THE ENERGETIC COSTS OF DIVING IN *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* PUPAE

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ABSTRACT. Undisturbed mosquito pupae rest at the water surface and respond to passing shadows or vibrations by diving. Pupae do not feed and rely solely on energy stored from the larval stage. The ability of a newly emerged adult mosquito to survive, and therefore to transmit disease, depends on these energy reserves. Earlier studies of diving behavior in *Aedes aegypti, Ae. albopictus,* and *Ae. triseriatus* pupae provided evidence that pupae sense their state of buoyancy and modify their diving behavior accordingly. With strong stimulation pupae tend to dive to a depth where they become neutrally or negatively buoyant and commonly rest on the bottom. This behavior, as well as the tendency to rest when not disturbed, may logically be viewed as energy-conserving. The results of these studies also generated the hypothesis that the diving behavior displayed by these container-breeding mosquitoes helps them avoid being washed from their container by overflowing water during rainfall. Rainfall stimulates diving and logically, prolonged, heavy rainfall stimulates excessive diving, a likely drain on energy reserves. Our objectives were to determine, in *Ae. aegypti* and *Ae. albopictus,* the energetic costs associated with resting behavior, with frequent diving, and with buoyancy reduction. Using survival rate, mean survival after adult emergence, and measurement of total calories, we found a clear energetic cost associated with frequent diving. In contrast, relative to diving, essentially no energy cost was associated with buoyancy reduction, that is, pupae behave in response to variations in buoyancy in a way that does not impact significantly on energy reserves.

KEY WORDS   Diving behavior, rainfall, pupa, energy use, *Aedes aegypti, Aedes albopictus*

INTRODUCTION

Mosquito pupae are positively buoyant because of the presence of gas in the ventral air space, an external cavity formed by the developing legs, wings, and mouthparts (Hurst 1890, Christophers 1960). Undisturbed pupae rest quietly at the water surface and in response to a passing shadow or vibrations, they dive. At the surface and while diving, *Aedes aegypti* (L.) pupae alternate between being slightly less dense to slightly more dense than water (Romoser 1975). Buoyancy varies inversely with time submerged and depth of dive, directly with temperature and ventral air space gas volume, and periodically while resting at the surface (Romoser 1975, 1978; Romoser and Nasci 1978).

Studies of the effects of artificial buoyancy reduction in *Ae. aegypti, Aedes albopictus* (Skuse), and *Aedes triseriatus* (Say) showed that pupae are able to sense their state of buoyancy and behave accordingly (Romoser and Lucas 1999). In all 3 species, normally buoyant pupae dive to greater depths and for longer periods of time than do reduced-buoyancy pupae. With strong stimulation, that is the stimulation associated with the transfer of a pupa from 1 container to another, the majority of both normal and reduced-buoyancy pupae dive to a depth at which they became neutrally or negatively buoyant and when in shallow containers, tend to rest on the bottom.

According to Romoser (1975)

Since pupal mosquitoes do not feed, the time spent in this stage constitutes a considerable drain on the energy stores accumulated during the larval stage. In addition to energy expended in maintenance of the life processes, energy must be expended during the profound tissue reorganization taking place. Further, mosquito pupae, unlike the pupae of most other holometabolous insects, are highly motile and depending upon circumstances, may be quite active. Given these categories of energy drain and realizing that the newly emerged adult mosquito must fly to a sugar or blood source before there can be any renewal of energy stores, any energy-conserving mechanism during the pupal period would have survival value.

Behavior that involves resting quietly at the air–water interface, or when diving, maximizing the time spent in a neutrally (hovering) or negatively buoyant state (including bottom-sitting) can logically be viewed as energy-conserving.

Romoser and Lucas (1999) suggested that the diving behavior displayed by pupae of *Ae. aegypti, Ae. albopictus,* and *Ae. triseriatus* represents an adaptation that helps keep them from being washed from their container habitat by overflowing water during prolonged, heavy rainfall. It follows that rainfall stimulates diving and that prolonged, heavy rainfall would stimulate frequent diving, which, in turn, could be expected to be associated with a drain on the pupa's energy reserves.

The research reported here has focused on *Ae. aegypti* and *Ae. albopictus.* Our objectives have been to determine the energetic costs associated with resting, with frequent diving, and with buoyancy reduction.

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MATERIALS AND METHODS

Rearing methods: Eggs of *Ae. aegypti* (Rockefeller strain) and *Ae. albopictus* were obtained from the U.S. Army Medical Research Institute of Infectious Diseases (Fort Detrick, Frederick, MD) and the Centers for Disease Control and Prevention (Fort Collins, CO), respectively. Larvae and pupae were raised under 16-h light: 8-h dark at 27 ± 1°C in 22 × 32-cm plastic pans containing 1,000 ml of water. Experiments were conducted at 27 or 23 ± 1°C. Larvae were reared on ground lab chow, liver powder, and live brewer's yeast (1:1:1). Approximately 100 mg of food was added to each pan of 150–160 larvae daily. Only female mosquitoes were used in these studies.

Experimental design: Duration of survival after adult emergence and total caloric expenditure were used as indirect and direct measures of energy stores, respectively, and were compared among the following 4 groups: normal pupae that were undisturbed throughout the pupal period (unstressed); normal pupae that were repeatedly stimulated to dive (stressed); pupae with reduced buoyancy that were undisturbed (unstressed); and pupae with reduced buoyancy that were repeatedly stimulated to dive (stressed) to the same extent as those in the normally buoyant, stressed group. Samples of normal and reduced positively buoyant pupae to be stressed were stimulated to dive by sharply tapping the container with the handle of a screwdriver at 5-min intervals for 10 h (120 dives). Pupae invariably dive in response to the tapping stimulus if they have come to rest at the surface after the preceding dive. Samples of normal and reduced positively buoyant pupae to be held at rest (unstressed) were transferred to identical tubes, in an identical water bath, and left undisturbed. Pupae with reduced ventral air space gas volume and hence reduced buoyancy were obtained as outlined in Romoser and Lucas (1999).

Survival studies: Post–adult emergence survival studies were done using only *Ae. aegypti*. To obtain the 4 experimental groups for determination of survival duration at approximately 23°C (ambient temperature in the laboratory), normal and reduced positively buoyant pupae, less than 3 h old, were placed individually in test tubes containing 10 cm of water. The tubes, in a rack, were placed in a plastic water bath (24 × 32 × 18 cm) containing 10 cm of water.

In a 2nd experiment involving determination of survival of adults after emergence at 27 ± 1°C, the 4 experimental groups described above were obtained in a different way. Stressed pupae were obtained by stimulating groups of normal or reduced-buoyancy pupae to dive in a Pyrex® column (170 cm deep × 3.8 cm diameter) (Corning Labware, Corning, NY) at 5-min intervals for 10 h (120 dives). To obtain unstressed groups, normal and re-

duced-buoyancy pupae were placed in an identical column and remained undisturbed.

To measure post–adult emergence survival, a pupa, after a given treatment, was held at 27 ± 1°C in a plastic vial (12 cm long × 1.5 cm diameter) containing 25 ml of water. Each vial was placed in an individual cardboard cage (9 cm deep × 8.5 cm diameter with a screen lid). Cages were numbered, coded, and mixed by an assistant, and placed in 50-gallon trash bags along with 4 moist sponges. Gauze pads saturated with water also were placed on the top of each cage and were replaced daily. Pupae were checked hourly for adult emergence. After adult emergence, survival was checked at 4-h intervals until all of the mosquitoes starved to death.

Measurement of energy expenditure: Caloric expenditure was compared in each of the species studied among the same 4 experimental groups as described for the 2nd experiment under *Survival studies,* plus 2 other groups (normal and reduced positively buoyant pupae within 1 h after pupation). After treatments, pupae were placed in individual cages and monitored hourly for adult emergence. Within 1 h after adult emergence, the mosquitoes were placed in Beem® capsules (BioQuip Products, Gardena, CA) and frozen at −90°C. The pupal and adult holding temperature was 27 ± 1°C.

The total caloric content of each mosquito in each experimental group was measured directly by the method of Van Handel (1988). One liter of concentrated sulfuric acid was heated to 70°C and 20 g of sodium bichromate was slowly added. The solution was allowed to cool and sit for several days and then decanted into a clean bottle. Mosquitoes were removed from the Beem capsules and placed individually in 16 × 100-mm test tubes that were then placed in a Thermodyne block heater. One milliliter of the reagent was added to each tube and the tubes were heated to 110 ± 5°C for 15 min. The tubes were then allowed to cool to room temperature and 1.5 ml of distilled water was added. The acid solution of bichromate oxidizes the proteins, lipids, carbohydrates, and chitin to CO_2, whereas Cr VI is reduced to Cr III ion. Optical density (OD) was measured at 620 nm against a reagent blank in a LKB Spectro II spectrophotometer in 12-mm square cuvettes. The color produced by bichromate oxidation of 1 mg of sugar (i.e., 0.1 ml of 1% sucrose solution) was used as a standard and is equivalent to 4 calories. The OD produced by 1 mg of sucrose was 0.378; therefore, 0.095 (i.e., 0.378/4) was taken as equivalent to 1 calorie. Some of the tubes containing *Ae. albopictus* turned green after heating, indicating that oxidation of the mosquito was incomplete (Van Handel 1988). To assure completion of oxidation, an additional 1 ml of reagent was added to each tube and 3 ml, rather than 1.5 ml, of distilled water was added to each tube. In these cases the OD was doubled to calculate the calories present in each mosquito.
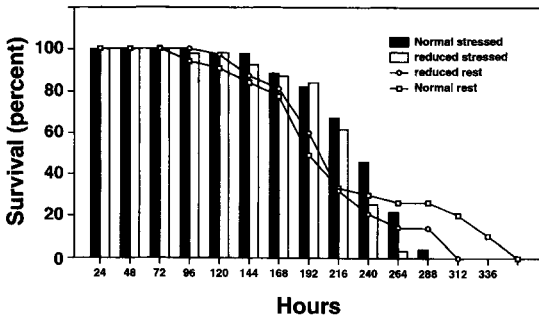
Fig. 1. Percent survival of *Aedes aegypti* in 24-h intervals after adult emergence at 23°C. Sample sizes: normal stressed, 36; reduced stressed, 31; reduced rest, 30; normal rest, 33.

Fig. 3. Percent survival of *Aedes aegypti* in 31-h intervals after adult emergence at 27°C. Sample sizes: normal stressed, 84; reduced stressed, 62; reduced rest, 65; normal rest, 84.

Data analysis: Data were analyzed according to methods outlined in Sokal and Rohlf (1969). Tests of homogeneity of variances were performed on each data set to determine if parametric analysis was appropriate. Where appropriate, analysis of variance was performed. Multiple comparisons tests (Student–Newman–Kuels multiple range test and Tukey's honestly significant difference test) were performed when analysis of variance revealed significant interaction among variables. Mantel–Haenszel comparison of survival curves was used to produce the resultant chi-square value.

RESULTS

Survival rates of adult *Ae. aegypti* at 24-h intervals and mean survival after adult emergence as a function of various treatments of pupae at 23°C are summarized in Figs. 1 and 2, respectively. A Mantel–Haenszel comparison of survival curves showed that the rate of survival was significantly higher among adults from resting pupae than among those repeatedly stimulated to dive (stressed) regardless of the state of buoyancy ($\chi^2 = 8.62$, df = 1, $P = 0.035$). Likewise, survival rates

among adults from stressed pupae, normal versus reduced buoyancy, were not significantly different. The survival rates of adults from normally buoyant pupae held at rest (unstressed) were significantly higher than the survival rates of adults from reduced buoyancy pupae held at rest ($\chi^2 = 5.0927$, df = 1, $P = 0.024$).

Mean survival of adults from pupae (± standard error) held at rest, from normal and reduced buoyancy groups, was 214.5 ± 9.5 and 196.5 ± 6.4 h, respectively. Mean survival of adults from stressed pupae, normal and reduced buoyancy groups, was 179.0 ± 8.7 and 166.9 ± 10.8 h, respectively. Adults from pupae held at rest survived significantly longer than stressed pupae regardless of their buoyancy (F = 13.214, df = 1, $P < 0.01$). No significant difference was found in the mean survival of adults from normal versus reduced-buoyancy pupae whether held at rest or stressed.

Survival rates of *Ae. aegypti* at 31-h intervals and mean survival after adult emergence at 27°C are summarized in Figs. 3 and 4. Adults from pupae held at rest survived significantly longer ($\chi^2 = 164.45$, df = 3, $P < 0.01$) than adults from stressed
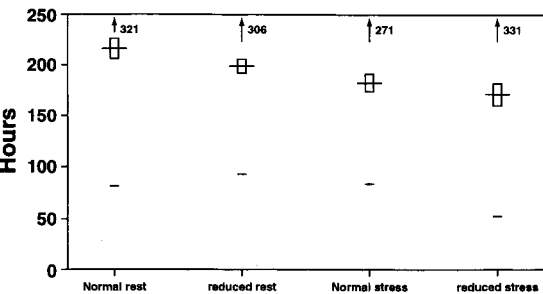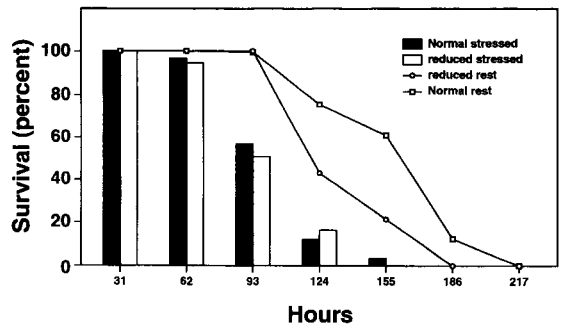

Fig. 2. Survival of *Aedes aegypti* after adult emergence at 23°C. Horizontal lines through vertical bars, mean survival; vertical bars, standard error; horizontal dashes, range (upper limit next to arrow). Sample sizes: normal rest, 33; reduced rest, 30; normal stressed, 36; reduced stressed, 31.
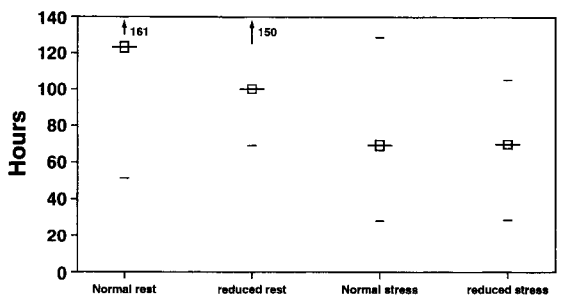

Fig. 4. Survival of *Aedes aegypti* after adult emergence at 27°C. Horizontal lines through vertical bars, mean survival; vertical bars, standard error; horizontal dashes, range (upper limit next to arrow). Sample sizes: normal rest, 84; reduced rest, 65; normal stressed, 84; reduced stressed, 62.
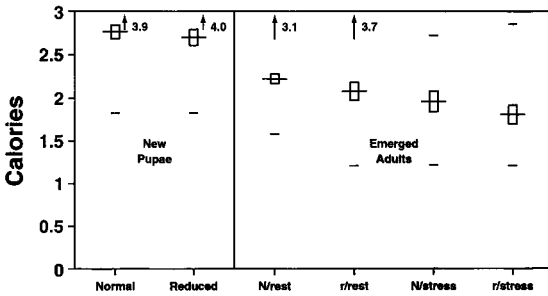
Fig. 5. Calories present in *Aedes aegypti* in new pupae and adults within 1 h after emergence. Horizontal lines through vertical bars, mean survival; vertical bars, standard error; horizontal dashes, range (upper limit next to arrow). New pupae (sample sizes): Normal, normal buoyancy (44); Reduced, reduced buoyancy (41). Newly emerged adults (sample sizes): N/rest, from normally buoyant pupae held at rest (34); r/rest, from reduced buoyancy pupae held at rest (34); N/stress, from normally buoyant pupae repeatedly stimulated to dive (16); r/stress, from reduced buoyancy pupae repeatedly stimulated to dive (17).
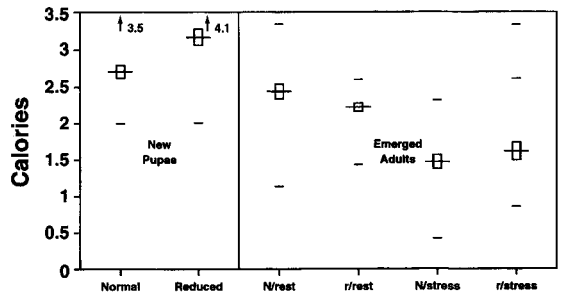
Fig. 6. Calories present in *Aedes albopictus* in new pupae and adults within 1 h after emergence. Horizontal lines through vertical bars, mean survival; vertical bars, standard error; horizontal dashes, range (upper limit next to arrow). New pupae (sample sizes): Normal, normal buoyancy (24); Reduced, reduced buoyancy (18). Newly emerged adults (sample sizes): N/rest, from normally buoyant pupae held at rest (23); r/rest, from reduced buoyancy pupae held at rest (19); N/stress, from normally buoyant pupae repeatedly stimulated to dive (20); r/stress, from reduced buoyancy pupae repeatedly stimulated to dive (18).

pupae regardless of buoyancy. No significant difference was found in the survival rate among the adults that were repeatedly stimulated to dive as pupae regardless of state of buoyancy. Among adults from pupae held at rest, the survival rate of those from normally buoyant pupae was significantly greater ($\chi^2 = 39.5882$, df $= 1$, $P < 0.01$) than among those from pupae with reduced buoyancy. Adults from pupae held at rest survived significantly longer (F $= 284.6$, df $= 1$, $P < 0.01$) than those repeatedly stimulated to dive, regardless of buoyancy. No significant difference was found in mean survival of adults from pupae that had been stressed, regardless of buoyancy. Adults from normal pupae held at rest (unstressed) survived significantly longer (F $= 13.2$, df $= 1$, $P < 0.01$) than those from pupae with reduced buoyancy held at rest.

Caloric content of newly emerged *Ae. aegypti* adults from pupae in the 4 experimental groups is shown in Fig. 5. Pupae held at rest had a significantly greater energy reserve than did stressed pupae regardless of buoyancy (F $= 7.1$, df $= 1$, $P < 0.01$). No significant difference was found in energy reserves among normal and reduced-buoyancy pupae, whether held at rest or stressed by repeated stimulation to dive.

Caloric content among newly emerged adult *Ae. albopictus* from pupae in the 4 experimental groups, plus normal and reduced-buoyancy pupae 0–1 h after pupation, is summarized in Fig. 6. Adults from pupae at rest had significantly greater nutrient reserves than did those from pupae that were stressed, regardless of buoyancy state (F $= 61.1$, df $= 1$, $P < 0.001$). A significant interaction of variables existed (F $= 6.2$, df $= 2$, $P > 0.01$), but multiple comparisons revealed no significant

difference in caloric reserves of adults from normal and reduced-buoyancy pupae, whether held at rest or stressed.

DISCUSSION

The ability of a mosquito to survive, and therefore to transmit disease, depends on nutrient reserves present at adult emergence. Compared with other holometabolous insects, the fact that mosquito pupae are motile places an additional drain on these reserves. Van Handel (1988) demonstrated a decline in glycogen during the pupal stadium of *Ae. aegypti*, *Culex nigripalpus* (Theobald), and *Culex quinquefasciatus* (Say). Nyar and Pierce (1977) demonstrated in *Cx. nigripalpus*, *Wyeomyia medioalbipes* (Lutz), and *Aedes taeniorhynchus* (Say) that glycogen and triglycerides decline exponentially after adult emergence, even at rest.

Analysis of the results of this study shows a clear energetic cost associated with frequent diving as compared to resting. Adult *Ae. aegypti* from normal and reduced-buoyancy pupae held at rest survived significantly longer than did adults from pupae repeatedly stimulated to dive (stressed) regardless of state of buoyancy or temperature. Likewise, caloric expenditure among adult *Ae. aegypti* and *Ae. albopictus* from normal and reduced-buoyancy pupae, held at rest, was significantly less than in adult *Ae. aegypti* and *Ae. albopictus* that were stressed as pupae, regardless of the state of buoyancy. The difference in mean caloric expenditure among adult *Ae. aegypti* from pupae with normal and reduced buoyancy at rest, and those stressed by being repeatedly stimulated to dive was 0.267 and 0.272 calories, respectively. These figures represent approximately 10% of the total calories present im-

mediately after pupation and show that frequent diving, which is likely to occur during prolonged, heavy rainfall, can be expected to have an effect on the duration of adult survival.

In contrast, relative to diving, no energy costs were detected in association with buoyancy reduction. Measurement of total calories revealed no significant differences between normal and reduced-buoyancy groups regardless of treatment (stressed or held at rest). Likewise, among adults from stressed pupae, no significant differences were detected relative to survival. As was suggested by Romoser and Lucas (1999), pupae of *Ae. aegypti* seem to sense their state of buoyancy and compensate behaviorally for changes. Our data are consistent with this idea and show that compensation occurs to the extent that even permanent buoyancy reduction does not significantly affect energy usage.

However, among groups held at rest, adults from normal positively buoyant pupae demonstrated a significantly greater survival rate than adults from pupae with reduced buoyancy held at 23°C and a significantly greater mean survival and survival rate than those from reduced-buoyancy pupae held at 27°C. Pupae with reduced buoyancy possibly dive spontaneously more frequently than do normally buoyant pupae, with the resultant energy drain.

Basal metabolism and tissue reorganization represent other drains on reserves at adult emergence. The difference in mean calories present in newly emerged adults produced from normally buoyant pupae held at rest and mean calories present in normally buoyant pupae at 1 h after pupation was approximately 0.50 calories, an estimate of the expenditure for basal metabolism and tissue reorganization. This figure represents more than 20% of the total caloric reserve accumulated during the larval stadium.

Any mechanism that would reduce caloric ex-penditure during the nonfeeding pupal stage would have a direct influence on a mosquito's ability to survive and hence to transmit the etiologic agents of disease. The ability to fill the ventral air space with gas that enables pupae, at least in the case of *Ae. aegypti* and *Ae. albopictus,* to alternate between being just slightly less dense than water to being just slightly more dense (Romoser 1975); the tendency, if undisturbed, to rest at the air–water interface; and diving behavior, which tends to maximize the time a pupa spends in a neutral state of buoyancy (hovering), or negatively buoyant and bottom-sitting, seem to be such energy-conserving mechanisms.

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