction procedure for GRF is in the description of Experiment VI.

Five replications were used in each of the four treat-

ments. The treatments were: plain Trager's solution (the control, T_1), Trager's solution passed through the extraction procedure (T_4), the GRF extract at full strength (T_3) and the GRF extract at four times its normal (full) strength (T_2). Ten milliliters of each solution were used with 15 larvae in each reaction vessel. Respiratory measurements were made for three hours. All other procedures followed were the same as in Experiment VI.

Experiment X

The purpose of this experiment was to ascertain the effects on overcrowded larvae of the growth retardant factors (GRF) extracted from overcrowded larval populations. Growth retardant factors were extracted from overcrowded populations comprised of 640 larvae per dish. The GRF were tested on overcrowded fourth instar larvae. The density of the populations from which these fourth instars were taken was 640 per dish. Each of four treatments had five replications. The treatments consisted of plain Trager's solution, the GRF extract at full strength, the GRF at four times its full strength and the GRF at ten times its normal strength. Fifteen larvae were placed in each reaction vessel containing ten milliliters of the appropriate solution. Oxygen uptake measurements were taken for three hours.

Experiment XI

In this experiment the comparative respiratory rates of various levels of overcrowded larval populations were

considered. Measurements were made from three population densities, 400, 640, and 1280 larvae per dish. The replicates consisted of fifteen larvae of the test populations. Fourth instars of all three population densities were used in respiration tests. The unequal numbers of replications in the three densities tested were the result of premature pupation occuring during the testing period. Respiratory measurements ran for three hours.

Experiment XII

This experiment was designed to evaluate the respiratory trends in pupal respiration of standard populations. Ten replications of fifteen pupae per reaction vessel were used. Twelve hours after initiation of pupation, all pupae were removed. All larvae pupating within the succeeding four hours were used in respiration tests. Thus, all pupae were from one to four hours old when respiratory measurements were begun. Respiratory observations were made for thirty-eight hours.

Experiment XIII

This experiment was identical to Experiment XII except that tests were made on pupae of overcrowded populations. The density of the overcrowded population was 640 larvae per dish.

CHAPTER IV

RESULTS

Experiment I Optimal Vessel Density

Of the treatments with 5, 7, 10, 15 and 20 larvae per vessel those with 5 and 7 larvae registered very low oxygen uptake values. Treatments with 10, 15 and 20 larvae per vessel showed higher and more easily registered oxygen consumption values. The treatment with 10 larvae per vessel yielded erratic respiratory values between replications. Treatments with 15 and 20 larvae per vessel showed relatively high values with uniform readings among vessels. Although treatments with both 15 and 20 larvae per vessel would serve well in future experiments, the smaller number of larvae offer greater procedural flexibility. This is particularly evident in experiments where a large number of larvae are required. Therefore, it was decided that 15 larvae per vessel would be preferable as an experimental standard in subsequent experimentation.

Experiment II Vessel Density Effects (Standard)

This experiment was terminated after three readings. Termination of the experiment was due to the approach of the micrometer to its upper limit (about 500ul.). The rapid approach to this upper limit would have prevented the measurement of oxygen uptake for the full four hour period. Treatment 3 (65 larvae per vessel) accounted for the major

part of the high respiratory values. This high rate averaged 60 to 70 ul of oxygen per 15 larvae per 15 minute intervals. Another experiment with identical goals was designed with consideration given to the design weaknesses of this experiment.

The results of both Experiments I and II were used as a basis for later and more extensive experimentation. They led to a fuller understanding of the limitations of the test animal and of the respirometer.

Experiment III Age of Eggs and Larval Respiration

Several interesting points were revealed in this experiment. The analysis of variance and treatment mean comparisons are shown in Tables 1, 2 and 3. A significant difference, at $p < 0.01$ was found in the oxygen uptake between treatments composed of larvae reared from eggs of various ages. Similarly, a significant difference, at $p<0.01$ was also found in respiration over the four hours of respiratory measurements. A significant treatment X hour interaction, at $p<0.01$ was also evident; which indicates either a synergistic or interference effect between hours and treatments.

Treatment mean differences were ascertained since these comparisons were germane to the goals of this experiment. Several interesting points were revealed using single-degree of freedom orthogonal comparisons. The first concerns treatments 2 and 3 which used eggs that were laid on the same day (September 20th). Although, fourth instar larvae of treat-

ments 2 and 3 were reared and tested at intervals of at least four days, no significant differences were indicated in the ul of oxygen respired per mg. dry weight per hour. However, the oxygen uptake of those treatments using larvae from eggs of different ages were found to differ from each other.

The oxygen uptake (treatment) means ranges as follows: T_2 (September 20) < T_3 (September 20) < T_1 (September 5) < T_4 (October 14). This ranking represents a gradient of absolute values without reference to significant differences. The oxygen uptake for T_1 (September 5) was not significantly lower than that of T_4 (October 14), at $p < 0.01$, although the difference in larval respiration between T_1 and T_4 was significant at $p < 0.05$. The major share of the significant treatment variance was attributable to the comparison of the average of T_2 and T_3 versus that of T_1 and T_4, at $p < 0.01$ (cf. Table 2).

Duncan's multiple range tests were used to make comparisons that could not be made with single-degree of freedom orthogonal comparisons. At $p < 0.05$, the oxygen uptake values for T_1 (eggs laid on September 5) were significantly higher than those for T_2 or T_3 (eggs laid on September 20). Yet, the values for T_4 (treatment using eggs laid on October 14) were significantly larger than those of T_1 which entailed larvae from eggs laid on September 5. Thus, there are strong indications that the age of mosquito eggs, can not be used to determine or predict any trend in the respiratory activity of

resulting fourth instar larvae, at least within the range tested. The range in age of the eggs used was not large. Therefore, it is feasible that a greater age difference in the eggs might affect the respiration of resultant larvae and exhibit a trend not evident in this experiment.

Experiment IV Respiration of Overcrowded, Starved and Standard Larvae

Results of all statistical analyses of data from Experiment IV are presented in Tables 4 and 5. Analyses of data on treatments indicated that there was a highly significant difference in the oxygen uptake, at $p < 0.01$, of mosquito larvae reared under either standard, overcrowded or starvation conditions. Each treatment was found to be significantly different from each other. It is also important to note the sequence of respiratory treatment means of the three populations. This respiratory activity gradient would thus give an indication of the qualitative populational sensitivity to the presence or absence of particular stresses. Results from range test analyses, at $p < 0.01$, indicate that the standard larvae showed higher oxygen consumption than larvae reared under overcrowded conditions and that the latter had a higher rate than starved larvae.

A significant difference at $p < 0.01$, was found in the changes in respiratory activity as a function of time. However, results do not show any trend. The hourly means per treatment are all significantly different from each other, at $p < 0.05$. The respiratory activity rises during the

second and third hour, then it drops during hour four. One could speculate that the oxygen consumption was levelling off because, at $p < 0.01$, the drop in respiration between hours three and four was non-significant.

In addition to the above significant differences, there existed a significant treatment X hour interaction. There seems to be a change in respiratory activity due to both treatments and time that differs from the individual effect of either treatment or time alone.

Experiment V Vessel Density Effects (Standard)

This experiment was designed to answer the question that logically arises from the results of Experiment IV. That is, if overcrowding during rearing significantly affects respiration, would higher densities within the reaction vessels cause any changes in standard larvae?

Tables 6 and 7 show that treatments ranging from 15 to 35 larvae per vessel significantly differ, at $p < 0.01$, from each other. Duncan's range tests indicated that, at $p < 0.05$, each treatment is significantly different from each other. However, at $p < 0.01$, no significant difference existed between T_1 (15 larvae/vessel) and T_2 (25 larvae/vessel) but T_3 (35 larvae/vessel) did differ significantly from T_1 and T_2. It is worth noting that while the mean difference between T_1 and T_2 was 0.167, the LSR, at $p < 0.01$, which had to be exceeded for significance, was 0.170.

Among the more interesting results was the distinct effect on respiration per unit weight due to the number of

larvae within each reaction vessel. Respiratory values increased from a mean for T_1 (15 larvae/vessel) of 6.908 to 7.075 for T_2 (25 larvae/vessel) and 7.921 for T_3 (35 larvae/vessel). Each treatment mean differed significantly from the others, at $p < 0.05$, as indicated above. Similarly, a significant difference was found in the treatment X hour interaction. The effect of time was dissimilar to the initial rising trend found in Experiment IV but similar to the dropping respiratory pattern in Experiment III. Hourly values for oxygen uptake followed the sequence of $H_1 > H_2 > H_3 > H_4$.

Generally, there is a significant decreasing pattern in respiration with the passage of time. At $p < 0.05$, the values for larval respiration during the first hour is higher than that of the following hour. The larval respiration during the second and third hours, however, did not differ statistically. A second significant drop was indicated during the fourth hour of respiration. At $p < 0.01$, while the respiratory values for the first hour were significantly higher the succeeding three values for the second, third and fourth hours did not differ statistically.

Experiment VI GRF On Standard Larvae

In this experiment, no significant difference ($p < 0.01$) existed in the respiration of fourth instar standard larvae exposed to overcrowding factors or plain Trager's solution. A significant difference in respiration, at $p < 0.05$, was demonstrated in larvae throughout the time period in which respiration was measured. A significant difference was found

in the treatment X hour interaction. The lack of any statistical difference in either treatments or hours but with a significant difference in the interaction connotes that the mutual effect of treatment and time on each other is synergistic. Analysis of variance data is shown in Table 8.

Experiment VII Changed Versus Unchanged Medium

The results of the statistical analysis of this experiment are presented in Table 9. No significant differences were indicated in the respiration of larvae whose medium was changed daily as compared to those whose medium was filtered, but remained unchanged. A similar lack of significance was exhibited in the interaction of treatments and hours. Conversely, hourly changes in oxygen uptake were revealed to be significantly different over the three hour duration of the experiment. An evaluation of hourly trends using Duncan's range test indicated that larval respiration rises significantly during the second hour. Although the values for respiratory activity increase from the second to the third hour, this rise is not statistically significant.

Experiment VIII Vessel Density Effect (Overcrowded)

Results of statistical analysis of data is indicated in Table 10. This analysis indicated that previously overcrowded larvae placed in reaction vessels at densities of 15, 25 and 35 larvae per 10 ml. of Trager's solution showed no significant differences in respiration. However, highly

significant results were found in the hourly respiratory readings. Significance also was indicated in the treatment X hour interaction. A further evaluation of hourly differences indicated an initial significant rise in respiration during the first hour. This was followed by a numerical but statistically insignificant increase during the second and third hours.

Experiment IX GRF On Standard Larvae

The analysis of variance and Duncan's range test for this experiment are shown in Tables 11 and 12. A significant difference, at $p < 0.05$, was found among the treatments of this experiment. These treatments were plain Trager's salt solution (T_1), Trager's solution passed through the GRF (growth retardant factors) extraction procedure (T_4), extracted GRF at ordinary concentration (T_3) and GRF at four times ordinary concentration (T_2). Ordinary concentration (X) being that concentration of GRF in 80 ml of Trager's with a larval density of 640 larvae. A closer scrutiny of the significant differences in treatments using Duncan's range tests (cf. Table 12) reveals that T_1, T_4 and T_3 do not significantly differ from each other. However, T_2, the treatment consisting of GRF at four times ordinary concentration, was significantly different ($p < 0.05$) from T_1, T_3 and T_4.

The increase indicated in the hourly changes in respiration was also highly significant. The respiration values for the first hour differed significantly from those

of the second and the third hour (cf. Table 12). No significance was attributed to the interaction of treatments and hours.

Experiment X GRF On Overcrowded Larvae

Tables 13 and 14 show least-squares analysis of variance and Duncan's multiple range tests, respectively for this experiment. A highly significant difference was found among treatments. A closer analysis of these differences using Duncan's range test indicated that while 4X and 10X concentrations of GRF did not differ from each other, at $p < 0.01$, they did differ from T_1 and T_2, i.e. plain Trager's solution and X concentration GRF. Plain Trager's and X concentration GRF did not differ from each other. A highly significant difference was found in hourly mean respiratory values at $p < 0.01$. The trend obtained by a Duncan's range test of these values indicated a rise in oxygen uptake from the first hour to the third. The mean values for hours one, two and three were all different from each other at $p < 0.01$. The interaction between hours and treatments was found to be significant at $p < 0.01$.

Experiment XI Respiration Of Three Overcrowded Density Levels

Table 15 indicates the least-squares analysis of variance for data from this experiment and Duncan's range tests on hourly means. No significant differences were found among the respiration rates of fourth instar larvae reared at 400, 640 or 1280 per 80 ml densities. Conversely, highly signif-

icant differences were found in hourly respiratory means at $p < 0.01$. In addition there was a significant difference, at $p < 0.01$, indicated in the treatment X hour interaction. Duncan's range tests of the differences among hourly means indicated that the mean for hours one, two and three, were highly significant.

Experiments XII and XIII Pupal Respiration In Overcrowded And Standard

Patterns indicated in the respiration of male pupae are illustrated in Figures 5 and 6. Irregular peaks of oxygen consumption were noted throughout the 38 hour observation period. No distinct rhythm in the occurrence of these peaks is evident. The intervals between these peaks also vary. Data on respiration of the two test populations indicate that the rates of respiration in overcrowded larvae are higher than those of standard larvae.

CHAPTER V

DISCUSSION

Respiratory Values Of Experimental Populations

In measuring the oxygen uptake of several types of populations under a variety of conditions, a range of respiratory values was obtained. The significance that is attributed to these numerical values should be considered and interpreted as to their biological significance. Three levels of activity that have been used as a base for metabolic measurements are described by Prosser and Brown (1961). Basal metabolism is considered to be the oxygen consumption used only for maintenance. This assumes a level of inactivity not achievable in larval mosquitoes. Standard metabolism is defined as oxygen consumption under minimal activity. The last type mentioned is activity metabolism which refers to measurement of oxygen uptake at a set level of forced activity such as flying, swimming or running. Considering the goals of the experiments performed, the above two types of conditions are not feasible or practical in tests using mosquitoes. These levels are those at which metabolism is frequently evaluated in physiological investigations.

The above situations do not adequately describe the conditions under which oxygen consumption values were obtained for standard larval populations. A more appropriate description of the conditions under which respiration of

these populations were measured is that expressed as existence metabolism. Odum et al. (1962) defines existence metabolism as the energy (reflected in respiration) "required by individuals not subjected to energy demands above that required for day to day maintenance of body weight and health at room temperature." As compared to three original definitions, existence metabolism has more significance in an ecological context and comes closest to the experimental conditions under which respiration was measured. If existence metabolism can be equated to the metabolic demands of standard larvae then shifts in the respiration of overcrowded and starved populations can be considered as energy demands differing from existence metabolism.

The mode of action of the factors inducing changes in overcrowded larvae have not been determined. The effect of these factors may be wholly intrinsic or may be manifested as changes of activity. These changes in activity may reflect changes in degree of stress under unfavorable conditions. The shifts in respiration of variously stressed populations may be thus a function of internal physiological reactions or simply direct induced changes in activity. Therefore, discussion of absolute numerical respiratory value does not add to an understanding of the problems investigated here. Absolute numerical respiratory values are rarely compared in this study since one of the conditions which determines respiratory rates, i.e. activity is not predetermined or constant in the experiments performed.

Experimental Results

Experiments I and II provide information as to the population density that would give replicable results within the limitations of the experimental equipment. In Experiment III the role of differences in the age of mosquito eggs and the potential effect on larval respiration was investigated. One major aspect of the results of this experiment led to the conclusion that the age of the egg can not be considered as a determining factor in respiratory trends of resulting larvae. The reason was evident in the comparison of respiration in T_1 (September 5 eggs), T_2 (September 20 eggs), T_3 (September 20 eggs) and T_4 (October 14 eggs). When T_4 and T_1 were compared, respiratory values for T_4 were greater than T_1. Larvae from younger eggs (relative to day of testing) had higher respiration values than larvae from older eggs. However, when T_1 and T_3 were compared T_1 was greater than T_3. Therefore, in this test older eggs produced higher values than younger eggs. This is just the opposite conclusion as found in the comparison of T_4 and T_1. An additionally significant result was that eggs of the same age (T_2 and T_3) produced identical results in larval respiration. This occurred even though respiration measurements were made on separate days and rearing of larvae from the two treatments was initiated on different days.

The comparison of the respiration of standard, overcrowded and starved populations (Experiment IV) was one of

the major aspects of this study. It clearly illustrates that the stresses imposed by both overcrowded and starved conditions cause a significant drop in metabolic activity. Although the double stress of overcrowded populations (overcrowding and starvation) was expected to reduce metabolism more than merely starvation, this did not occur. It would seem that the attempt to compensate for the starvation conditions existing in overcrowded populations as described in Materials and Methods, was not as successful as desired. It is possible that due to the mortality in overcrowded populations a greater amount of food was available for overcrowded larvae. Dead larvae were rarely seen in overcrowded populations. Actual consumption of dead larvae and pupae has been noted. Thus, the greater stress of starvation existing in starved populations may explain why their metabolic rate was lower than that of overcrowded populations.

Ikeshiji and Mulla (1970a) suggest that GRF produced by third and fourth instar larvae affect younger instars but are not affected themselves. This is not indicated by these results since the overcrowded larvae whose respiration dropped to a very low level were fourth instars.

Both Experiments V and VIII investigated the effects of increasing numbers within reaction vessels (Figure 1). Populations of fourth instar larvae were tested in both, but each experiment used populations reared differently. In Experiment V larvae were reared in accordance to a standard technique. In Experiment VIII larvae were reared in overcrowded conditions. In Experiment V the respiratory values were directly

proportional to the increasing density. However, in Experiment VIII there were no significant differences in the oxygen uptake regardless of the density, whether 15, 25 or 35 larvae per reaction vessel. Thus, while increasing numbers stimulated the respiratory activity of standard larvae it had no effect on the respiration of overcrowded fourth instars. There is a possibility that overcrowded larvae may not be as easily affected once respiration has dropped to a low level due to overcrowded rearing conditions. Also see page 66 for another aspect of this hypothesis.

The most important aspect of this experiment is its significance in terms of the surface law. The surface law states that the metabolic rate per unit weight decreases with increasing size. The overcrowded and starved larvae tested in this experiment were approximately half the weight of standard larvae. Standard larvae would be expected to exhibit a lower respiratory rate per unit weight but did not. Thus the effect of stress in larval populations is to reverse the expected (surface law) pattern of metabolic activity.

Experiments VI, IX and X investigated the effects of exposure to various concentrations of growth retardant factors on standard and overcrowded larvae (Figure 2). Experiments VI and IX tested the effects GRF exposure to fourth instar standard larvae while Experiment X utilized fourth instar overcrowded larvae. While Experiment VI tested Trager's solution and an ordinary (X) concentration of GRF, Experiment IX tested Trager's solution, Trager's solution

passed through the GRF extraction, X concentration GRF and 4X concentration GRF. Experiment X tested Trager's passed through GRF extraction, X concentration GRF, 4X concentration GRF and 10X concentration GRF. As expected, both Trager's solution treatments did not affect standard larvae but, in addition they were unaffected by X concentration GRF. This lack of susceptibility was also demonstrated by overcrowded larvae. However, standard fourth instars were susceptible to a 4X concentration of GRF. Overcrowded larvae were similarly affected by a 4X concentration as well as a 10X concentration, although the values for 4X and 10X concentrations did not differ significantly. In all experiments for both standard and overcrowded larvae, the significant differences were expressed as a stimulation of respiratory rate.

In Experiment VII a comparison was made of an overcrowded larval population whose medium was changed daily with an overcrowded population whose medium remained unchanged. The only difference between treatments was the quantities of yeast (food) in the dishes of larvae. When the water from the unchanged treatment was centrifuged at the end of the experiment, large amounts of yeast were evident. Thus even with a greater amount of food available to individuals of overcrowded, unchanged populations respiratory exchange did not differ from the changed treatment.

This seems to be good indirect evidence that food availability is not the total cause of changes in overcrowded populations. These results strengthen the possibility of the

involvement of chemical factors in the water.

Experiment XI compared the oxygen uptake of three levels of overcrowded larval populations of Aedes aegypti (400, 640 and 1280). Within the range tested, no differences were exhibited by larvae of any of the three densities. The experiment reaffirms the idea that larvae become insensitive to further density stresses once respiration reaches low levels as postulated in the discussion of Experiments V and VIII.

In Experiments XII and XIII the most important result was the higher respiratory rate indicated in overcrowded pupae as compared to standard pupae. Although the overcrowded pupae exhibit a higher respiratory rate this difference is probably not due to stress during rearing. The differences in respiratory rates of overcrowded and standard pupae seem merely to follow the dictates of the surface law. Since the replications of 15 overcrowded pupae averaged 3.01 mg. as compared to 9.43 mg. per 15 standard pupae their respiratory rate would be expected to be higher.

Peaks of respiratory activity were evident in both overcrowded and standard larvae. These peaks of oxygen uptake did not exhibit any rhythmical pattern. Olifan (1949) in measuring the respiration of pupae of Anopheles found that periodic fluctuations occurred at intervals of about five to seven hours. However, the smallest measuring interval used was approximately three hours. No such clear interval between fluctuations was discernible for A. aegypti pupae.

Pupae of mosquitoes are generally active and therefore the fluctuations in respiration found in Experiments XII and XIII represent periods of increase oxygen uptake due to activity. These peaks may also reflect internal metamorphic processes. Punt (1950) suggests that there are two types of CO_2 production. One type is continuous but in certain species CO_2 production occurs in bursts. The interval of bursting can vary in different species and can include frequencies of ten minute bursts or one burst per 24 hours. Thus, this type of CO_2 production may help determine the pattern of respiratory activity and may be a possible explanation of pupal respiratory fluctuations in Aedes aegypti.

Several overall speculations seem feasible at this point. Results indicate that two types of responses are evident under unfavorable conditions represented by over-crowded-starved populations. These two types of density factors are chemical factors such as those found in over-crowded conditions and mechanical agitation such as that found in an enclosed space e.g. reaction vessel (cf. Figure 4). In overcrowded larvae a rise in respiration is induced by chemical factors in "overcrowded" water while a second different response is induced by mechanical agitation. In comparison, the respiratory rate of standard larvae is stimulated by both mechanical agitation and chemical factors.

A certain hypothesis was originally considered to be the most feasible scheme of compensation under unfavorable conditions (cf. Figure 3). It suggested that when stress

causes an imbalance in the homeostatic condition of a population, compensation can occur in either of two ways. The path taken in this compensation is dependent on the physiological state of the population. If the population has been under stress, compensation may occur by lowering metabolism i.e., hypometabolic compensation. If a population has not been under stress, then compensation may occur by an increase in metabolic activity i.e., hypermetabolic compensation. Thus, by two routes the population is returned to a dynamic optimal range.

Upon a thorough evaluation of the experimental results this hypothesis, although far from confirmed, still seems plausible (Figure 4). Only one modification must be made in the scheme of the hypothesis. As previously mentioned, Experiments VIII, X and XI would seem to indicate that once the lower respiratory levels are reached by stressed populations, they are relatively insensitive to small increases in density.

CHAPTER VI

CONCLUSIONS

1. Sequential increasing or decreasing trends in the respiratory rates of standard fourth instar larvae are not dependent on age of eggs.
2. Stresses such as overcrowding and starvation cause significant drops in metabolic activity (as indicated by respiratory activity).
3. Post-rearing density stress stimulates oxygen uptake of standard larvae but does not affect the rate of respiration of overcrowded larvae.
4. Exposure of both overcrowded and standard larvae to high concentrations of growth retardant factors (GRF) stimulates respiration.
5. Respiration of overcrowded larvae is not easily affected by further density stress.
6. Overcrowding causes no major changes in the respiratory rate of pupae from overcrowded populations as compared to standard pupae.
7. Further experimentation will be productive if:
   a) The effects of overcrowding and starvation can be isolated
   b) The limitations of the respirometer can be by-passed.

LITERATURE CITED

Allee, W. C. 1931. Animal Aggregations, University of Chicago Press, Chicago, Illinois. 431 pp.

_____. 1934. Recent studies in mass physiology. Camb. Phil. Soc. Biol. Rev. Biol. Proc. 9:1-48.

Allen, M. D. 1959. Respiration rates of worker honeybees of different ages and at different temperatures. J. Exp. Biol. 36:91-101.

Barbosa, P. and T. M. Peters. 1969. A comparative study of egg hatching techniques for Aedes aegypti (L.). Mosq. News 29:548-551.

Barbosa, P., T. M. Peters and N. C. Greenough. Overcrowding of mosquito populations: responses of larval Aedes aegypti (L.) in lit.

Bar-Zeev, M. 1957. The effects of density on the larvae of a mosquito and its influence on fecundity. Bull. Res. Counc. Israel 6B:220-228.

Bat-Miriam, M. and R. Galun. 1962. Effect of age and sex on oxygen uptake by adult mosquitoes. Entomol. Exp. et Appl. 5:244-248.

Bellucci, R. 1939. The respiratory metabolism of starved Japanese beetle larvae (Popillia japonica Newman) at different relative humidities. Physiol. Zool. 12:50-56.

Bertalanffy, L. von. 1951. Metabolic types and growth types. Amer. Nat. 85:111-117.

Blackith, R. E. and G. F. Howden. 1961. The food preserves of hatchling locusts. Comp. Biochem. Physiol. 3:108-124.

Bodine, J. H. 1921. Factors influencing the water content and the rate of metabolism of certain orthoptera. J. Exp. Zool. 32:137-164.

Buck, J. and M. Keister. 1956. Diffusion phenomena in the tracheae of Phormia larvae. Physiol. Zool. 29:137-146.

Buffington, J. D. 1968. A circadian pattern in the respiration of larvae of the mosquito Culex pipiens. Mosq. News 28:95-98.

_____. 1969. Temperature acclimation of respiration in Culex pipiens (Diptera: Culicidae) and the influence of seasonal selection. Comp. Biochem. Physiol. 30:865-878.

Butler, C. G. and J. M. Innes. 1936. A comparison of the rate of metabolic activity in the solitary and migratory phases of Locusta migratoria. Proc. Roy. Soc. Lond. 119:296-304.

Clarke, K. U. 1957. The relationship of oxygen consumption to age and weight during the post-embryonic growth of Locusta migratoria L. J. Exp. Biol. 34:29-41.

Clements, A. N. 1963. The Physiology of Mosquitoes. Pergamon Press, London, 393 p.

Chauvin, R. 1967. The World of an Insect. World Univ. Library, London. 254 p.

Czerpak, R. and B. Czeczuga. 1969. The respiratory rate of Eylais hamata (Koenike 1897) (Hydracarina, Arachnoidea). Zool. Poloniae 19:351-360.

Dixon, M. 1951. Manometric Methods. Cambridge Univ. Press, London. 165 p.

Drummond, R. O. and W. F. Chamberlain. 1961. Studies on respiration of cattle grubs. Ann. Entomol. Soc. Amer. 54:524-526.

Ebeling, W. 1964. The permeability of insect cuticle. (In Rockstein, 1964) p. 507-556.

Edwards, G. A. 1946. The influence of temperature upon the oxygen consumption of several arthropods. J. Cell. Comp. Physiol. 27:53-64.

_____. 1953a. Respiratory Mechanisms. (In Roeder, 1953) pp. 55-95.

_____. 1953b. Respiratory Metabolism. (In Roeder, 1953) pp. 96-146.

Edwards, R. W. 1958. The relation of oxygen consumption to body size and to temperature in the larvae of Chironomus riparius Meigen. J. Exp. Biol. 35:383-395.

Engelmann, M. D. 1966. Energetics, terrestrial field studies and animal productivity. In Advances in Ecological Research 3:73-115.

_____. 1968. The role of soil arthropods in community energetics. Amer. Zool. 8:61-69.

Fox, H. M. and B. G. Simmonds. 1932. Metabolic rate and habitat. Nature 130:277-278.

Fraenkel, G. and G. V. B. Herford. 1938. The respiration of insects through the skin. J. Exp. Biol. 15:266-280.

Galun, R. 1960. Respiration of decapitated mosquitoes. Nature 185:391-392.

Gardiner, B. G. 1958. Some observations on the respiration of young nymphs of Schistocerca gregaria (Forskal) in relation to phase and rearing density. Proc. Roy. Entomol. Soc. Lond. 33:159-166.

Gilson, W. E. 1963. Differential respirometer of simplified and improved design. Science 141:531-532.

Ginsburg, J. M. 1929. Relation between toxicity of oil and its penetration into respiratory siphons of mosquito larvae. Proc. 16th Ann. Mtg. N. J. Mosq. Exterm. Assoc. 15 p. (cf. Richards, 1941).

Golley, F. B. 1960. Energy dynamics of a food chain of an old-field community. Ecol. Monogr. 30:187-206.

Golley, F. B. and J. B. Gentry. 1964. Bioenergetics of the southern harvester ant, Pogonomyrmex badius. Ecology 45:217-225.

Goma, L. K. H. 1966. The Mosquito. Hutchinson & Co., Ltd., London. 144 p.

Hacker, H. P. 1925. How oil kills Anopheline larvae. Fed. Malay St. Malaria Bur. Repts. III:1-62 (cf. Richards, 1941).

Hagstrum, D. W. 1970. Simultaneous measurement of aerial and aquatic respiration and study of mode of action of petroleum oils. Ann. Entomol. Soc. Amer. 63:1466-1467.

Hedeen, R. A. 1953. The biology of the mosquito Aedes atropalpus Coquillett. Kans. Entomol. Soc. 26:1-10.

Hyman, L. H. 1919. Physiological studies on Planaria I. Oxygen consumption in relation to feeding and

starvation. Amer. J. Physiol. 49:377-402.

Ikeshoji, T. and M. S. Mulla. 1970a. Overcrowding factors of mosquito larvae. J. Econ. Entomol. 63:90-96.

_____. 1970b. Overcrowding factors of mosquito larvae. 2. Growth-retarding and bacteriostatic effects of the overcrowding factors of mosquito larvae. J. Econ. Entomol. 63:1737-1743.

Ingram, R. L. 1954. A study of the bionomics of Aedes polynesiensis Marks under laboratory conditions. Amer. J. Hyg. 60:169-185.

Ishii, T. 1963. The effect of population density on the larval development of Culex pipiens pallens. Jap. J. Ecol. 13:128-132.

Ito, Y. 1964. Preliminary studies on the respiratory energy loss of a spider, Lycosa pseudoannulata. Res. Pop. Ecol. 6:13-21.

Jones, W. L. 1960. The effects of crowding on the larvae of Aedes aegypti when reared under aseptic and non-aseptic conditions. Dissertation Abstr. 21:3204-3205. Ph.D. dissertation, Ohio State University.

Keister, M. and J. Buck. 1964. Respiration: Some exogenous and endogenous effects on rate of respiration. (In Rockstein, 1964), pp. 617-658.

Kleiber, M. 1947. Body size and metabolic rate. Physiol. Rev. 27:511-541.

Knight, A. W. and A. R. Gaufin. 1966. Oxygen consumption of several species of stoneflies (Plecoptera). J. Insect Physiol. 12:347-355.

Kolesov, O. F. 1956. Changes in respiration in Aedes larvae after DDT poisoning. Meditsinskaia parazitologiia i parazitarnye bolezni 27:99-100.

Krafsur, E. S. and C. L. Graham. 1970. Spiracular responses of Aedes mosquitoes to carbon dioxide and oxygen. Ann. Entomol. Soc. Amer. 63:691-696.

Krafsur, E. S., J. R. William, C. L. Graham and R. E. Williams. 1970. Observations on spiracular behavior in Aedes mosquitoes. Ann. Entomol. Soc. Amer. 63:684-691.

Krishnan, K. S., V. N. Bhatnager and N. G. S. Raghavan. 1959. Effects of overcrowding of Culex fatigans larvae in laboratory breeding pans. Bull. Nat. Soc. Ind. Malaria Mosquito Dis. 7: 4:131-132.

Krogh, A. 1941. The Comparative Physiology of Respiratory Mechanisms. Univ. of Penn. Press, Philadelphia. 172 p.

Kurihara, T. 1963. Laboratory experiments on the effect of some environmental conditions on the growth of larvae of the mosquito Culex pipiens s.l. Jap. J. Sanit. Zool. 14:7-15.

Lindeman, R. L. 1942. The trophic-dynamic aspect of ecology. Ecology 23:399-417.

Ludwig, D. 1937. The effect of different relative humidities on respiratory metabolism and survival of the grasshopper Chortophaga viridifasciata De Geer. Physiol. Zool. 10:342-351.

Macfie, J. W. S. 1917. The limitations of kerosine as a larvicide, with some observations on the cutaneous

respiration of mosquito larvae. Bull. Entomol. Res. 7:277-295.

Melampy, R. M. and E. R. Willis. 1939. Respiratory metabolism during larval and pupal development of the female honeybee (Apis mellifica L.). Physiol. Zool. 12:302-311.

Menhinick, E. F. 1967. Structure, stability and energy flow in plants and arthropods in a sericea lespedeza stand. Ecol. Monogr. 37:255-272.

Mercado, T. I., H. L. Trembley and T. von Brand. 1956. Observations on the oxygen consumption of some adult mosquitoes. Physiol. Comp. et Oecol. 4:200-208.

Moore, C. G. and B. R. Fisher. 1969. Competition in mosquitoes. Density and species ratio effects on growth, mortality, fecundity and production of growth retardant. Ann. Entomol. Soc. Amer. 62:1325-1331.

Nayar, J. K. 1968. Biology of Culex nigripalpus Theobald (Diptera: Culicidae). Part 1: Effects of rearing conditions on growth and the diurnal rhythm of pupation and emergence. J. Med. Entomol. 5:39-46.

_____. 1969. Effects of larval and pupal environmental factors in biological status of adults at emergence in Aedes taeniorhynchus. Bull. Entomol. Res. 58:811-827.

Nayar, J. K. and D. M. Sauerman, Jr. 1968. Larval aggregation formation and population density interrelations in Aedes taeniorhynchus, their effects on pupal ecdysis and

adult characteristics at emergence. Entomol. Exp. Appl. 11:423-442.

_____. 1969. Flight behavior and phase polymorphism in the mosquito Aedes taeniorhynchus, Entomol. Exp. Appl. 12:365-375.

_____. 1970a. A comparative study of growth and development in florida mosquitoes. Part 1: Effects of environmental factors on ontogenetic timings, endogenous diurnal rhythm and synchrony of pupation and emergence. J. Med. Entomol. 7:163-174.

_____. 1970b. A comparative study of growth and development in florida mosquitoes. Part 2: Effects of larval nurture on adult characteristics at emergence. J. Med. Entomol. 7:235-241.

_____. 1970c. A comparative study of growth and development in florida mosquitoes. Part 3: Effects of temporary crowding on larval aggregation formation, pupal ecdysis and adult characteristics at emergence. J. Med. Entomol. 7:521-528.

Obreshkove, V. and A. M. Banta. 1930. A study of the rate of oxygen consumption of different Cladocera clones derived originally from a single mother. Physiol. Zool. 3:1-8.

Odum, E. P., C. E. Connell and L. B. Davenport. 1962. Population energy flow of three primary consumer components of old-field ecosystems. Ecology 43:88-96.

Olifan, V. I. 1947. Periodicity in the postembryonic development of Anopheles maculipennis. Comp. Rend. Acad. Sci. USSR 60:169-172.

_____. 1949. Some regularities in the changes of gas exchange in the Anopheles pupae. Comp. Rend. Acad. Sci. USSR 65:577-580.

Orr, P. R. 1937. Physiological studies on respiratory metabolism. Physiol. Zool. 10:235-243.

Pener, M. P. 1964. Comparative studies on oxygen consumption in the albino and normal strains of Schistocerca gregaria bred under crowded and isolated conditions. Proc. 12th Intern. Congr. Entomol.:222-223.

Peters, T. M., B. I. Chevone and R. A. Callahan. 1969a. Interactions between larvae of Aedes aegypti (L.) and Culex pipiens L. in mixed experimental populations. Mosq. News 29:435-438.

Peters, T. M., B. I. Chevone, N. C. Greenough, R. A. Callahan and P. Barbosa. 1969b. Intraspecific competition in Aedes aegypti (L.) larvae: I. Equipment, techniques and methodology. Mosq. News 29:667-674.

Petersen, J. A. 1960. Experimental studies on population physiology. I. Respiratory metabolism on Brazilian sibling species of the willistoni group of Drosophila. Rev. Bras. Biol. 20:205-216.

Petitpren, M. F. and A. W. Knight. 1970. Oxygen consumption of the dragonfly Anax junius. J. Insect Physiol. 16: 449-459.

Piper, W. D. and K. E. Maxwell. 1971. Mode of action of surfactants on mosquito pupae. J. Econ. Entomol. 64:601-606.

Prosser, C. L. and F. A. Brown, Jr. 1961. Comparative Animal Physiology. 2nd ed. W. B. Saunders Co., Philadelphia, Penn. 688 p.

Querra, A. A. and D. G. Cochran. 1970. Respiration during the life cycle of the face fly. J. Econ. Entomol. 63:918-921.

Rajagopal, P. K. and E. Bursell. 1966. The respiratory metabolism of resting tsetse flies. J. Insect Physiol. 12:287-297.

Richards, A. G., Jr. 1941. Differentiation between toxic and suffocating effects of petroleum oils on larvae of the house mosquito (Culex pipiens L.) Trans. Amer. Entomol. Soc. 67:161-193.

_____. 1964. The generality of temperature effects on developmental rate and on oxygen consumption in insect eggs. Physiol. Zool. 26:199-211.

_____. 1969. Oxygen consumption of the american cockroach under complete inanition prolonged to death. Ann. Entomol. Soc. Amer. 62:1313-1316.

Richman, S. 1958. The transformation of energy by Daphnia pulex. Ecol. Monogr. 28:273-291.

Rockstein, M. 1964. ed. The Physiology of Insecta. Vol. III. Academic Press, New York. 692 p.

Roeder, K. D. 1953. ed. Insect Physiology. John Wiley & Sons, Inc., New York. 1100 p.

Sayle, M. H. 1928. Factors influencing the rate of metabolism of Aeshan umbrosa nymphs. Biol. Bull. 54:212-230.

Schuett, J. F. 1933. Studies in mass physiology: the effects of numbers upon the oxygen consumption of fishes. Ecology 14:106-122.

_____. 1934. Studies in mass physiology: the activity of goldfish under different conditions of aggregation. Ecology 15:258-262.

Sen, S. K. 1914. Observations on respiration of Culicidae. Ind. J. Med. Res. 2:681-697.

Shannon, R. C. and P. Putnam. 1934. The biology of Stegomyia under laboratory conditions. I. The analysis of factors which influence larval development. Proc. Entomol. Soc. Wash. 36:185-216.

Shibazaki, A. and Y. Ito. 1969. Respiratory rates of green and black larvae of the army-worm, Leucania separata (Lepidoptera: Noctuidae) Appl. Entomol. Zool. 4:100-101.

Shlaifer, A. 1938. Studies in mass physiology: effect of numbers upon the oxygen consumption and locomotor activity of Carassius auratus. Physio. Zool. 11:408-424.

_____. 1939. An analysis of the effect of numbers upon the oxygen consumption of Carassius auratus. Physiol. Zool. 12:381-392.

Surtees, G. 1959. Influence of larval population density on fluctuation in mosquito numbers. Nature 183:269-270.

Terzian, L. A. and N. Stahler. 1949. The effects of larval population density on some laboratory characteristics of Anopheles quadrimaculatus, Say. J. Parasitol. 35: 487-495.

Trager, W. 1935. On the nutritional requirements of mosquito larvae. Amer. J. Hyg. 22:475-493.

Valente, D. 1943. O efeito de numero sobre o consumo de oxigenio por Crustaceos Decapodos. Bol. Zool. Sao Paulo Univ. 32:305-310.

Wada, Y. 1965a. Effect of larval density on the development of Aedes aegypti (L.) and the size of the adults. Quaest. Entomol. 1:223-249.

_____. 1965b. Overcrowding of Culex pipiens complex in relation to dispersal. World Health Organ. Vector Control. 125.65:119-125.

Wallis, R. C. 1954. The effect of population density and NaCl concentrations in test series in laboratory experiments with ovipositing Aedes aegypti. Mosq. News 14: 200-204.

Wang, L. 1938. A comparative study of the oxygen requirement of mosquito larvae. Chinese Med. J. Suppl. 2:487-493.

Wiegert, R. G. 1964. Population energetics of meadow spittlebugs (Philaenus spumarius L.) as affected by migration and habitat. Ecol. Monogr. 34:217-241.

Wiegert, R. G. and D. C. Coleman. 1970. Ecological significance of low oxygen consumption and high fat accumulation by Nasutitermes costalis (Isoptera: Termitidae) Bio Science 20:663-665.

Zeiss, F. R., Jr. 1963. Effects of population densities on zooplankton respiration rates. Limnol. Oceanogr. 8:110-115.

Zeuthen, E. 1953. Oxygen uptake as related to body size in organisms. Quart. Rev. Biol. 28:1-12.

Figure 1. The effects of increasing reaction vessel density on the respiratory rates of overcrowded and standard larvae of Aedes aegypti.

Population	Density Levels (Numbers/Vessel)				
Standard	15	<	25	<	35
Overcrowded	15	=	25	=	35

Figure 2. Comparison of respiratory rates of previously overcrowded or standard larvae exposed to various concentrations of growth retardant factors (GRF).

Population*	Experiment
Standard	
Trager's Solution = [X] - GRF	VI
(Trager's Solution = Extracted Trager's = [X] - GRF) ≠ [4X] - GRF	IX
Overcrowded	
(Extracted Trager's = [X] - GRF) ≠ ([4X] -GRF = [10X] -GRF)	X

*Note: Parenthesis denotes that as a group there is no significant difference.

Note: [X], [4X] and [10X]- GRF denote concentrations of growth retardant factors.

Figure 3. Scheme for the dynamics of compensation due to stress in Aedes aegypti.

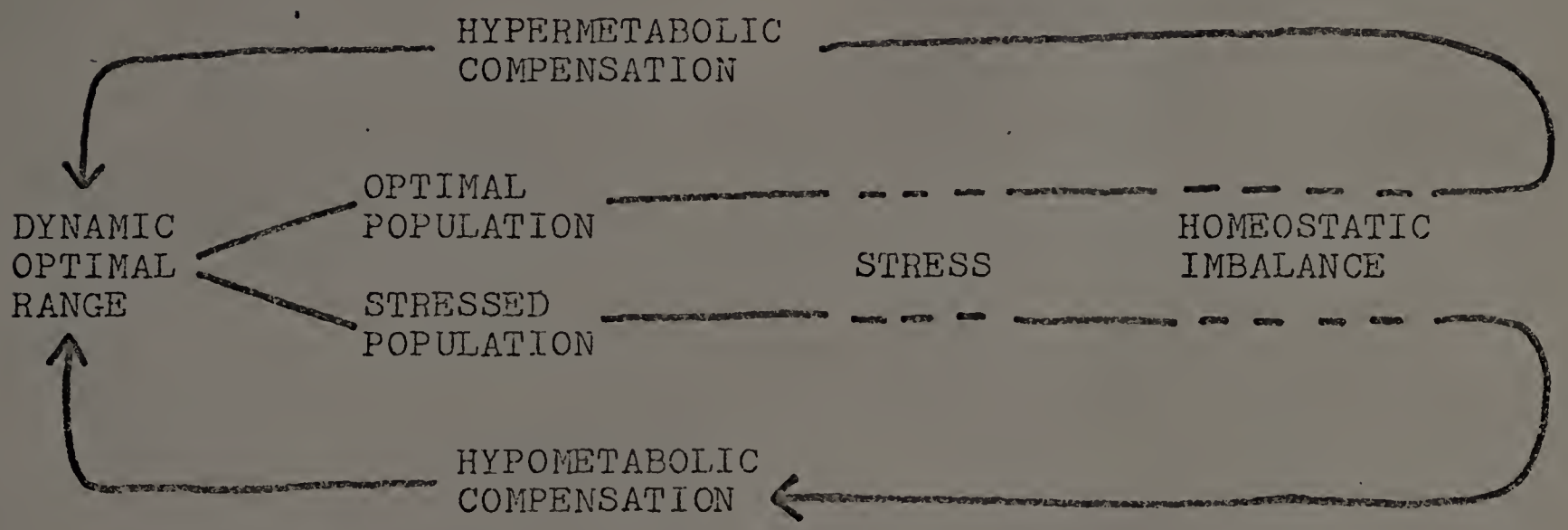
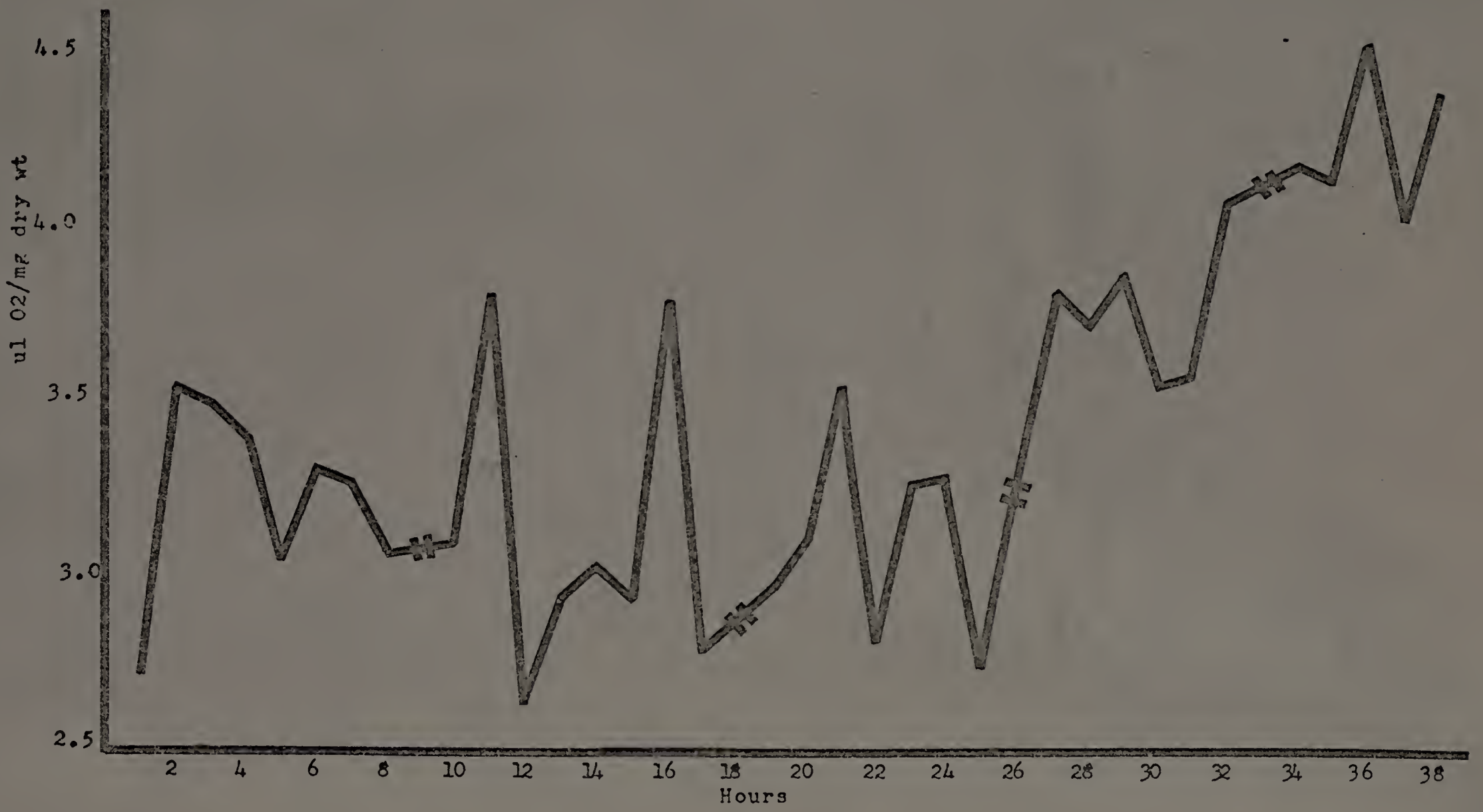


Figure 4. Summary of respiratory effects of starvation and overcrowding.

		Population		Respiratory Reaction	Experimental Source
Density Effects	Mechanical	Standard	---	Increase	V, VIII
		Overcrowded	---	None	
	Chemical	Standard	---	Increase	VI, IX, X
		Overcrowded	---	Increase	
Relative Respiratory Rates Due to Stress		Standard	---	High	
		Overcrowded	---	Low	IV
		Starved	---	Lower	
Sensitivity To Stress*		Standard	---	High	V
		Overcrowded	---	Low	VIII, XI

* This is reflected by shifts in respiratory rates.

Figure 5. Respiration of pupae from standard Aedes aegypti larvae.*



*Cross bars on lines indicate missing value due to instrument limitations.

APPENDIX A

Statistical Tables

Table 1. Coefficients for single degree of freedom orthogonal comparisons of treatment differences in Experiment III (Age of eggs and larval respiration).

Treatments	T_1	T_2	T_3	T_4
Means	90.900	81.020	86.389	99.849
Comparisons				
T_1 vs. T_4	1	0	0	-1
T_2 vs. T_3	0	1	-1	0
$(T_2 + T_3)$ vs. $(T_1 + T_4)$	-1	1	1	-1

Table 2. Least-squares analysis of variance for data in Experiment III (Age of eggs and larval respiration).

Source	df	SS	MS	F
Treatments (T)	3	11.916163	3.972054	12.689**
T_1 vs T_4	1	2.502643	2.502643	7.995*
T_2 vs T_3	1	0.900817	0.900817	2.877 n.s.
(T_2+T_3) vs (T_1+T_4)	1	8.51180	8.51180	27.193**
Vessels within Treatments (V:T)	12	3.756188	0.31301	
Hours (H)	3	1.269688	0.423229	21.199**
Interaction (TH)	9	1.222150	0.135794	6.802**
Error (HV:T)	36	0.718712	0.019964	

n.s. No significance
* Significance at $p < 0.05$
** Significance at $p < 0.01$

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Table 3. Duncan's multiple range tests for treatment and hourly means in Experiment III (Age of eggs and larval respiration).

		Treatments			
	Confidence Level	Sept. 20 T_2	Sept. 20 T_3	Sept. 5 T_1	Oct. 14 T_4
Mean	95	5.0637	5.3993	5.6812	6.2406
	99	5.0637	5.3993	5.6812	6.2406

	Hour**			
	H_1	H_2	H_3	H_4
Mean	5.834	5.542	5.541	5.466

** Significance at $p < 0.01$

Table 4. Least-squares analysis of variance for Experiment IV (Respiration of overcrowded, starved and standard larvae).

Source		df	SS	MS	F
Treatment	(T)	2	42.472153	21.236076	28.081949**
Vessels: Treatment	(V:T)	15	11.343270	0.756218	
Hour	(H)	3	8.233915	2.744638	45.597**
Treatment X Hour Interaction	(TH)	6	28.146981	4.691163	77.936**
Error		45	2.708679	0.060193	

** Significance at $p < 0.01$

Table 5. Duncan's multiple range tests for treatment and hourly means in Experiment IV (Respiration of overcrowded, starved and standard larvae).

	Treatment**		
	T_1 (Standard)	T_2 (Overcrowded)	T_3 (Starved)
Mean	8.231	7.304	6.350

		Hour			
	Confidence Level	H_1	H_2	H_4	H_3
Mean	95	6.762	7.262	7.498	7.657
	99	6.762	7.262	7.498	7.657

** Significance at $p < 0.01$

Table 6. Least-squares analysis of variance for data in Experiment V (Vessel density effects - standard).

Source		df	SS	MS	F
Treatment	(T)	2	9.454579	4.727290	41.69973**
Vessels: Treatment	(V:T)	9	1.020288	0.113365	
Hours	(H)	3	8.787767	2.929256	19.647**
Treatment X Hour Interaction	(TH)	6	28.895371	4.815895	32.302**
Error		27	4.025462	0.149091	

** Significance at $p < 0.01$

Table 7. Duncan's multiple range tests for treatment and hourly means in Experiment V (Vessel density effects - standard).

		Treatment		
	Confidence Level	T_1 (15 larvae/ vessel)	T_2 (25 larvae/ vessel)	T_3 (35 larvae/ vessel)
Mean	95	6.908	7.075	7.921
	99	6.908	7.075	7.921

		Hour			
		H_1	H_2	H_3	H_4
Mean	95	7.992	7.214	7.180	6.819
	99	7.992	7.214	7.180	6.819

Table 8. Least-squares analysis of variance for Experiment VI (GRF on standard larvae).

Source		df	SS	MS	F
Treatment	(T)	1	0.386123	0.386123	1.544918 n.s.
Vessels: Treatment	(V:T)	8	1.999450	0.249931	
Hours	(H)	3	0.819028	0.273009	4.474*
Treatment X Hour Interaction	(T_H)	3	2.879427	0.959809	15.730**
Error		24	1.464470	0.061020	

n.s. No Significance
* Significance at $p<0.05$
** Significance at $p<0.01$

Table 9. Least-squares analysis of variance and Duncan's multiple range tests for hours in Experiment VII (Changed versus unchanged medium).

ANOVA

Source		df	SS	MS	F
Treatment	(T)	1	0.045630	0.045630	0.031309 n.s.
Vessels: Treatment	(V:T)	8	11.659066	1.457383	
Hours	(H)	2	6.635180	3.317590	34.621**
Treatment X Hour Interaction	(TH)	2	0.103940	0.051970	0.542 n.s.
Error		16	1.533213	0.095826	

Duncan's multiple range test**

	Hour		
	H_2	H_3	H_1
Mean	5.060	4.816	3.963

n.s. No Significance
** Significance at $p<0.01$

Table 10. Least-squares analysis of variance and Duncan's multiple range tests for hours in Experiment VIII (Vessel density effect - overcrowded).

ANOVA

Source		df	SS	MS	F
Treatment	(T)	2	1.058147	0.529074	1.206485 n.s.
Vessels: Treatment	(V:T)	13	5.759511	0.438525	
Hours	(H)	2	15.264326	7.632163	90.151**
Treatment X Hour Interaction	(TH)	4	2.601368	0.650342	7.682**
Error		26	2.201149	0.084660	

Duncan's multiple range test**

	Hour		
	H_1	H_2	H_3
Mean	3.285	4.478	4.493

n.s. No Significance
** Significance at $p<0.01$

Table 11. Least-squares analysis of variance for Experiment IX (GRF on standard larvae).

Source		df	SS	MS	F
Treatment	(T)	3	3.181727	1.060576	3.632146*
Vessels: Treatment	(V:T)	16	4.448974	0.291997	
Hours	(H)	2	17.522080	8.761040	249.428**
Treatment X Hour Interaction	(TH)	6	0.123733	0.020622	0.587 n.s.
Error		32	1.123987	0.035125	

n.s. No Significance
* Significance at $p<0.05$
** Significance at $p<0.01$

Table 12. Duncan's multiple range test for treatment and hourly means in Experiment IX (GRF on standard larvae).

	Treatment**			
	T_1 (Plain Trager's)	T_4 (Trager's Extract)	T_3 (X-GRF)	T_2 (4X-GRF)
Mean	6.5153	6.5326	6.5486	7.0633

	Hour**		
	H_1	H_2	H_3
Mean	5.943	6.809	7.243

** Significance at $p<0.01$

Table 13. Least-squares analysis of variance for Experiment X (GRF on overcrowded larvae).

Source		df	SS	MS	F
Treatment	(T)	3	17.948942	5.982981	13.686553**
Vessel: Treatment	(V:T)	12	5.245717	0.437143	
Hours	(H)	2	24.424879	12.212440	212.717**
Treatment X Hour Interaction	(TH)	6	1.246571	0.207762	3.619*
Error		24	1.377883	0.057412	

* Significance at $p<0.05$
** Significance at $p<0.01$

Table 14. Duncan's multiple range test for treatment and hourly means in Experiment X (GRF on overcrowded larvae).

	Treatment**			
	T_1 (Trager's)	T_2 (X-GRF)	T_3 (4X-GRF)	T_4 (10X-GRF)
Mean	3.020	3.455	4.284	4.521

	Hour**		
	H_1	H_2	H_3
Mean	2.998	3.704	4.745

** Significance at $p<0.01$

Table 15. Least-squares analysis of variance and Duncan's multiple range tests for hours in Experiment XI (Respiration of three overcrowded density levels).

ANOVA

Source		df	SS	MS	F
Treatment	(T)	2	2.624916	1.312458	0.675782 n.s.
Vessel: Treatment	(V:T)	15	29.131980	1.942132	
Hours	(H)	2	29.475875	14.737937	127.918**
Treatment X Hour Interaction	(TH)	4	2.897366	0.724341	6.287**
Error		30	3.456423	0.115214	

Duncan's multiple range test**

	Hour		
	H_1	H_2	H_3
Mean	2.720	3.747	4.542

n.s. No Significance
** Significance at $p<0.01$

APPENDIX B

Procedural Example of Respiratory Experiment

Example: Experiment IV

Goal

To compare the respiratory activity of standard, overcrowded and starved populations.

Rearing

Procedures for rearing the three populations are available in Materials and Methods.

Respirometry

a) Procedures for the preparation of vessels and mosquitoes are available in Materials and Methods section.

b) Intervals at which respirometric readings are taken is dependent upon the magnitude of oxygen uptake. Fifteen minutes is usually an appropriate interval. If oxygen uptake is very small readings may be taken at one hour intervals.

Respirometric Readings*

Overcrowded

ul O_2/hr./15 larvae

Hour	1	2	3	4	5	6
1	217.2	212.4	215.4	217.4	217.0	218.4
2	239.4	230.0	235.2	238.4	239.4	240.8
3	264.8	251.3	258.7	263.6	266.4	266.2
4	291.0	273.5	282.3	288.4	293.0	291.2

Standard

ul O_2/hr./15 larvae

Hour	1	2	3	4	5	6
1	258.2	254.6	259.0	361.0	262.4	258.0
2	317.0	310.8	317.5	320.8	323.6	314.1
3	370.0	363.7	377.2	376.9	381.6	369.0
4	424.6	416.8	426.8	433.0	439.4	421.8

Starved

ul O_2/hr./15 larvae

Hour	1	2	3	4	5	6
1	216.9	217.2	239.2	215.4	217.8	217.4
2	233.5	234.9	258.0	230.3	237.0	234.0
3	251.2	254.2	278.2	245.8	255.4	253.2
4	268.6	271.0	297.2	260.4	272.7	270.1

* Initial micrometer setting at 200.0

Dry Weight (in mg.) per replicate (15 larvae/vessel)

Population Replication	Weight Standard	Overcrowded	Starved
1	6.9	3.1	3.0
2	6.4	2.5	3.1
3	6.8	2.8	2.9
4	7.0	3.3	2.4
5	7.4	2.9	2.9
6	7.0	3.2	2.3

Computer Program: Conversion to ul O_2/mg/hr.

```
Name    Mosq.
List
05  Print "UL/MG"
10  Read A, B, C, D, E, F
20  LET  G = A - B
30  LET  H = B - C
40  LET  I = C - D
50  LET  J = D - E
60  PRINT
70  LET  K = INT ((G/F)*100+ .5)/100
80  LET  L = INT ((H/F)*100+ .5)/100
90  LET  M = INT ((I/F)*100+ .5)/100
100 LET  N = INT ((J/F)*100+ .5)/100
110 PRINT K, L, M, N
120 Go to 05
200 END
```

Procedure for data input

```
Name    DATA
OK
USE  BASIC
OK
DATA
RUN DATA AS MOSQ
```

Print Out

ul/mg dry wt./hr.

Overcrowded

Vessel	Hour 1	Hour 2	Hour 3	Hour 4
1	5.55	7.16	8.16	8.45
2	4.96	7.04	8.52	8.88
3	5.50	7.07	8.39	8.43
4	6.00	7.24	8.69	8.55
5	5.31	7.00	8.44	8.31
6	5.58	6.79	7.70	7.58

Standard

1	8.49	8.46	7.68	7.91
2	8.53	8.78	8.27	8.30
3	8.67	8.60	8.04	8.03
4	8.71	8.54	8.01	8.01
5	8.43	8.27	7.84	7.81
6	8.78	8.01	7.84	7.54

Starved

1	5.63	5.53	5.90	5.80
2	5.55	5.71	6.23	5.42
3	5.90	6.48	6.97	6.55
4	6.42	6.21	6.46	6.08
5	6.14	6.62	6.34	5.97
6	7.57	7.22	8.35	7.35

Statistical Analysis - See Tables 4 and 5.

APPENDIX C

Raw Data

Experiments I and II

No calculations were made due to the nature of the experiments.

Experiment III

	ul O_2/mg. dry wt./hr.				Treatment
Vessel	Hr. 1	Hr. 2	Hr. 3	Hr. 4	
1	6.11	5.55	5.55	5.58	T_1
2	6.10	5.48	5.79	5.78	
3	6.04	5.43	5.52	5.59	(Sept. 5)
4	5.91	5.40	5.50	5.57	
5	5.05	5.08	5.24	4.80	T_2
6	4.89	5.28	5.18	4.85	
7	5.09	5.14	5.03	5.14	(Sept. 20)
8	5.16	5.01	4.98	5.10	
9	5.36	4.85	5.45	5.10	T_3
10	5.40	5.02	5.35	5.03	
11	5.73	5.32	5.44	5.31	(Sept. 20)
12	6.06	5.81	5.94	5.22	
13	6.82	6.63	6.01	6.41	T_4
14	7.22	6.94	6.48	6.32	
15	5.85	5.58	5.38	5.65	(Oct. 14)
16	6.56	6.16	5.82	6.02	

Experiment IV

See Appendix B.

Experiment V

Vessel	ul O2/mg. dry wt. Hr. 1	Hr. 2	Hr. 3	Hr. 4	Treatment
1	6.53	7.28	7.36	7.01	T_1
2	6.85	7.46	7.33	6.69	
3	6.21	6.85	7.09	6.58	(15 larvae/
4	6.32	7.06	7.12	6.79	vessel)
5	7.24	7.41	7.26	6.89	T_2
6	7.12	7.35	7.27	6.82	
7	6.56	7.07	7.26	6.88	(25 larvae/
8	7.08	7.21	7.08	6.70	vessel)
9	9.00	7.62	7.35	7.21	T_3
10	11.20	7.16	7.01	6.68	
11	10.43	6.95	6.91	6.66	(35 larvae/
12	11.37	7.15	7.13	6.92	vessel)

Experiment VI

Vessel	ul O2/mg. dry wt./hr. Hr. 1	Hr. 2	Hr. 3	Hr. 4	Treatment
1	6.22	6.61	6.46	6.44	T_1
2	6.47	6.41	6.82	6.84	
3	6.66	7.08	7.06	7.05	(Trager's
4	6.65	7.21	7.09	7.24	Solution)
5	6.89	6.00	6.90	6.74	
6	7.68	7.36	6.94	6.73	T_2
7	7.48	7.01	6.68	6.94	
8	7.47	7.11	6.74	6.93	([X] - Con-
9	7.55	7.28	6.42	6.27	centration GRF)
10	7.36	7.00	5.91	5.91	

Experiment VII

Vessel	ul O_2/mg. dry wt./hr. Hr. 1	Hr. 2	Hr. 3	Treatment
1	4.92	6.83	5.71	T_1
2	3.26	4.29	3.71	
3	3.79	4.54	4.43	(Changed
4	3.34	4.34	4.19	medium)
5	4.52	5.91	6.00	
6	3.73	4.40	4.40	T_2
7	4.17	4.93	4.90	
8	3.44	4.85	4.67	(Unchanged
9	4.71	5.14	4.86	medium)
10	3.75	5.37	5.29	

Experiment VIII

Vessel	ul O_2/mg. dry wt./hr. Hr. 1	Hr. 2	Hr. 3	Treatment
1	3.21	5.29	4.82	T_1
2	2.96	4.35	4.23	
3	3.71	5.00	4.71	(15 larvae/
4	2.85	4.62	4.69	vessel)
5	2.88	5.12	5.92	
6	2.16	4.08	4.56	
7	4.05	4.73	4.82	T_2
8	3.41	4.11	4.39	
9	2.84	3.84	3.72	(25 larvae/
10	2.59	4.33	4.51	vessel)
11	2.78	3.87	4.09	
12	3.32	4.18	4.00	T_3
13	3.38	4.28	4.06	
14	4.08	4.65	4.54	(35 larvae/
15	3.89	4.67	4.63	vessel)
16	4.13	4.80	4.53	

Experiment IX

	ul O_2/mg. dry wt./hr.			Treatment
Vessel	Hr. 1	Hr. 2	Hr. 3	
1	5.72	6.98	7.16	T_1
2	6.08	6.93	7.12	
3	6.51	6.94	7.31	(Trager's
4	5.38	6.38	6.92	Solution)
5	5.22	6.30	6.78	
6	6.20	7.08	7.73	T_2
7	6.65	7.39	7.81	
8	6.68	7.29	7.85	([4X] -GRF)
9	6.28	7.17	7.58	
10	5.85	6.92	7.47	
11	5.02	6.22	6.67	T_3
12	6.45	6.72	7.27	
13	5.75	6.71	7.45	([X] -GRF)
14	6.30	7.13	7.65	
15	5.57	6.39	6.93	
16	5.87	6.87	7.35	T_4
17	5.87	6.72	7.21	
18	5.48	6.75	6.53	(Extracted
19	6.14	6.63	6.88	Trager's
20	5.84	6.66	7.19	Solution)

Experiment X

Vessel	ul O_2/mg. dry wt./hr. Hr. 1	Hr. 2	Hr. 3	Treatment
1	2.39	2.26	3.87	T_1
2	2.11	2.47	4.03	
3	2.70	2.89	4.35	(Trager's
4	2.42	2.61	4.03	Solution)
5	2.20	2.70	3.73	
6	2.65	3.30	4.19	T_2
7	2.86	3.03	4.65	
8	2.25	3.31	4.44	([X] -GRF)
9	2.66	3.50	4.62	
10	2.47	3.00	4.29	
11	3.81	4.46	5.73	T_3
12	3.53	4.68	5.22	
13	2.97	4.58	5.10	([4X] -GRF)
14	3.74	4.85	5.59	
15	3.56	4.23	4.64	T_4
16	3.65	4.30	5.54	
17	3.10	3.27	3.92	([10X] -GRF)
18	4.03	5.00	6.17	
19	3.35	5.00	5.38	

Experiment XI

Vessel	ul O_2/mg. dry wt./hr. Hr. 1	Hr. 2	Hr. 3	Treatment
1	2.94	3.97	4.91	T_1
2	2.59	3.68	4.16	
3	4.36	4.82	5.44	(400/80ml)
4	2.90	3.48	4.48	
5	2.84	3.74	4.44	
6	2.94	3.22	3.82	
7	3.57	4.24	4.52	
8	2.20	2.57	3.46	T_2
9	3.23	4.26	5.03	
10	2.25	3.12	3.94	
11	2.58	3.33	3.85	(640/80ml)
12	3.03	3.46	3.97	
13	2.56	3.21	4.53	
14	2.11	3.86	4.75	T_3
15	2.46	4.21	5.11	
16	2.73	4.57	5.70	(1280/80ml)
17	1.79	4.79	4.79	
18	2.70	2.76	4.45	

Experiment XII

ul O_2/mg. dry wt./hr.

Replication

Hour	1	2	3	4	5	6	7	8	9	10
1	2.55	3.13	2.77	2.59	2.59	2.76	2.97	2.68	2.75	2.32
2	3.51	4.02	3.68	3.52	3.19	3.43	3.73	3.57	3.43	3.21
3	3.39	3.93	3.73	3.40	3.05	3.36	3.88	3.53	3.45	3.12
4	3.47	4.00	3.60	3.43	3.04	3.29	3.58	3.31	3.23	2.98
5	2.88	3.49	3.23	2.96	2.73	2.96	3.30	3.16	2.98	2.82
6	3.26	3.74	3.57	3.23	2.79	3.25	3.56	3.29	3.28	3.06
7	3.13	3.77	3.37	3.15	2.98	3.13	3.60	3.29	3.21	3.06
8	3.09	3.56	3.33	3.03	2.60	3.00	3.21	3.02	2.87	2.84
9	2.02	2.43	2.15	2.17	1.90	2.17	2.40	2.12	2.18	1.94
10	3.01	3.62	3.49	3.08	2.74	3.00	3.23	3.02	3.03	2.69
11	3.91	4.54	4.07	3.71	3.38	3.64	3.98	3.83	3.59	3.39
12	2.62	3.11	2.70	2.59	2.21	2.64	2.80	2.73	2.55	2.42
13	2.91	3.29	3.18	2.98	2.60	2.82	3.11	2.89	2.83	2.72
14	2.98	3.55	3.24	2.92	2.72	2.93	3.29	3.08	2.97	2.63
15	3.04	3.31	3.11	2.83	2.51	2.86	3.11	2.98	2.91	2.75
16	3.72	4.38	4.13	3.82	3.27	3.76	4.05	3.90	3.51	3.25
17	2.77	3.17	3.08	2.68	2.46	2.63	2.97	2.87	2.80	2.47
18	1.52	1.90	1.79	1.73	1.56	1.95	2.02	1.51	1.79	1.68
19	2.96	3.26	3.05	2.88	2.60	2.93	3.32	3.04	2.92	2.69
20	3.13	3.49	3.40	3.05	2.72	3.03	3.23	3.17	3.00	2.83
21	3.40	3.99	3.71	3.34	3.08	3.48	3.86	3.67	3.54	3.32
22	2.85	3.19	3.02	2.82	2.47	2.80	3.07	2.81	2.65	2.60
23	3.14	3.73	3.45	3.22	2.83	3.25	3.47	3.31	3.21	2.98
24	3.24	3.73	3.64	3.13	2.85	3.11	3.54	3.39	3.20	3.03
25	2.69	3.13	2.92	2.78	2.46	2.77	2.88	2.73	2.65	2.42
26	2.39	2.94	2.61	2.83	2.15	2.82	2.53	2.54	2.23	1.91
27	3.63	4.39	4.13	3.74	3.32	3.79	4.04	3.83	3.70	3.40
28	3.77	4.25	3.95	3.68	3.19	3.64	3.95	3.74	3.68	3.31
29	3.71	4.40	4.14	3.88	3.31	3.86	4.13	4.00	3.68	3.45
30	3.51	4.01	3.89	3.40	3.08	3.51	3.77	3.50	3.43	3.35
31	3.41	4.10	3.76	3.46	3.15	3.60	3.91	3.73	3.49	3.23
32	3.94	4.43	4.44	3.93	3.52	4.00	4.43	4.21	4.01	3.67
33	3.16	3.54	3.54	3.22	2.87	3.34	3.63	3.22	3.19	2.97
34	4.06	4.44	4.44	4.02	3.63	4.01	4.48	4.13	4.05	3.83
35	4.00	4.37	4.37	4.01	3.53	4.02	4.46	4.16	3.93	3.71
36	4.37	4.80	4.80	4.26	3.79	4.35	4.77	4.54	4.28	4.24
37	3.87	4.36	4.36	4.01	3.59	3.88	4.42	4.04	3.88	3.59
38	4.24	4.69	4.69	4.22	3.94	4.29	4.73	4.52	3.97	4.28

Experiment XIII

ul O2/mg. dry wt./hr.

Hour	Replication 1	2	3
1	2.14	1.67	1.75
2	5.20	4.36	4.56
3	3.59	3.43	3.75
4	4.69	4.00	4.75
5	5.59	5.03	5.03
6	5.28	4.40	4.94
7	3.83	4.10	3.81
8	3.93	3.60	3.59
9	4.34	4.20	4.56
10	5.07	3.90	4.37
11	4.07	3.77	4.16
12	4.66	4.60	4.41
13	3.66	3.07	3.38
14	3.48	3.40	3.44
15	3.62	2.87	3.00
16	3.97	3.83	3.62
17	4.38	3.93	4.31
18	4.69	4.00	3.87
19	4.62	4.30	4.25
20	5.93	5.30	5.03
21	4.07	3.73	3.72
22	4.93	4.27	4.25
23	4.48	4.17	4.09
24	4.34	4.13	4.16
25	3.72	3.33	3.44
26	5.21	5.00	4.75
27	4.41	4.17	3.91
28	2.72	2.87	3.91
29	3.55	3.63	3.44
30	4.10	4.33	3.91
31	4.38	4.33	4.22
32	4.14	4.00	3.84
33	4.97	4.83	4.37
34	4.14	4.30	4.37
35	5.17	5.10	4.81
36	4.76	4.53	4.37
37	4.28	4.40	4.12
38	6.34	6.16	5.81