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BUOYANCY AND DIVING BEHAVIOR IN MOSQUITO PUPAE

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ABSTRACT. Mosquito pupal diving behavior has been studied mostly in *Aedes aegypti* and in this species pupal buoyancy varies relative to several factors. The research reported herein addresses the 2 following questions. Does diving behavior vary among different mosquito genera and species? How is diving behavior influenced by variation in buoyancy? Depth and duration of dive, and dive pattern, were compared among *Ae. aegypti, Culex pipiens, Anopheles stephensi, Aedes albopictus,* and *Aedes triseriatus.* In response to the stimulation associated with transferring pupae between containers, diving behavior varied dramatically among the different genera studied. *Culex pipiens* and *An. stephensi* make short-duration, shallow dives and remain positively buoyant. The 3 aedine species studied make longer-duration dives, typically to a depth at which they become neutrally or negatively buoyant. Buoyancy reduction effects were studied in the 3 aedine species. Normally buoyant pupae tend to dive to greater depths and for longer periods of time than reduced-buoyancy pupae. Aedine pupal diving behavior clearly is closely regulated relative to buoyancy variation. To the earlier hypotheses that pupal behavior may help avoid predation and be energy-conserving, we add the suggestion that the diving behavior displayed by the container-breeding aedine pupae we studied represents an adaptation that helps keep them from being washed from their container habitat by overflowing water during rainfall. We also suggest that the diving behavior of all the species studied may help pupae survive heavy, pelting rainfall by enabling them to avoid the mechanical shock of a direct hit by a raindrop, which could cause disruption of the gas in the ventral air space, thereby causing the loss of hydrostatic balance and drowning.

KEY WORDS Pupa, diving behavior, rainfall, energy conservation

INTRODUCTION

In response to a passing shadow or vibrations, mosquito pupae break from the water surface and dive. They are positively buoyant because of the presence of gas in the ventral air space (VAS), an external cavity formed by the developing legs, wings, and mouthparts (Hurst 1890, Christophers 1960).

In *Aedes aegypti* (L.), buoyancy has been found to vary inversely with time submerged, in irregular cycles at the surface, inversely with depth of submergence, and directly with the volume of gas in the VAS (Romoser 1975, 1978; Romoser and Nasci 1978). Buoyancy also can be expected to vary directly with temperature. Variation in buoyancy with time submerged and periodically at the surface are considered to be associated with passive-suction ventilation (Romoser 1978). Variation in buoyancy with depth of submergence and temperature is due to the expansion or contraction of gases in the tracheal system and VAS, for example, increasing depth (i.e., increasing water pressure) and decreasing temperature both would cause contraction of gases and hence a decrease in buoyancy (Romoser 1978). Romoser (1975) demonstrated that even just below the water surface, an *Ae. aegypti* pupa alternates between being slightly less dense than water to slightly more dense.

Behavioral elements associated with positive buoyancy are as follows (Romoser 1975). In an active dive, a pupa actively dives against the tendency to float to the surface; in a passive rise, a pupa rises passively with no abdominal movement; and in an active–passive rise, a pupa alternately flicks its abdomen and passively rises. Behavioral elements associated with negative buoyancy are as follows. In a passive sink, a pupa sinks without abdominal movement; in an active–passive dive, a pupa alternates active diving and passive sinking; in bottom-resting, a pupa rests on the bottom on the tips or flattened regions of the abdominal paddles and sometimes on the posterior regions of the dorsum of the abdomen; and in an active rise, a pupa actively moves toward the surface by flicking movements of the abdomen. A pupa at neutral buoyancy may hover motionless in the water. A given dive is a composite of 2 or more of these behavioral elements, which together make up the dive pattern. Undisturbed pupae tend to remain at rest at the surface. A pupa with severely reduced buoyancy is unable to maintain normal balance in the water, becomes disoriented, moves about erratically, and eventually sinks to the bottom and drowns.

The adaptive value of pupal behavior may be in avoiding predation and conserving energy (Romoser 1975). Shuey, Bucci, and Romoser (1987) demonstrated that laboratory-reared pupae of *Ae. aegypti, Aedes triseriatus* (Say), and *Culex restuans* Theobald tended to rest in concave menisci associated with emergent vertical surfaces and suggested that pupae in such locations, in addition to conserving energy, are less visible and accessible to predators. Two additional adaptive roles for pupal behavior are proposed in this paper.

Mosquito pupal diving behavior has been studied mostly in *Ae. aegypti* and many questions remain unanswered. The purpose of this research has been to address the following 2 of these questions. Does diving behavior vary among different mosquito genera and species? How is diving behavior influenced by variation in buoyancy?

MATERIALS AND METHODS

Larval rearing: Laboratory-reared *Ae. aegypti, Culex pipiens* L., *Anopheles stephensi* Liston, *Aedes albopictus* (Skuse), and *Ae. triseriatus* were studied. *Aedes aegypti* (Rockefeller strain), *Cx. pipiens* (El Gabal strain), and *An. stephensi* were obtained from the U.S. Army Medical Research Institute of Infectious Diseases (Fort Detrick, Frederick, MD). R. S. Nasci (Centers for Disease Control and Prevention, Fort Collins, CO) and E. D. Walker (Department of Entomology, Michigan State University, East Lansing, MI) provided eggs of *Ae. albopictus* and *Ae. triseriatus,* respectively. Only female mosquitoes were used in our studies. Sex was determined initially by pupal size and confirmed at adult emergence.

Larvae and pupae were raised under 16 h light–8 dark at 27 ± 1°C in 22 × 32-cm plastic pans containing 1,000 ml of water. All experiments were conducted at 27 ± 1°C. One hundred fifty to 160 larvae were reared in each pan on a diet consisting of equal parts of ground lab chow, liver powder, and yeast. One hundred milligrams of food was added to each pan daily.

Observation of diving behavior: Depth and duration of dive and dive pattern were compared among the 5 species. Diving behavior was observed in a Pyrex column, 3.81 cm in diameter, containing 165 cm of water, and marked in 1.0-cm vertical increments. If a pupa dove to the bottom of the column, depth of dive was recorded as 165 cm. All other dives were measured to the nearest centimeter. Duration of dive was measured as the time between breaking from and reestablishing contact with the atmosphere at the water's surface. The dive pattern for each pupa was observed and recorded in one of the following categories: the pupa remained positively buoyant throughout the dive, the pupa reached neutral buoyancy during the dive, or the pupa reached negative buoyancy during the dive. The stimulation associated with transferring a pupa from the holding container to the observation column was used in all studies of diving behavior.

Buoyancy reduction experiments: Buoyancy reduction experiments were done only with *Ae. aegypti, Ae. albopictus,* and *Ae. triseriatus.* To obtain reduced buoyancy pupae, late 4th-stage larvae were examined for the presence of dendritic tufts and darkened trumpets visible beneath the larval cuticle, both indications that feeding had stopped (Klowden and Chambers 1990) and that pupation would take place within approximately 2 h. Pupae with reduced but still positive buoyancy were obtained by allowing larvae to pupate in small inverted test tubes (7 mm in diameter, 120 mm long) (Romoser 1978). The mechanism of buoyancy reduction is described in Romoser and Nasci (1978).

To confirm buoyancy reduction, pupae within 1 h of pupation were evaluated for time to passive descent under forced submergence conditions, a relative measure of buoyancy (Romoser 1978). Time to passive descent in pupae with reduced but still positive buoyancy ranged from 10 sec to 5 min 55 sec. All normally buoyant pupae (those that had pupated at an upright air–water interface) failed to reach passive descent within 20 min.

Following confirmation of state of buoyancy, pupae were placed in individual holding vials. The behavior of pupae with reduced buoyancy in the vertical column was compared to the behavior of normally buoyant pupae.

Data analysis: Data were analyzed according to methods outlined in Sokal and Rohlf (1969). After tests for homogeneity of variances, analysis of variance was performed on each data set. Multiple comparisons tests (Student–Newman–Keuls and Tukey's honestly significant difference method) were performed when analysis of variance revealed significant interaction among variables.

RESULTS

Diving behavior in 5 species

In response to the stimulation associated with the transfer of a pupa from the holding container to the observation column, mean depth of dive (Fig. 1) varied significantly among all 5 species ($F = 99.848$, $df = 4$, $P < 0.01$). Multiple comparisons tests revealed that all means were significantly different. *Aedes aegypti* dove deeper than the other 4 species (mean depth ± standard error, 112 ± 4.4 cm). *Anopheles stephensi* was the shallowest diver (mean depth ± standard error, 3.8 ± 0.3 cm) followed by *Cx. pipiens* (mean depth ± standard error, 20.4 ± 2.03 cm). Depths of dive in the other 2 aedine species fell between that of *Ae. aegypti* and *Cx. pipiens.* Depth of dive showed much more variation in the aedine species than in *Culex* or *Anopheles.*

Duration of dive (Fig. 2) also varied significantly among the 5 species ($F = 58.97$, $df = 4$, $P < 0.01$). Multiple comparisons tests revealed that all means were significantly different. *Aedes aegypti* had the greatest duration of dive with a mean duration of 179.5 ± 10.6 sec. *Anopheles stephensi* dove for the shortest duration with a mean of 49.8 ± 0.8 sec followed by *Cx. pipiens* with a mean of 22.7 ± 2.6 sec. As with depth of dive, duration of dive in the other 2 aedine species fell between the values of *Ae. aegypti* and *Cx. pipiens.* Duration of dive showed much more variation in the aedine species than in *Culex* or *Anopheles.*

Dive pattern in each of the 5 species is shown in Fig. 3. Dive patterns are depicted as the proportion displaying only behavior associated with positive

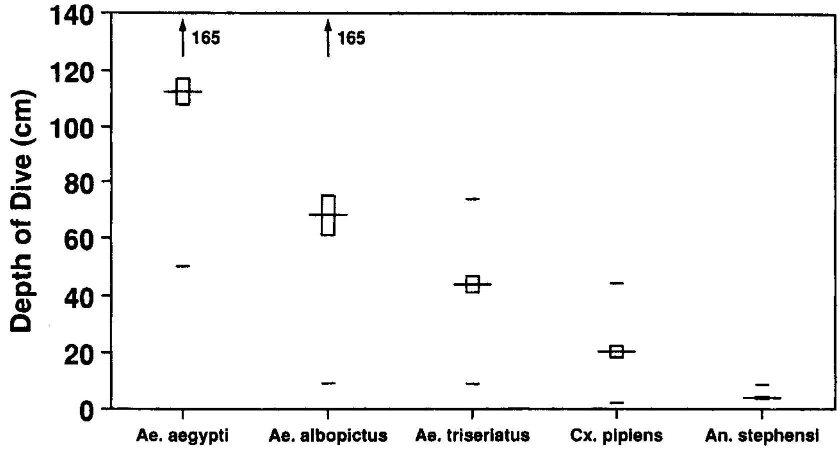

Fig. 1. Depth of dive. Horizontal lines through vertical bars, mean depth; vertical bars, standard error; horizontal dashes, range (upper limit next to arrow). Sample sizes: *Aedes aegypti,* 55; *Aedes albopictus,* 36; *Aedes triseriatus,* 50; *Culex pipiens,* 30; *Anopheles stephensi,* 30.

buoyancy, or dive including hover, or dive including behavior associated with negative buoyancy. The majority of the individuals in all 3 aedine species dove to a depth at which they became neutrally or negatively buoyant. Under the conditions of this study, none of *Cx. pipiens* or *An. stephensi* dove to a depth at which they became neutrally or negatively buoyant.

Diving behavior and buoyancy reduction

In all 3 species (*Ae. aegypti, Ae. albopictus,* and *Ae. triseriatus*) normally buoyant pupae dove significantly deeper ($F = 183.28$, $df = 1$, $P < 0.1$) than those with reduced buoyancy (Fig. 4). Multiple comparisons tests revealed that *Ae. aegypti* dove deeper than the other 2 species, in both normal and reduced states of buoyancy. *Aedes albopictus* dove significantly deeper than *Ae. triseriatus.*

In all 3 species, mean duration of dive (Fig. 5) among normal pupae was significantly longer than that among pupae with reduced buoyancy ($F = 56.22$, $df = 2$, $P < 0.01$). *Aedes aegypti* displayed a significantly longer duration of dive than *Ae. albopictus* or *Ae. triseriatus* in both normal and reduced states of buoyancy.

Assessment of the dive pattern (Fig. 6) of the 3 species revealed that the percent of pupae displaying behavior associated with neutral and negative buoyancy increased when buoyancy was reduced.

DISCUSSION

Examination of depth and duration of dive and dive pattern in *Ae. aegypti, Ae. albopictus, Ae. triseriatus, Cx. pipiens,* and *An. stephensi* revealed that under the strong stimulation associated with transferring pupae from one container to another

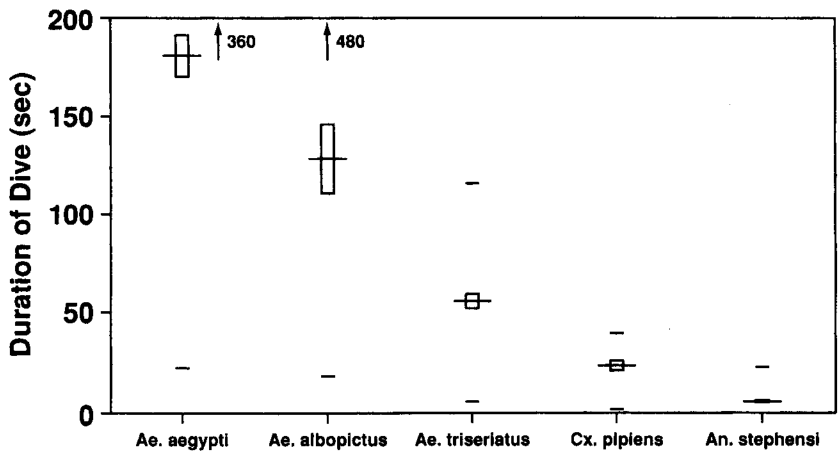

Fig. 2. Duration of dive. Horizontal lines through vertical bars, mean duration; vertical bars, standard error; horizontal dashes, range (upper limit next to arrow). Sample sizes as in Fig. 1.

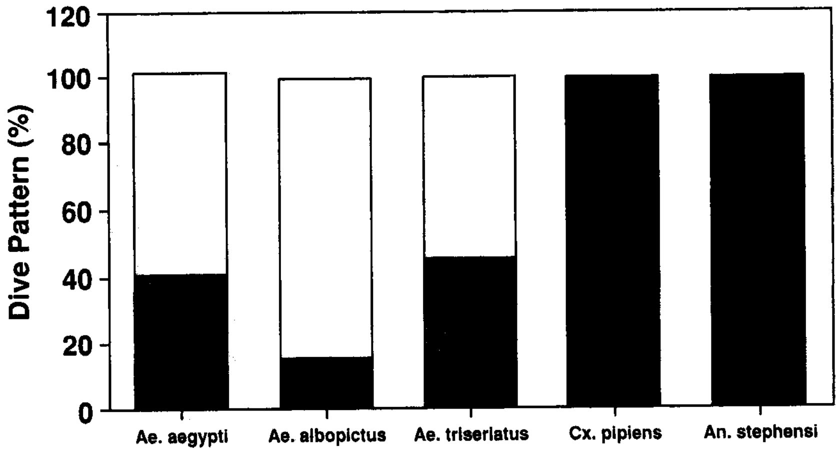

Fig. 3. Dive pattern. Black areas, remained positively buoyant throughout dive; gray areas, reached neutral buoyancy during dive; light areas, reached negative buoyancy during dive. Sample sizes as in Fig. 1.

diving behavior varies dramatically among the different genera and species studied. Such differences no doubt reflect adaptations to somewhat different aquatic habitats. *Culex pipiens* and *An. stephensi* generally make dives of comparatively short duration to a shallow depth and remain positively buoyant. The 3 aedine species studied make dives of comparatively long duration, and usually to a depth at which they become neutrally or negatively buoyant. Under conditions of weak stimulation such as a passing shadow or a gentle tap on their container, behavior of the aedine pupae resembles that of *Anopheles* and *Culex.*

In *Ae. aegypti, Ae. albopictus,* and *Ae. triseriatus,* a greater proportion of normally buoyant pupae dove to significantly greater depths, for significantly longer periods of time, than did pupae with reduced buoyancy. The majority of pupae with normal and reduced buoyancy of all 3 species dove to a depth at which they became neutral or negatively buoyant. Thus, depth and duration of dive and dive pattern in all 3 species vary with buoyancy. Typically, the more buoyant a pupa, the deeper and longer it dives.

When aedine pupae dive, a lower limit of buoyancy is reached that induces active rise and a still lower limit exists where balance and orientation are lost. Buoyancy varies continuously with several factors and it is clear that a maximum safe depth exists for a given pupa at a given time and temperature. The pupae are somehow able to sense their state of buoyancy and behave such that the safe depth is not exceeded. If the bottom happens to lie about the safe depth, pupae tend to dive

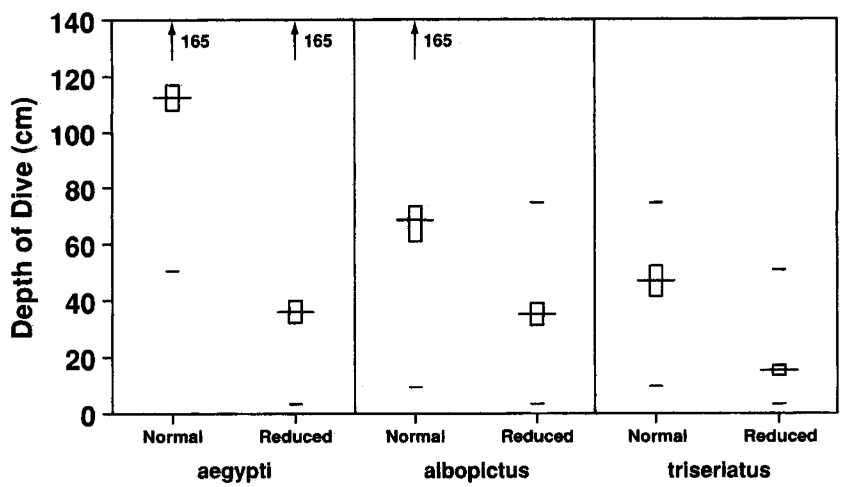

Fig. 4. Depth of dive in aedine pupae with normal and reduced buoyancy. Horizontal lines through vertical bars, mean depth; vertical bars, standard error; horizontal dashes, range (upper limit next to arrow). Sample sizes (normal and reduced buoyancy): *Aedes aegypti,* 55 and 46; *Aedes albopictus,* 36 and 31; *Aedes triseriatus,* 50 and 37.

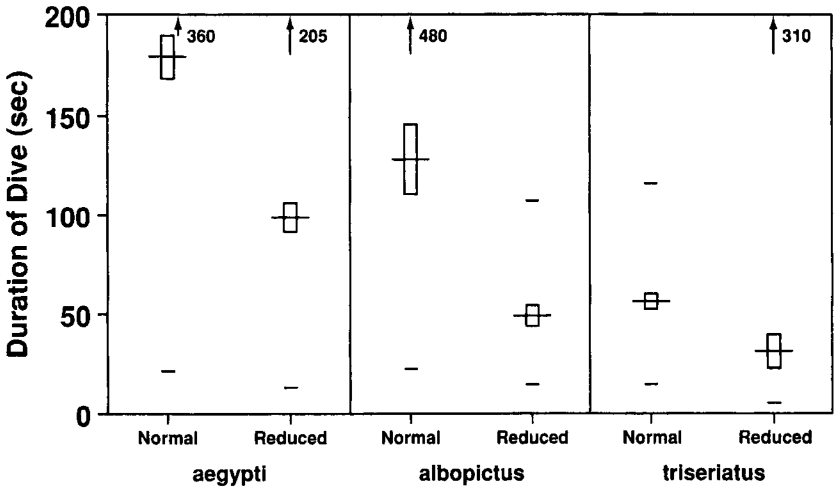

Fig. 5. Duration of dive in aedine pupae with normal and reduced buoyancy. Horizontal lines through vertical bars, mean duration; vertical bars, standard error; horizontal dashes, range (upper limit next to arrow). Sample sizes (normal and reduced buoyancy): *Aedes aegypti,* 55 and 46; *Aedes albopictus,* 36 and 26; *Aedes triseriatus,* 50 and 35.

against the bottom, producing a bouncing motion, until neutral or negative buoyancy is reached. Once a pupa becomes negatively buoyant, it rests on the bottom for a period of time before returning to the surface. In *Ae. aegypti, Ae. albopictus,* and *Ae. triseriatus,* diving behavior evidently is closely regulated relative to buoyancy changes.

The tendency to rest if undisturbed, as displayed by all species in this study, is clearly energy-conserving. In the aedine species, the tendency to dive to the point of neutral or negative buoyancy, which enables a pupa to remain submerged without continual, energy-using body movement, can also be viewed as energy-conserving.

Romoser (1975) showed that pupae forced to remain submerged until they have become neutrally or negatively buoyant, and those that do so spontaneously, can regain positive buoyancy with only brief contact (less than 1 sec) with the surface. This was interpreted as being due to the negative pressure that builds up in the tracheal system in association with passive-suction ventilation. This also implies that pupae are essentially able to take a quick breath at the water's surface and submerge once again.

Escape from potential predators and energy-conservation as explanations for the adaptive value of aedine pupal behavior make sense, and are proba-

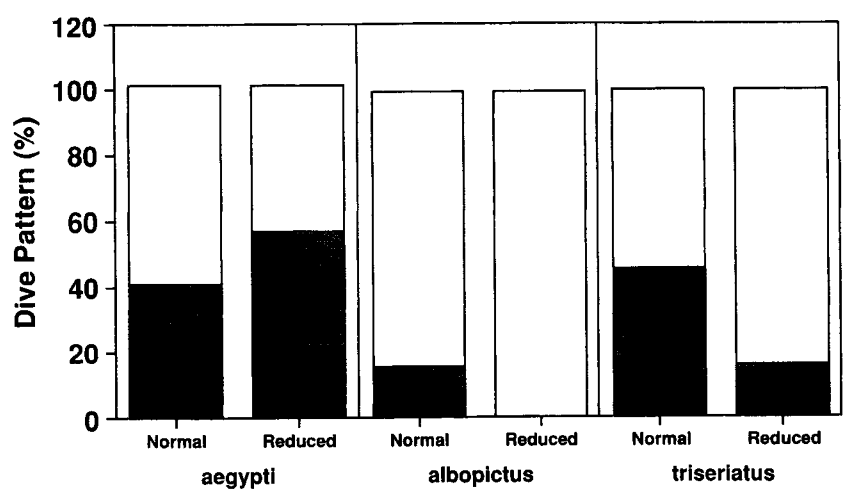

Fig. 6. Dive pattern in aedine pupae with normal and reduced buoyancy. Black areas, remained positively buoyant throughout dive; gray areas, reached neutral buoyancy during dive; light areas, reached negative buoyancy during dive. Sample sizes (normal and reduced buoyancy): *Aedes aegypti,* 55 and 46; *Aedes albopictus,* 36 and 35; *Aedes triseriatus,* 50 and 26.

bly true, but we would like to offer an additional hypothesis regarding aedine pupal diving behavior. The 3 species we studied are all container breeders and their diving behavior may well represent an adaptation to this sort of habitat. The strong sensitivity and responsiveness to surface vibrations (Shuey et al. 1987), the ability to take a quick breath at the surface (Romoser 1975), and the ability to make long-duration dives dominated by the neutrally or negatively buoyant state, including bottom-resting, would be of value in a situation where pupae are liable to be washed out of their container habitat during rains heavy enough or frequent enough to cause the container to overflow. By responding to the surface vibrations caused by rain drops and being able to remain submerged for long periods with only brief, periodic exposure to the surface, pupae would tend to stay below the level of the water flowing from the container and thereby avoid being washed away. Diving behavior could thus represent an adaptation that helps keep container-breeding mosquito pupae in their habitat. This probably applies to aedine larvae as well. For example, Washburn and Anderson (1993) showed that larvae of the western tree hole mosquito, *Aedes sierrensis* (Ludlow), were flushed by rainfall from artificial containers and from treeholes. These authors suggested that such flushing may be a significant source of larval mortality.

More study will be necessary to generate hypotheses to explain the behavioral differences we observed between aedine species and *Cx. pipiens* and *An. stephensi.* Although *Culex* mosquitoes are often found in containers, they also frequent storm sewer catch basins, poorly drained street gutters, polluted ground pools, and other similar habitats (Pratt and Moore 1993) and so are not as specifically adapted to containers as are the aedine species we studied. Anopheline mosquitoes are found mainly in permanent bodies of freshwater (Pratt and Moore 1993).

Diving behavior of all the species studied may facilitate survival in heavy, pelting rainfall. Romoser et al. (1994) showed that mechanical shock can cause disruption of the gas in the VAS and thereby cause the loss of hydrostatic balance and drowning. A direct hit by a raindrop may well be sufficient to cause this sort of damage and by diving in response to rainfall, pupae may tend to avoid such damage. The clear differences in diving behavior between *Aedes, Culex,* and *Anopheles* mosquitoes make it evident that a great deal more remains to be learned about behavior of mosquito pupae.

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