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FIELD AND LABORATORY COMPARISON OF HATCH RATES IN *AEDES ALBOPICTUS* (SKUSE)

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ABSTRACT. Laboratory experiments attempting to elicit a response based on a natural condition rely on the assumption that the laboratory treatment accurately mimics field conditions. With *Aedes albopictus* (Skuse), laboratory experiments analyzing hatch rates assume that the laboratory stimuli resemble those received by the eggs in field conditions. With the use of a colonized strain of *Ae. albopictus*, an analysis of the hatch rates comparing both field and laboratory settings was conducted. Additionally, hatch rates were compared for mosquitoes exposed to regular, periodic hatch stimulation (as usually seen in laboratory experiments) and random hatch stimulation (as seen in the field). In both experiments, laboratory treatments were not found to differ significantly from the field treatments, indicating that experimental results achieved in the lab are relevant to field situations.

KEY WORDS Hatch stimuli, *Aedes albopictus* (Skuse), hatch rate

INTRODUCTION

Ecological experimentation in the field is more desirable than attempting to replicate field conditions in a laboratory. It is difficult to control all noncritical variables in the field, however, so laboratory experimentation becomes necessary. With laboratory experimentation, accuracy of laboratory methods is essential for any well-designed experiment attempting to replicate field conditions. Without an accurate representation of field conditions, experimental results will always be subject to question. Ecological studies conducted in the lab should first verify that the techniques and methods used accurately mimic field conditions.

Conducting hatch studies involving container-breeding mosquitoes presents just such a dilemma. Even under ideal field conditions, many environmental variables cannot be controlled. Under laboratory conditions, environmental factors can be controlled to minimize their influence on the hatch rates of experimental egg batches. The method of hatching eggs in the laboratory may differ from the hatching stimulus that an egg will encounter in the field. Previous work has indicated that the hatch stimulus for container-breeding mosquitoes in the genera *Aedes* and *Ochlerotatus* is a decline in the oxygen concentration after the eggs are submerged in a liquid medium (Gjullin et al. 1941, Judson 1960, Barbosa and Peters 1969, Fallis and Snow 1983). In the field, this oxygen concentration drop is caused by a bloom in the bacteria population within a tree hole, brought on by an influx of nutrients during a rainfall as nutrients flow down the tree trunk into the tree hole (Walker and Merritt 1988, Walker et al. 1991). In the laboratory, the drop in oxygen concentration can be achieved by a variety of methods. Physical methods including artificially aerating the liquid medium prior to egg immersion, chemical means, or biological means can all result in a drop in the oxygen concentration that can be detected by an egg. These and other methods of hatch stimulation will result in different hatch rates under laboratory conditions (Horsfall 1956, Novak and Shroyer 1978, Livdahl et al. 1984). Hatching eggs using the biological method of inducing a bacterial bloom may be the most similar to the hatch stimulus in the field. In the laboratory, bacteria in the hatch medium have been shown to have a positive effect on egg hatch (Rozeboom 1934, Gillett et al. 1977). Presumably, this positive effect on hatch rate is the result of an oxygen concentration drop in the medium, and the same effect occurs in the field. However, the response to these stimuli in the laboratory may depart from natural stimulus conditions.

There has been little field research conducted on hatching of *Aedes albopictus* (Skuse) eggs, nor on the *Aedes* and *Ochlerotatus* spp. in general. One field study involved the inhibition of hatching of *Ochlerotatus triseriatus* (Say) eggs by a high density of larvae (Livdahl and Edgerly 1987), but for the most part field studies of hatch rates have been ignored. Additionally, there is little research on the installment hatching of *Ae. albopictus*. Installment hatching, or delayed hatch of a fraction of eggs through successive stimuli, has been examined in a number of other tree hole mosquitoes, notably *Oc. triseriatus* and *Aedes aegypti* (Linnaeus) (Gillett 1955). The majority of the hatching experiments involving *Ae. albopictus* have focused on the actual hatch stimulus (Matsuzawa and Kitahara 1966, Mogi 1976, Imai 1993) and interactions with other species (Edgerly et al. 1993).

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Our goal was to test laboratory hatching methods with the use of *Ae. albopictus* and compare these methods to conditions an egg is likely to encounter in the field. By verifying that the hatch procedure under controlled laboratory conditions is similar to field conditions, it is possible to conduct further experiments in the laboratory and hypothesize that results would be similar to those in the field. Two aspects of laboratory hatching were examined: the hatch medium that is used in the laboratory, and the pattern of hatching stimulation the eggs receive over time.

The initial experiment compared the hatch rates of eggs subjected to a variety of hatch media. The field hatch conditions consisted of hatching eggs in both tree hole and tire dump settings. In the laboratory, the hatch media consisted of a variety of nutrient broth concentrations. The second experiment tested the influence of 2 temporal patterns of hatch stimulus. In the laboratory, eggs are stimulated on a regular, predictable basis over time, whereas in the field rainfall occurs in a much more random pattern. Eggs were subjected to stimuli either on a regular or random basis in the laboratory to examine this effect. The actual probability of stimulation was the same for both the random and regular intervals between stimulation. The intervals of stimulation for the random treatment were determined based on the same frequency of stimulation as the regular treatment. From these data, it is possible to determine if laboratory methods of hatching eggs accurately mimic field conditions. If it is determined that laboratory conditions can accurately reflect what is happening in the field, the relevance of laboratory conditions is established for further experiments.

MATERIALS AND METHODS

For both of these experiments, a strain of laboratory-reared *Ae. albopictus* was used. This population originated in the early 1990s from locations in North Carolina, and had been reared for many successive generations in the laboratory. Fertilized eggs from this population of *Ae. albopictus* were collected on wooden tomato stakes placed in the laboratory cages. These stakes were made from pine, and cut into 5-inch-long segments. Following oviposition, they were cut into slivers, with similar numbers of mosquito eggs on each sliver of wood creating an egg batch. Within 24 h of oviposition, these egg batches were stored at a 16 h:8 h light:dark cycle, and kept damp in plastic vials until a sufficient number of eggs was obtained. For both experiments, the eggs were randomly divided into the experimental groups, so the age of the eggs would not affect the outcome (although age was included in the analysis).

Hatching medium: For the first experiment, egg batches were subjected to one of a variety of nutrient broth concentrations, in an effort to modify the strength of stimulus. Concentrations included 1.0 g/liter; 0.5 g/liter; 0.25 g/liter, 0.1 g/liter, and 0.0 g/liter (distilled water). The nutrient broth mixtures and distilled water were aerated for 30 min prior to submerging the eggs for 24 h.

Field stimuli were included for comparisons. Egg batches were placed in both tree hole and tire habitats. Egg batches were contained within vials that had a fine plastic mesh on both ends of the vial, allowing liquid and nutrients to pass through the vial but not allowing the eggs or hatched larvae to escape. The tree hole vials were fixed horizontally to the sides of tree holes in 1 of 4 beech trees immediately prior to a rainfall, just above the waterline of the tree holes. The tree holes were selected based on the presence of container-breeding mosquitoes following previous rainfalls. Four vials were each placed in 3 different tree holes, and removed after 24 h. A similar procedure was used for the tire-dump setting, with 4 vials placed above the waterline in 1 of 2 discarded tires prior to a rainfall, and removed 24 h later.

In all cases, hatched larvae were counted, and all unhatched eggs were dissected to determine viability, as indicated by the presence of a developed larva. Once the total number of remaining viable eggs was determined, a hatch percentage consisting of the number of hatched larvae divided by the total number of viable eggs (hatched eggs plus remaining viable eggs) was determined.

Age of egg batches was suspected to influence hatch rate. Because it was not possible to use eggs of uniform age throughout the experiment, age was included as an independent variable. Treatment (hatch stimulation method) was randomized across the various ages. Within each egg batch, the age of the eggs was the same. Specific, planned comparisons were conducted with the use of least-square means contrasts available within JMP v. 4.0 software (SAS Institute, Inc., SAS Campus Drive, Cary, NC).

Hatching pattern and frequency: The second experimental procedure was conducted by exposing eggs to sequential hatch stimuli, either at random intervals or at regular intervals. As with the previous experiment, a hatch stimulus consisted of submerging the egg batch in an aerated nutrient broth medium consisting of 0.5 g/liter of nutrient broth mixed with distilled water. Three different frequencies for regular stimulation were examined: once every 3 days, once every 6 days, and once every 9 days. For the random hatching intervals, this frequency (once every 3 days, once every 6 days, and once every 9 days) was

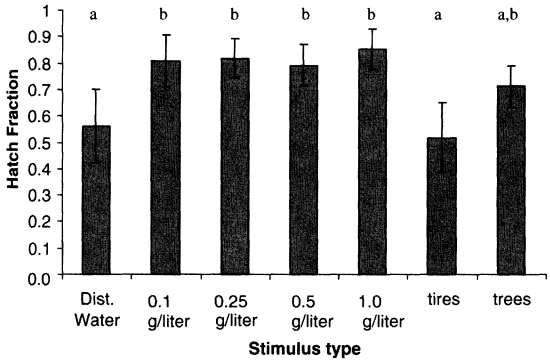

Fig. 1. Mean hatch fractions for each of the hatch media (± SE). Each treatment was contrasted with all others. Hatch fraction was calculated by dividing the total number of hatched eggs after the hatch stimulus by the total number of viable eggs. Different letters correspond with significant differences in the mean hatch fractions ($P < 0.05$).

converted to a daily probability of being stimulated (0.33, 0.17, and 0.11, respectively). This probability was then used to construct a daily hatch schedule, using a draw of random numbers to determine days for stimuli on the random stimulus groups. All experimental treatments were halted after 10 hatch stimuli. A median hatch stimulus number was calculated for each replicate using a logistic regression. This median is the stimulus number at which 50% of the delayed-hatching eggs had hatched. A delayed-hatching egg is one that hatched after the 1st stimulus. Within each egg batch, cumulative hatch rates for each stimulus represented the data points, with a hatch being a nominal value (either "y" or "n"). Because all eggs were subjected to the initial stimulus at the same time, the logistic regression was applied only to eggs that hatched after the first stimulus, counting the first stimulus as time 0, the second stimulus as time 1, etc. These batch medians constituted observations for comparisons of hatch delay for experimental treatments.

Pattern of frequency (random or regular intervals) and the actual frequency of stimulation provided nominal independent variables for comparisons based on regression analysis. As with the previous experiment, the age of the egg batches was included as a covariate, to attempt to reduce unexplained variation.

RESULTS

Hatching medium

Figure 1 shows the average hatch fractions for each of the treatments. Both hatching medium and initial age of the eggs were significant factors affecting the hatch rate of the eggs, and there was no evidence for an interaction between the 2 factors (Table 1). A detailed comparison of each of the treatments with the use of a least-squares means contrasts (JMP, v 4.0; SAS) indicates that there were differences in the hatch response to some of the treatments (indicated on Fig. 1). Hatch rates in a broth medium were not significantly different at any concentrations ($F = 0.17$, $P = 0.91$). A regression analysis was also run on only the broth concentrations (0 g/liter through 1 g/liter) and was nonsignificant ($F=1.97$, $P = 0.17$), confirming that there was no difference in any of the broth treatments.

Table 1. Analysis of variance for the effects of initial age and hatching medium on the hatch rates of *Aedes albopictus* eggs. The dependant variable of hatch rate was calculated by dividing the total number of hatched eggs by the total number of viable eggs for each egg batch.

Source	df	SS	F	P
Age	1	2.50	53.91	< 0.0001
Hatch medium (HM)	6	1.13	4.04	< 0.01
Age × HM	6	0.39	1.41	0.23
Error	54	2.50		

Stimulus pattern and frequency

There was no significant difference in the hatch rates on the first stimulus due to either frequency or treatment (Fig. 2). The only factor that had a significant influence on the hatch rate during the first stimulus was the initial age of the eggs (Table 2). Age was also included in all subsequent analyses for this reason.

After a critical stimulus number was calculated for each replicate, an analysis of covariance indicated that there was no significant difference between the critical stimuli of the random or the regular treatments (Table 3). Stimulus frequency did have a significant influence on the critical stimulus. Egg age was also a significant factor affecting the critical stimulus. There was no

Table 2. Analysis of variance for the first hatch fraction. The dependant variable hatch fraction was calculated by dividing the number of hatching larvae by the total number of viable eggs. The independent variables are the stimulus pattern (subjecting the eggs to a hatch stimulus on a random or regular pattern), stimulus frequency (how frequently the eggs are subjected to a hatch stimulus), and the initial age of the eggs.

Source	df	SS	F	P
Stimulus pattern	1	3.20×10^{-3}	0.13	0.72
Stimulus frequency	1	5.40×10^{-3}	0.23	0.64
Age of the eggs	1	2.11×10^{-1}	8.78	< 0.01
Error	34	8.16×10^{-1}		

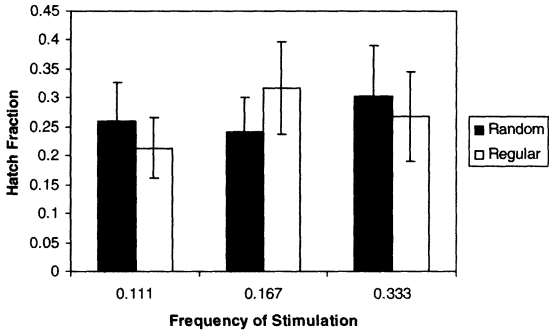

Fig. 2. Hatch fractions of the egg batches on the first hatch stimulus (± SE). Frequency of stimulation represents the probability of an egg batch being stimulated each day, and the treatment (*random* or *regular*) represents whether the stimulation occurred on a regular or random basis.

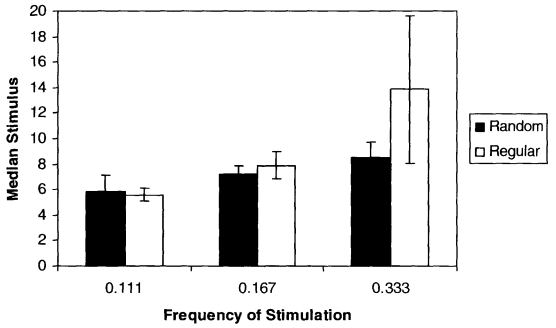

Fig. 3. The median stimulus values for the different treatments. The bars represent the mean values for each treatment combination (± SE). *Random* and *regular* represent the stimulus pattern, and *stimulation frequency* is the probability of stimulation on a daily basis.

significant interaction between any of the variables, and the interaction term was removed from the ANOVA analysis.

Figure 3 shows the critical stimulus number graphically. The extreme difference in the 0.33 frequency means for random and regular treatments was due to 1 median stimulus value of 42.57 in the regular treatment. If this value was removed, the averages were much more similar (8.56 vs. 8.11).

DISCUSSION

Based on these results, it seems to be possible to replicate field conditions in laboratory hatch rates accurately. Using the appropriate hatch medium to replicate the desired setting is the first step. The tree hole setting was not significantly different from any of the laboratory media (any of the broth concentrations or the distilled water). In addition, stimuli in tree holes seem to produce a mean hatch rate intermediate between the distilled water and the nutrient broth media. When attempting to replicate a tire stimulus, it seems that using distilled water is the most realistic method tested. They both produced a similar mean hatch rate, and were not significantly different from one another. All of the nutrient broth concentrations had a higher mean hatch rate than a tree hole setting (although none of them were significantly different from tree holes). Sex as a potential influence on hatch rates was not included in the analysis, but it may be a relevant factor. Whereas males generally hatch earlier and develop faster than females, there may be a sex specific response to the different hatch treatments that warrants further study.

Table 3. Analysis of variance for the influences on the median stimulus. The dependent variable median stimulus was calculated with the use of a logistic regression, and represents the predicted stimulus at which 50% of the delayed-hatching eggs would hatch. The independent variables represent the stimulus pattern (stimulating the eggs on a regular or random basis), stimulus frequency (the frequency at which the eggs are stimulated), the age of the eggs, and the first hatch fraction (the hatch rate on the first stimulus).

Source	df	SS	F	*p*
Stimulus pattern	1	0.71	0.40	0.53
Stimulus frequency	1	60.72	34.15	< 0.0001
Age of the eggs	1	63.02	35.44	< 0.0001
First hatch fraction	1	2.57	1.45	0.24
Error	54	2.50		

The media tested are not the only possible methods that can be used to hatch eggs. Other methods described include using a chemical agent (usually ascorbic acid or bubbling nitrogen gas) to cause a reduction of oxygen concentration, or using other biological means (such as a media of corn broth, rat chow, or mouse pellets) to cause a bacterial bloom that will then lower the oxygen concentration (Horsfall 1956, Mogi 1976, Novak and Shroyer 1978, Livdahl et al. 1984). Some of these methods, or a combination of the described methods, may provide a more accurate replication of tree hole hatching. Treatment of eggs prior to submersion or stimulation can also influence the subsequent hatch rate (Horsfall 1956). In our experiment, eggs were stored a minimum of 24 h following oviposition, and not subjected to any wetting prior to actual hatch stimulation. Larvae presence may also alter hatch rates; some stimulatory effects have been found at low densities, although as the density of larvae increases, egg hatch is inhibited (Livdahl and Edgerly 1987, Livdahl et al. 1984, Edgerly and Marvier 1992.

The colony mosquitoes used in our experiment had been reared under controlled conditions since the early 1990s. A colony strain of mosquitoes is exposed to environmental conditions that are

vastly different from a wild population, including but not limited to fixed photoperiods, controlled humidity and temperatures, and a readily available food supply. Previous work has shown that colony strains rapidly adapt to laboratory conditions (Mogi 1976). In many cases, this is due to artificial selection that results from only subjecting eggs from colony populations to 1 hatch stimulus. Under these conditions, eggs that avoid hatching during the first stimulus are discarded, which results in a strong selection pressure, minimizing any hatch delay. In our colony, we routinely subjected eggs to multiple hatch stimuli, in an attempt to avoid this specific selection pressure. However, because of the length of colonization, it is conceivable that our colony has been subjected to other forms of artificial selection that may have altered their hatch response. As such, although our colony population responded similarly to both field and laboratory hatch stimuli, it may be possible that other colony strains or wild mosquitoes may not respond in the same fashion.

The regression analysis conducted on the laboratory stimuli produced the same results as the least squares means contrasts. Both indicated there were no significant differences between any of the broth concentrations. Of the methods examined, using a 0.5-g/liter nutrient broth mixture was the closest to a tree hole setting, based on the average hatch fraction.

The second aspect of replicating hatching in the field is the pattern of stimulation. These data indicate there is no difference between stimulating the eggs at regular or random intervals. In field conditions, stimulation via rainfall may occur randomly, but in laboratory settings, regular stimulation is often more convenient. Interestingly, the stimulation frequency seems to have an effect on the median number of stimuli required by delayed hatchers. This may indicate some sort of plasticity or ability of the mosquitoes to respond to environmental cues. It is known that container-breeding mosquitoes can detect and respond to certain cues, including photoperiod, larval crowding, and a decrease in oxygen (previously discussed as the actual hatch stimulus) (Shroyer and Craig 1983, Focks et al. 1994). However, laboratory populations that have been reared for a high number of generations may evolve different responses to hatching cues due to inadvertent selection pressures. Previous work with *Ae. albopictus* has shown that a laboratory population, initially collected from a forest setting and reared for an extended length of time, developed an overall higher hatch rate (Mogi 1976), and this could have resulted from the removal of delayed-hatching eggs from the population. For these and other reasons, recently captured populations should be used to examine plasticity of egg responses to environmental cues.

The treatment of regular stimulation at a 0.33 probability resulted in what appears to be a large difference in the average median stimulus number (Fig. 3). However, this difference is primarily due to 1 replicate that had an estimated median value of 42.57, approximately 4 times higher than any of the median stimulus of any other treatment. This extreme value may have resulted from a failure to correctly identify nonviable eggs, which may have appeared as viable larvae on dissection. If this value is removed, the mean value is much more similar (8.56 for random stimulation and 8.11 for regular stimulation).

The influence of age on the hatch rates was not surprising. The eggs of *Ae. albopictus* are resistant to but not immune to desiccation (Sota and Mogi 1992a, 1992b). Increasing the time period between oviposition and hatching increases the amount of time the egg is at risk of desiccation. As the time period between oviposition and hatching increases, the sensitivity of the egg to a hatch stimulus may also increase to minimize the egg's vulnerability to desiccation. Our results show that increasing the time between oviposition and the first hatch stimulus does result in a higher hatch rate. Desiccation resistance varies from species to species, and different species may respond differently to increased aging of the eggs prior to hatching.

This information is vital for replicating field conditions in the laboratory. Given the inherent difficulty of conducting field studies and the difficulty of controlling all the variables in the field, calibration of laboratory methods to obtain stimuli that are relevant to field conditions is essential. These results indicate it is possible to accurately mimic field conditions in the laboratory. In addition to the factors we studied, other variables may influence the hatch rates of eggs and should be examined as well. Although it seems likely that other container-breeding species would respond similarly, wild populations of *Ae. albopictus* and other species may respond differently than our colony strain of *Ae. albopictus* to the hatch media and pattern of stimulation we examined. A different treatment or combination of treatments may be needed to mimic field hatching for other populations or species of container-breeding mosquitoes.

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REFERENCES CITED

Barbosa P, Peters TM. 1969. A comparative study of egg hatching techniques for *Aedes aegypti* (L.). *Mosq News* 29:548–551.

Edgerly JS, Marvier MA. 1992. To hatch or not to hatch? Egg hatch in response to larval density and to larval contact in a tree hole mosquito. *Ecol Entomol* 17:28–32.

Edgerly JS, Willey MS, Livdahl TP. 1993. The community ecology of Aedes egg hatching: implications for a mosquito invasion. *Ecol Entomol* 18:123–128.

Fallis SP, Snow KR. 1983. The hatching stimulus for eggs of *Aedes punctor* (Diptera: Culicidae). *Ecol Entomol* 8:23–28.

Focks DA, Linda SB, Craig Jr. GB, Hawley WA, Pumpuni CB. 1994. *Aedes albopictus* (Diptera: Culicidae): a statistical model of the role of temperature, photoperiod, and geography in the induction of egg diapause. *J Med Entomol* 31:278–286.

Gillett JD. 1955. The inherited basis of variation in the hatching response of Aedes eggs (Diptera: Culicidae). *Bull Entomol Res* 46:255–265.

Gillett JD, Roman EA, Phillips V. 1977. Erratic hatching in Aedes eggs: a new interpretation. *Proc R Soc London Ser B* 196:223–232.

Gjullin CM, Hegarty CP, Bollen WB. 1941. The necessity of a low oxygen concentration for the hatching of Aedes mosquito eggs. *J Cell Comp Physiol* 17:193–202.

Horsfall WR. 1956. Eggs of floodwater mosquitoes III (Diptera, Culicidae): conditioning and hatching of *Aedes vexans*. *Ann Entomol Soc Am* 49:66–71.

Imai C. 1993. Hatching of *Aedes albopictus* eggs for a strategy on life history. Record of paper for workshop on Quiescence and Moving of Sanitation Insects. In: Wada Yand Tsuji H, eds.

Judson CL. 1960. The physiology of hatching Aedine mosquito eggs: hatching stimulus. *Ann Entomol Soc Am* 53:688–691.

Livdahl TP, Edgerly JS. 1987. Egg hatching inhibition: field evidence for population regulation in a tree hole mosquito. *Ecol Entomol* 12:395–399.

Livdahl TP, Koenekoop RK, Futterweit SG. 1984. The complex hatching response of Aedes eggs to larval density. *Ecol Entomol* 9:437–442.

Matsuzawa H, Kitahara N. 1966. Some knowledge on the biology of *Aedes albopictus* (Skuse). *Jpn J Sanit Zool* 17:232–235.

Mogi M. 1976. Variation in oviposition, hatching rate, and setal morphology in laboratory strains of *Aedes albopictus*. *Mosq News* 42:196–201.

Novak RJ, Shroyer DA. 1978. Eggs of *Aedes triseriatus* and *Ae. hendersoni*: a method to stimulate optimal hatch. *Mosq News* 38:515–521.

Rozeboom LE. 1934. The effect of bacteria on the hatching of mosquito eggs. *Am J Hyg* 20:496–501.

Shroyer DA, Craig Jr. GB. 1983. Egg diapause in *Aedes triseriatus* (Diptera: Culicidae): geographic variation in photoperiod response and factors influencing diapause termination. *J Med Entomol* 20:601–607.

Sota T, Mogi M. 1992a. Interspecific variation in desiccation survival time of *Aedes (Stegomyia)* mosquito eggs correlated with habitat and egg size. *Oecologia* 90:353–358.

Sota T, Mogi M. 1992b. Survival time and resistance to desiccation of diapause and non-diapause eggs of temperate *Aedes (Stegomyia)* mosquitoes. *Entomol Exp Appl* 63:155–161.

Walker ED, Lawson DL, Klug MJ. 1991. Nutrient dynamic, bacterial populations, and mosquito productivity in tree hole ecosystems and microcosms. *Ecology* 75:1529–1546.

Walker ED, Merritt RW. 1988. The significant of leaf detritus to mosquito (Diptera: Culicidae) productivity from tree holes. *Environ Entomol* 17:199–206.