Winter Activity and Diapause of *Aedes albopictus* (Diptera: Culicidae) in Hanoi, Northern Vietnam

TAKASHI TSUNODA,[1,2] LUIS FERNANDO CHAVES,[1,3] GIANG THI TRA NGUYEN,[4] YEN THI NGUYEN,[4] and MASAHIRO TAKAGI[1]

J. Med. Entomol. 52(6): 1203–1212 (2015); DOI: 10.1093/jme/tjv122

ABSTRACT We studied the winter activity of *Aedes albopictus* (Skuse) from November 2008 to April 2009 in Bat Trang village of Hanoi, Vietnam. We selected 12 houses and collected: 1) adults with BG sentinel traps, 2) pupae from household water containers, and 3) eggs with ovitraps. *Aedes albopictus* adults, pupae, and eggs were not collected from early January to early February. Though the egg hatching probability tended to be initially high at longer day length, the maximum probability gradually shifted to shorter day length, as the observation period elapsed. When females were reared under long day length and their eggs were immersed 1 or 5 wk after oviposition, >50% of eggs hatched within 20 days. However, when females were reared under short day length and their eggs were immersed after 1 wk, hatching was suppressed for 60 days. When females were reared under short day length, the median hatching day occurred earlier in eggs kept dry for 5 and 10 wk after oviposition than in those dried for only 1 wk. This indicates that the extended dry periods accelerate egg hatching. Our results showed that hatchability gradually changed with day length, suggesting that selection for overwintering is not as strong relative to *Ae. albopictus* living in the temperate zone, where winter conditions are less favorable than in tropical and subtropical areas.

KEY WORDS bet hedging, photoperiod, subtropic, hatchability

Many major insect vectors of human pathogens have become “domesticated,” breeding in close proximity to humans and seeking human blood-meals (Powell and Tabachnick 2013). The Asian tiger mosquito, *Aedes albopictus* (Skuse), is a major “domesticated” vector of Dengue and Chikungunya viruses (Reiter et al. 2006). *Aedes albopictus* colonizes artificial containers, such as used tires and bamboo stalks (Medlock et al. 2012, Bonizzoni et al. 2013), and this species is now cosmopolitan throughout tropical and temperate zones.

The occurrence of *Ae. albopictus* across temperate (Mori et al. 1981) and tropical (Suwonkerd et al. 1996) latitudes likely implies a diverse set of strategies to deal with changing environments (Levins 1968). For example, *Ae. albopictus* females from temperate latitudes, where winter temperatures limit insect development and activity, lay diapausing eggs when pupae and/or adults are exposed to short day length at 25°C (Mori and Wada 1978, Mori et al. 1981). Moreover, *Ae. albopictus* diapausing eggs have an increased stress resistance that might enhance survival during long-distance transport, and could partially explain the remarkable success of this species as a successful an invasive species (Denlinger and Armbruster 2014). In contrast, *Ae. albopictus* populations from subtropical environments lay eggs during the winter, with a small proportion of eggs hatching without undergoing any diapause (Higa et al. 2007). Thus, the study of *Ae. albopictus* overwintering in the transition area between subtropical and temperate environments is critical to understand how life history strategies could have shaped its invasion of new habitats worldwide (Lounibos 2002).

Hanoi is located in northern Vietnam, where minimum winter temperatures sometimes fall below 10°C (Weatherbase 2013). Temperatures below 10°C imply that *Ae. albopictus* here could often be below its developmental zero point (Chen and Huang 1988). *Aedes albopictus* is believed to have been originally restricted to Southeast Asian forests (Smith 1956). Wing morphometrics suggest that Hanoi *Ae. albopictus* populations are more closely related to those of Japan and Korea than to southern Vietnam (Morales et al. 2013). Given this, we asked whether *Ae. albopictus* in Hanoi enters diapause during winter. Specifically, we investigated the field activity of *Ae. albopictus* immatures and adults during the winter and also examined the effect of photoperiod on egg diapause in the laboratory.

[1] Department of Vector Ecology and Environment, Institute of Tropical Medicine (NEKKEN), Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan.

[2] Corresponding author, e-mail: tsunoda@nagasaki-u.ac.jp.

[3] Programa de Investigación en Enfermedades Tropicales (PIET), Escuela de Medicina Veterinaria, Universidad Nacional, Apartado Postal 304-3000, Heredia, Costa Rica.

[4] Department of Medical Entomology and Zoology, National Institute of Hygiene and Epidemiology, 1 Yersin str., Hai Ba Trung dist., Hanoi, Vietnam.

Materials and Methods

Meteorological Data. Daily temperature and rainfall records for Hanoi, from November 2008 to April 2009 were obtained from the national Center for Hydro Meteorological Forecasting, Vietnam.

Monitoring of *Ae. albopictus*. From November 2008 to April 2009, we sampled *Ae. albopictus* in Bat Trang, a suburb of Hanoi. We monitored 12 houses, which were sampled biweekly. During each sampling session: 1) All the water-filled containers in each house were examined for the presence/absence of larvae and pupae. "Container index" was defined as (the number of positive container × 100)/the number of total containers. Small containers such as flower vases were emptied into a cup containing clean water to collect the immature mosquitoes. Jars and concrete tanks were collected with the quantitative sampling method that estimates the number of immatures (Knox et al. 2007). Though Bat Trang is famous for ceramics, we defined pottery left outside over 2 wk as discarded. Pupae were collected with pipettes and reared to adults, which were identified using standard mosquito identification keys (Stojanovich and Scott 1966). 2) One BG sentinel trap (BioGents, Regensburg, Germany) was placed in a storeroom of each house for 22 h (from 1000 to 0800 hours) to collect adults. 3) An ovitrap was placed within a 3-m radius of each house. The trap consisted of a plastic bucket (20 cm diameter, 17 cm height) filled with 3 liters of water for 1 wk. The number of eggs from eight paper strips (30 by 7 cm) placed inside each ovitrap was counted under a dissecting microscope.

Experiment 1: Egg Hatching from *Ae. albopictus* Grown under Different Photoperiods. *Aedes albopictus* larvae were collected from Bat Trang in August 2009 and kept at room temperature. Second-instar larvae were divided into six incubators at 25°C (treatments) with different photoperiods: 1) 9 hr light:15 hr dark, 2) 10 hr light:14 hr dark, 3) 11 hr light:13 hr dark, 4) 12 hr light:12 hr dark, 5) 13 hr light:11 hr dark, 6) 14 hr light:10 hr dark to examine the threshold photoperiod of diapause. All adults used in this experiment were confirmed as *Ae. albopictus* before oviposition started. Eggs were collected from females reared in each incubator. Eggs were also dried for 2 d, and kept in each incubator for 5 d prior to the hatching experiment. For the experiment, we prepared 8 cups that contained 20 eggs each per treatment, eggs were submerged in deionized water, and repeated twice. During the 100-d observation period for egg hatching, water was changed every day and eggs were kept at 25°C. Unhatched eggs were dissected under a microscope to examine embryonation.

Experiment 2: Egg Hatching From *Ae. albopictus* Grown at Different Photoperiods, With Different Post-oviposition Age and Hatching Photoperiod. Eggs from *Ae. albopictus* raised at 25°C in the following two photoperiods: 1) 10 hr light:14 hr dark (i.e., short day) and 2) 14 hr light:10 hr dark (i.e., long day) conditions were removed and dried. They were submerged in plain water 1, 5, and 10 wk after oviposition, and hatched under the two photoperiods defined previously, i.e., short day and long day. The resulting treatments, i.e., the combination of *Ae. albopictus* development photoperiod until oviposition and post-oviposition age, were conducted using 20 eggs per cup. Hatching was observed for 100 d in all the treatments, each treatment comprising 12 cups. After 100 d immersion, unhatched eggs were then dissected under a microscope to examine embryonation. As in the previous experiment, all adults used in the experiment were confirmed as *Ae. albopictus* prior to oviposition.

Statistical Analysis. To study the critical photoperiod of *Ae albopictus*, we fitted a negative binomial generalized linear model (Venables and Ripley 2002) to the number of eggs that hatched in 25 d intervals during the 100 d of Experiment 1, as a function of both day length and the day when unhatched eggs were counted. We chose a negative binomial model to account for the over-dispersion in the number of hatched eggs (Mangel 2006). The model and parameter estimates are presented in Supp Table 1 (online only).

A Kruskal–Wallis rank sum test was used to compare the hatching day of eggs with different postoviposition ages in the four different light treatments resulting from the combination of growing photoperiod and hatching photoperiod of Experiment 2. For Experiment 2, we also developed an egg hatching hazard model. *Aedes albopictus* egg hatching (eh_x) was estimated daily using the equation $eh_X = Eh_X/eh_0$, where Eh_X indicates the cumulative number of eggs that remained without hatching up to day X, and eh_0 the initial number of eggs in each treatment, i.e., 120. Since eh_X can be seen as analogous to a survival schedule, daily egg hatching was analyzed using a Cox proportional hazard model. The Cox hazard model considered the additive effects of light treatment and the number of weeks eggs were dormant (i.e., post-oviposition age) as covariates driving the hatching hazards. In the Cox hazard model, egg hatching was modeled using a baseline hazard function h_0, so that the hazard function $h(t) = h_0\exp(\mathrm{f}(covariates(t)))$ measured the proportional increase in egg hatching. When implementing the Cox hazard model, we only counted the eggs that were still viable (alive) at day 100 when the experiment finished. We compared whether unhatched eggs across the range of postoviposition times and light treatments had similar dead to alive odds ratios at the end of the experiment (day 100), using a binomial generalized linear model (Faraway 2006).

Results

Field Survey of *Ae. albopictus*. Mean temperature dropped below 17°C from late December to early March, with the lowest in late January (Fig. 1A). Precipitation decreased suddenly from mid-November (Fig. 1B). Though precipitation was above 80 mm per day in early November, there was little rain from mid-November to mid-March. Discarded containers were the most abundant container in most households (Table 1). One household (No. 6) had the highest

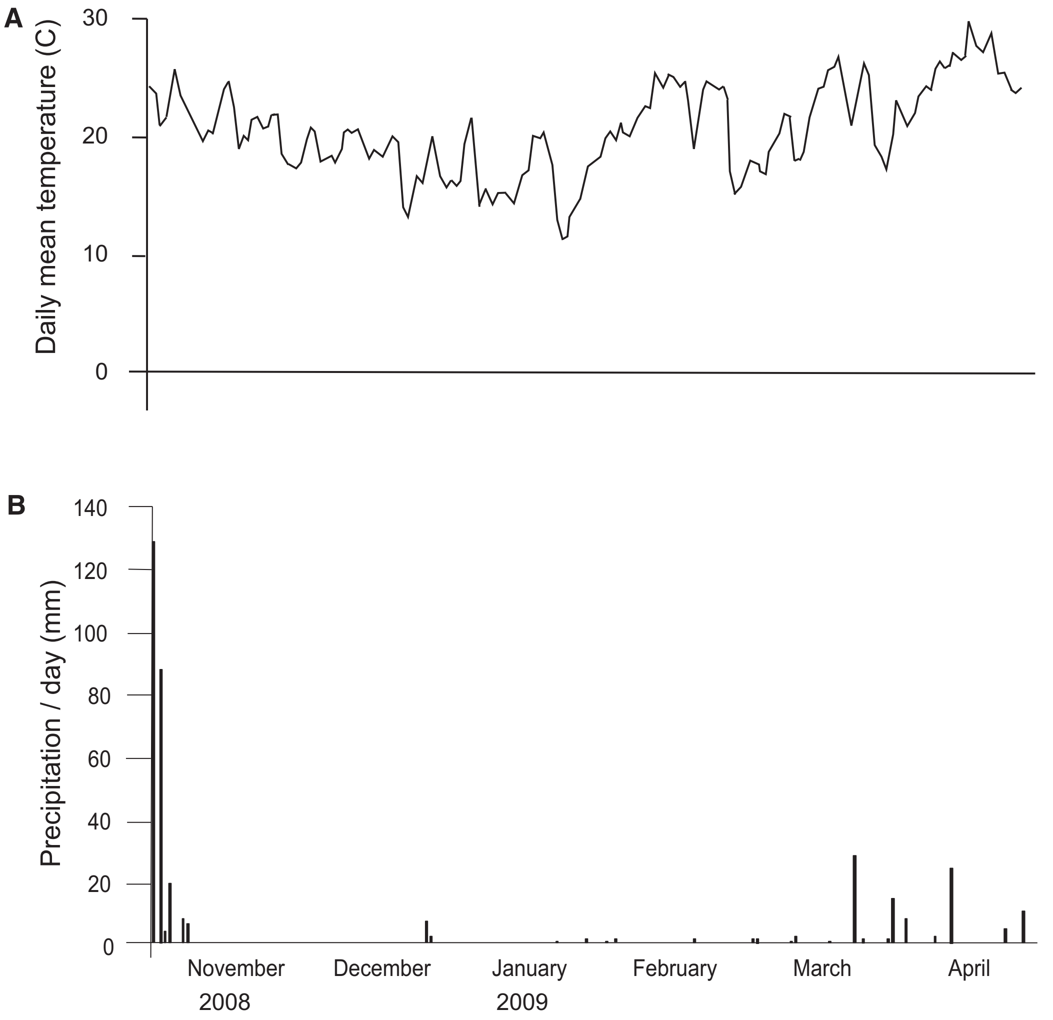

Fig. 1. Meteorological data from November 1, 2008, to April 30, 2009, in Hanoi. (A) Daily mean temperature. (B) Daily precipitation.

Table 1. The mean number ± SE of water containers in the houses surveyed from November 2008 to April 2009

HH[a]	1	2	3	4	5	6	7	8	9	10	11	12
Jar	0.3 ± 0.1	0.4 ± 0.1	0	0.2 ± 0.1	0.2 ± 0.2	0	0	0	2.0 ± 0.6	0	0.1 ± 0.1	0
RCT[b]	0.8 ± 0.2	0.3 ± 0.1	1.2 ± 0.2	0.3 ± 0.1	1.7 ± 0.1	0	0.8 ± 0.1	0	0.5 ± 0.3	1.1 ± 0.5	0.8 ± 0.2	0.1 ± 0.1
OCT[c]	0.3 ± 0.1	1.9 ± 0.1	2.9 ± 0.4	1.2 ± 0.2	0.3 ± 0.1	0	0.2 ± 0.1	1.1 ± 0.1	1.5 ± 0.3	1.0 ± 0.4	0.8 ± 0.3	0.9 ± 0.1
PD[d]	0.2 ± 0.1	0	0	0	0	0	0	0.1 ± 0.1	0	0	0	0
Bucket	2.3 ± 0.3	0.3 ± 0.1	0	2.2 ± 0.9	0	0.1 ± 0.1	0	0	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
FV[e]	0	0.2 ± 0.2	0.8 ± 0.7	0.5 ± 0.3	0.3 ± 0.2	5.3 ± 3.1	0	0.8 ± 0.6	0	0.8 ± 0.4	0.4 ± 0.4	0
PT[f]	0	0	0	0	0.2 ± 0.2	0	0	0	0	0	0	0
Bonsai	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	3.7 ± 1.3	0.4 ± 0.3	6.8 ± 3.7	0.4 ± 0.1	3.0 ± 2.0	0.7 ± 0.5	2.3 ± 0.8	0.5 ± 0.2	0.6 ± 0.1
TCT[g]	0.1 ± 0.1	0	0	0	0	0	0	0	0	0.3 ± 0.1	0	0
DC[h]	0.6 ± 0.3	5.3 ± 1.5	2.3 ± 0.6	5.3 ± 1.5	3.9 ± 0.7	23.1 ± 9.4	1.1 ± 0.5	1.7 ± 0.5	0.4 ± 0.3	2.2 ± 0.8	0.9 ± 0.4	0.8 ± 0.4
Others	0.1 ± 0.1	0	0.1 ± 0.1	0	0.2 ± 0.1	5.5 ± 5.5	0.2 ± 0.2	0.3 ± 0.3	0	0.1 ± 0.1	0.1 ± 0.1	0
Total	4.7 ± 0.2	8.7 ± 0.5	7.4 ± 0.3	13.2 ± 0.5	7.1 ± 0.4	40.8 ± 2.1	2.6 ± 0.1	7.0 ± 0.3	5.3 ± 0.2	7.9 ± 0.3	3.7 ± 0.1	2.4 ± 0.1

[a]House hold, [b]Round concrete tank, [c]Other concrete tank, [d]Plastic drum, [e]Flower vase, [f]Pig through, [g]Toilet concrete tank, [h]Discarded.

number of flower vases, Bonsai, and discarded containers. The mean number of containers with water was 3.0 to 10.6 from November to February and it was above 10 from March to April (Fig. 2A). There was no correlation between mean number of containers with water and the precipitation from the previous survey date to the current survey ($P > 0.05$). The container index was highest in early November and then

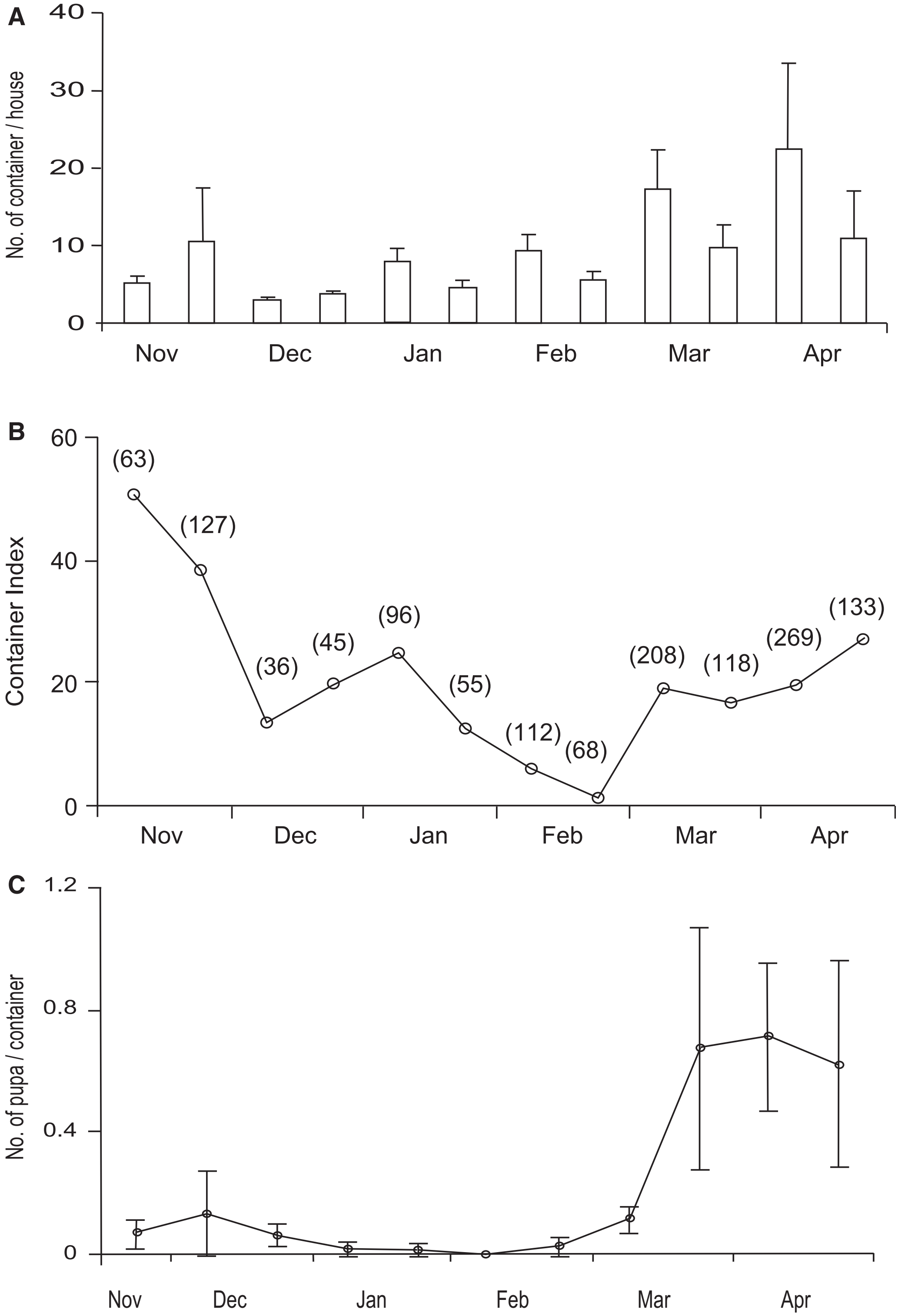

Fig. 2. (A) Mean number of containers with water in a household of Bat Trang from early November 2008 to late April 2009. (B) Container index of *Aedes* mosquitoes in Bat Trang from early November 2008 to late April 2009. Number in parenthesis is total number of containers holding water. (C) Mean number of *Ae. albopictus* pupae per container of Bat Trang from late November 2008 to late April 2009. Bars indicate SE.

decreased gradually, reaching a minimum in late February (Fig. 2B). The container index gradually increased from March onward. Density of pupae per container was low from November to February and increased from March onward (Fig. 2C).

We collected 3,244 *Culex pipiens quinquefasciatus* Say, 102 *Ae. albopictus*, 38 *Armigeres subalbaltus* (Coquillett), 21 *Cx. vishuni* Theobald, 14 *Anopheles sinensis* Wiedemann, 11 *Cx. tritaeniorhynchus* Giles, and 6 *An. tessellatus* Theobald from November to April by BG sentinel trap. The number of *Ae. albopictus* adults per trap decreased from November, reaching a minimum in January, and increased from February onward (Fig. 3). Ovitraps were positive at a rate of over 0.5 until mid-December, then less than 0.4 from late December to early March (Fig. 4A). The number of eggs decreased from November onward, nearly

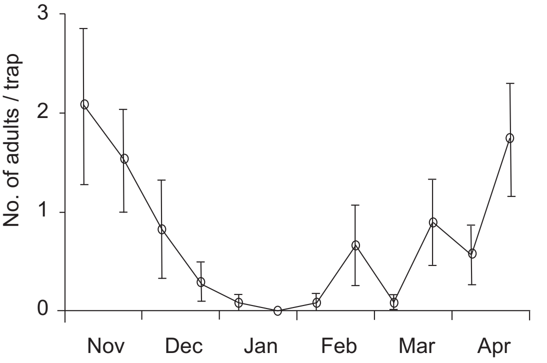

Fig. 3. Mean number of *Ae. albopictus* adults collected by BG Sentinel trap in Bat Trang from early November 2008 to late April 2009. Bars indicate SE.

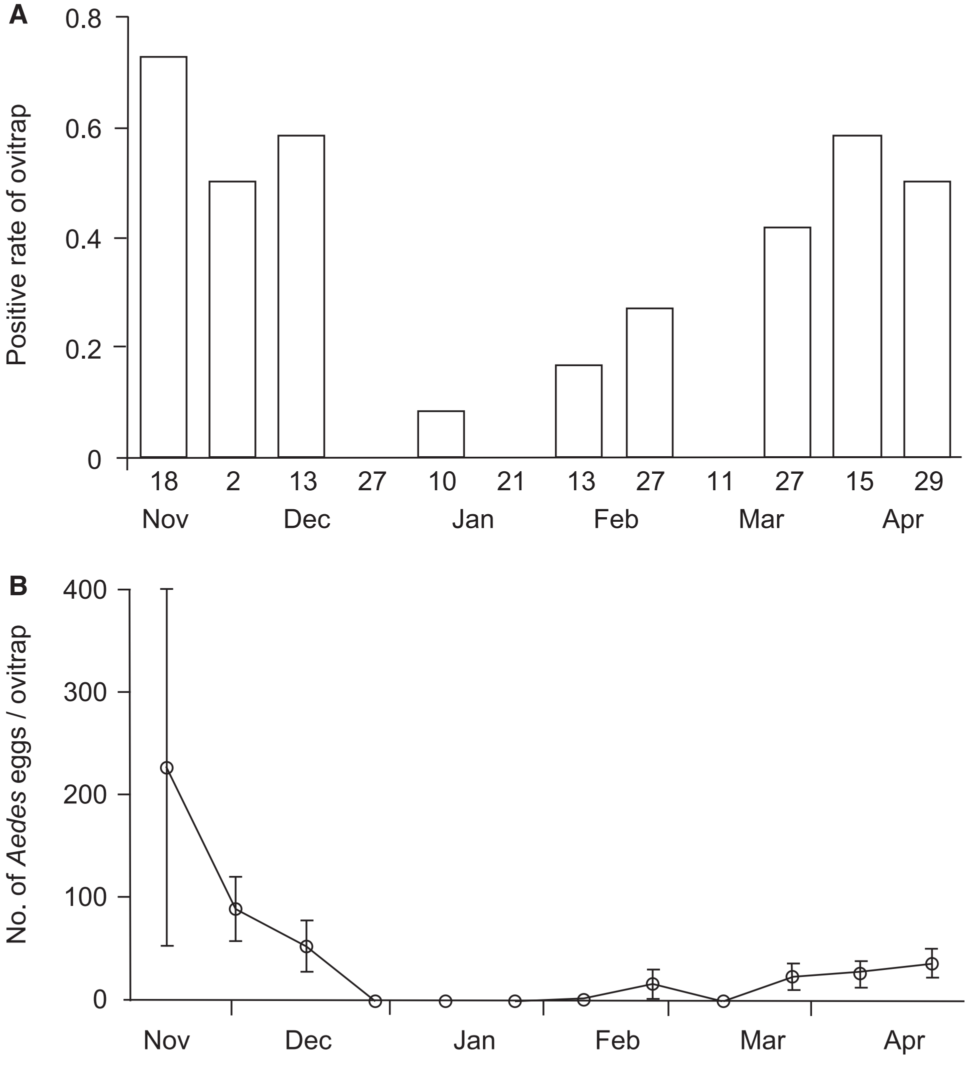

Fig. 4. (A) Ovitrap positive rate of ovitrap in Bat Trang from late November 2008 to late April 2009. Numbers under x-axis are the collection date. (B) Density of *Aedes albopictus* eggs collected from ovitraps in Bat Trang from late November 2008 to late April 2009. Bars indicate SE.

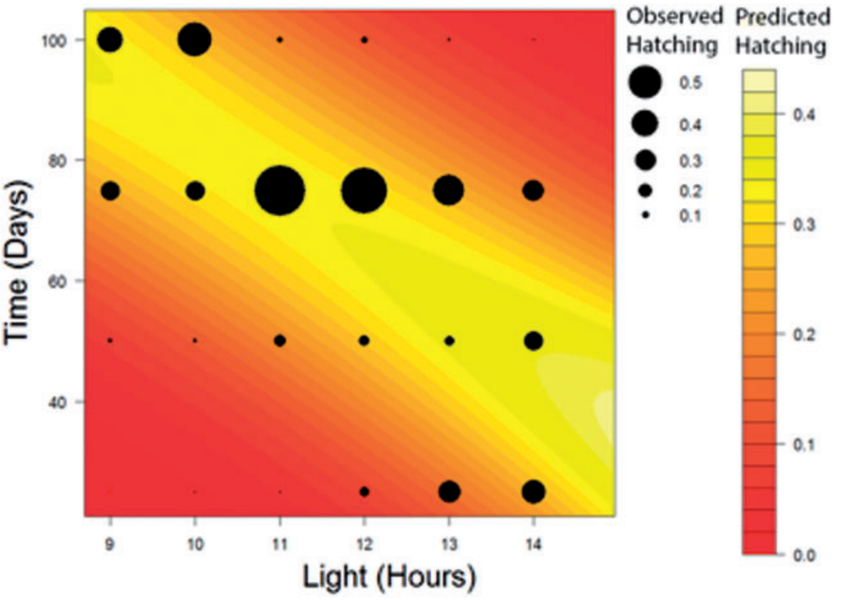

Fig. 5. Critical photoperiod for *Aedes albopictus* egg hatching. The *x* axis is the egg day-length during Experiment 1, the *y* axis the day cumulative hatching was recorded, colors indicate egg predicted hatching probability for different times and day lengths (a color graded scale is at the right of the main plot). Circles represent the observed data, and circle size is proportional to the observed hatching probability, and a reference scale is provided to the right of the main plot. Parameters employed for the probability surface construction are presented in Suppl Table 1 (online only).

reaching zero from late December to early March, except for late February (Fig. 4B). Both positive rate and the mean number of eggs increased after late March, though there were fewer eggs in spring than in autumn.

Laboratory Diapause Experiment. The egg hatching probability tended to be high at longer day length for the 25 d observation period (Fig. 5). However, as the observation period was extended, the day length at the maximum hatching probability was reduced. The hatching probability was highest at an 11 hr day during 75 d of the observation period in the Experiment 1.

When females were reared under long day length (Long–Long, Long–Short) and their eggs immersed 1 and 5 wk postoviposition, >50% of eggs hatched within 20 d (Fig. 6A and B). Hatchability of eggs from females reared under long-day conditions and immersed 10 wk postoviposition was between 0.6 and 0.7 after 100 d. When females were reared under short-day (Short–Long, Short–Short) and their eggs immersed after 1 wk, hatching were suppressed for about 60 days (Fig. 6C and D). When eggs of short-day females were immersed 5 or 10 wk postoviposition, hatchability was higher than that of 1-wk postoviposition eggs. The Cox Proportional Hazards model showed that both time post-oviposition and light treatment had an effect on egg hatching (Table 2). When we examined unhatched eggs for embryonation at the end of the 100-d observation period, both time postoviposition and light treatment had an effect on embryonation status (Table 3).

The median day of hatching was also significantly different when females were reared under long day (Long–Long, $\chi^2 = 91.00$, df = 1, $P < 0.001$; Long–Short, $\chi^2 = 133.21$, df = 1, $P < 0.001$) (Fig. 7A and B). When females were reared under short-day, the median day of hatching was earlier in eggs dry for 5 and 10 wk than those dry 1 wk (Short–Long, $\chi^2 = 165.67$, df = 1, $P < 0.001$; Short-Short, $\chi^2 = 318.02$, df = 1, $P < 0.001$) (Fig. 7C and D). This indicates that extended time until immersion accelerates hatching of eggs in diapause.

Discussion

Our results indicate that reproduction of *Ae. albopictus* was markedly suppressed during winter in Hanoi, although there was some oviposition. The primary reason for low winter reproduction is that flight activity is reduced, as seen *Ae. aegypti* (Christophers 1960), as the average air temperature is 17°C and the minimum average temperature is 12°C in January (Weatherbase 2013). Unlike *Ae. aegypti* which prefers concrete tanks underground, *Ae. albopictus* prefers containers put outside (e.g., garbage and flowerpots) (Tsunoda et al. 2014), exposing them to colder temperatures. Data indicate that *Ae. aegypti* is sluggish below 17°C and flies with difficulty at 12–14°C (Christophers 1960). Thus, flight activity would seem problematic for *Ae. albopictus* in January.

A second consideration is egg diapause in winter. Geographic variation of the photoperiodic response is known for North American and East Asian populations of *Ae. albopictus*, and populations collected from

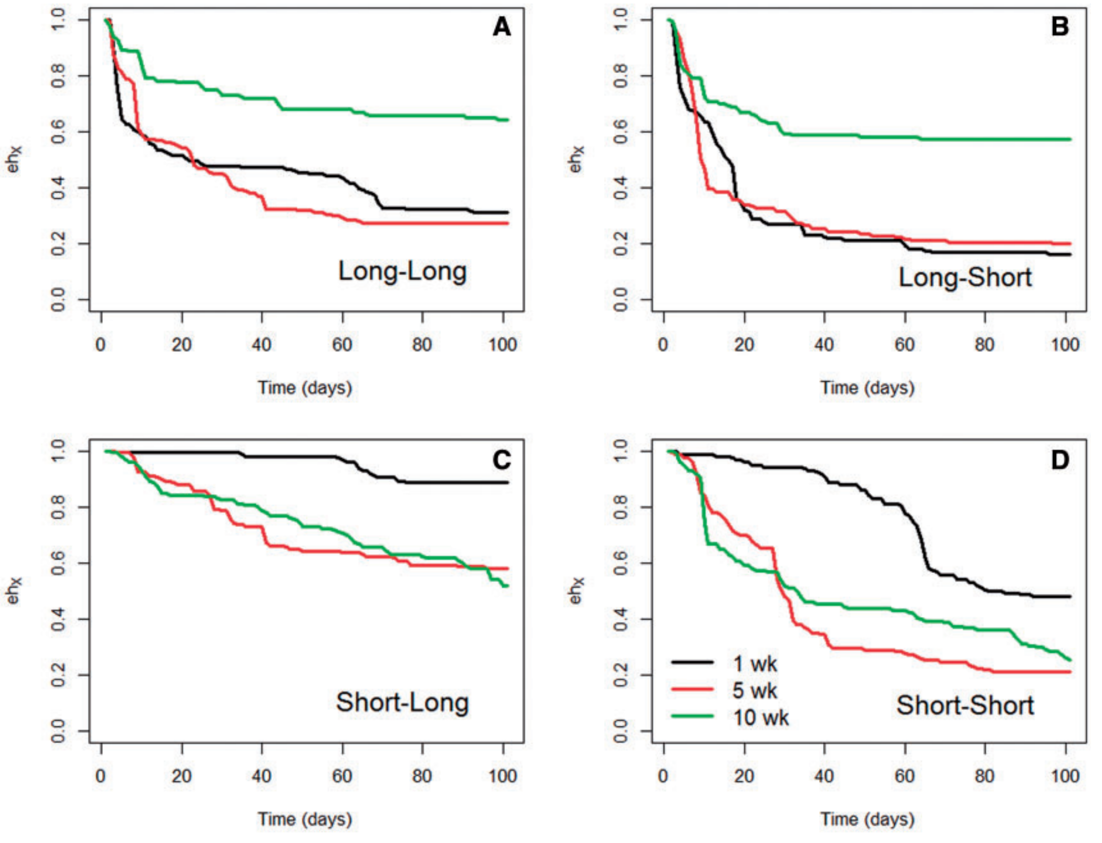

Fig. 6. *Aedes albopictus* egg hatching trajectories under different light treatments and post-oviposition times (A) Long–Long light treatment (lt), (B) Long–Short lt, (C) Short–Long lt and (D) Short–Short lt. For the postoviposition times, refer to the inset legend in (D).

Table 2. Cox Proportional Hazards for *Aedes albopictus* egg hatching as a function of day length (Light Treatment) and time since oviposition (Time)

Factor	Hatching hazard	Estimate	SE	Z	Pr (>|z|)
Time (wk)					
1	1	–	–	–	–
5	1.478	0.391	0.073	5.332	<0.0005*
10	1.165	0.153	0.082	1.861	0.0627
Light treatment					
Long–Long	1	–	–	–	–
Long–Short	0.519	−0.656	0.082	−8.021	<0.0005*
Short–Long	0.388	−0.948	0.103	−9.227	<0.0005*
Short–Short	1.388	0.328	0.082	4.017	<0.0005*

*Statistically significant, $P < 0.05$.

Table 3. Odds for unembryonated versus embryonated eggs at the end of the experiment (day 100) as a function of time since oviposition and light treatment

Factor	Odds Ratio	Estimate	SE	Z	Pr(>|z|)
Time (wk)					
1	1	–	–	–	–
5	9.764	2.279	0.247	9.221	<2e−16*
10	4.273	1.452	0.237	6.139	8.33E−10*
Light treatment					
Long–Long	1	–	–	–	–
Long–Short	0.182	−1.704	0.213	−7.997	1.28E−15*
Short–Long	0.386	−0.951	0.213	−4.455	8.38E−06*
Short–Short	0.186	−1.679	0.240	−7.009	2.39E−12*

*Statistically significant, $P < 0.05$.

Taiwan and Hong Kong do not exhibit diapause (Hawley et al. 1987, Philippi and Seger 1989). However, though Hanoi is located at a lower latitude than Taiwan and Hong Kong, our data show that hatchability of *Ae. albopictus* eggs collected here were affected by day length, as eggs laid under short-day delayed hatching. Since the hatching rate of *Ae. albopictus* eggs is low during winter in Okinawa Island despite a high embryonation rate, they are considered in "light dormancy" (Higa et al. 2007). Our results suggest the same situation in Hanoi.

Bet-hedging is defined as a strategy, where unpredictably variable environments favor genotypes with lower variance in fitness at the cost of lower mean fitness (Cohen 1966, Philippi and Seger 1989, Hopper 1999, Ripa et al. 2010). When species do not have reliable cues for the start of unfavorable environmental conditions, natural selection will favor either genotypes

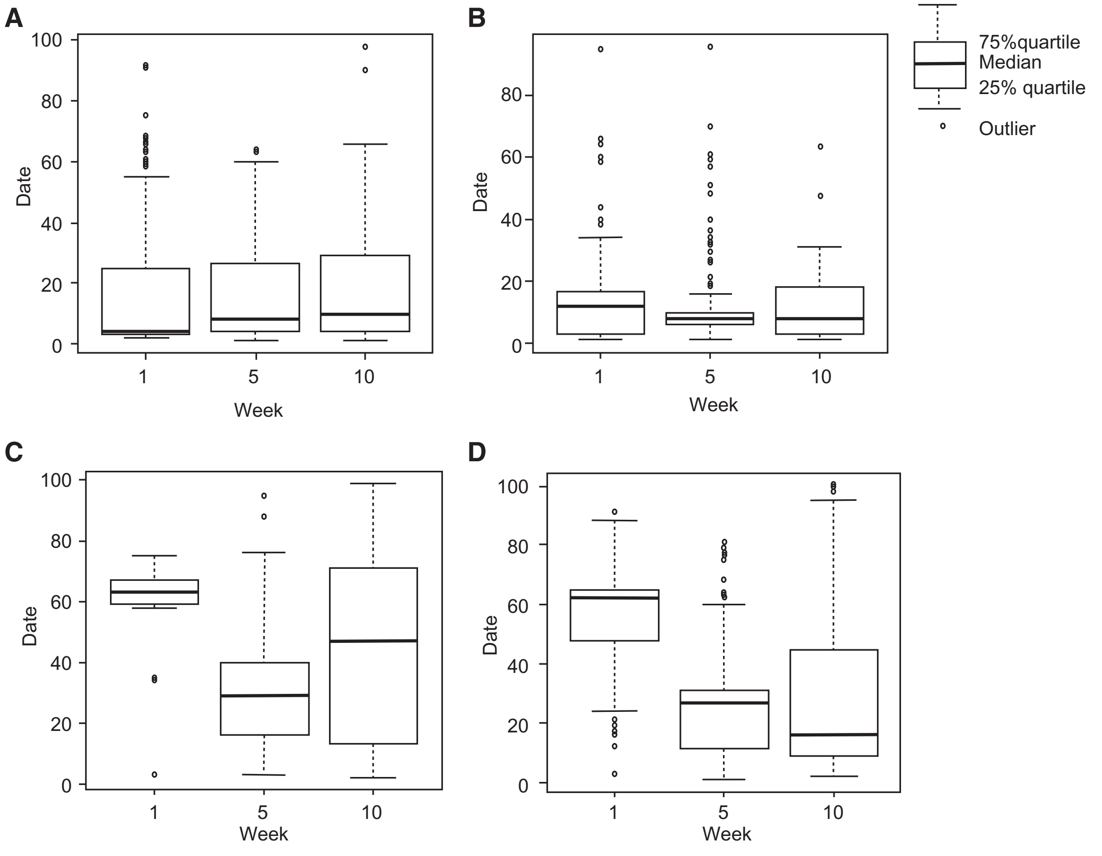

Fig. 7. Median day of *Aedes albopictus* egg hatching after immersion in water under different day length. (A) Long–Long, (B) Long–Short, (C) Short–Long, (D) Short–Short. 'Long' means 14 hr light and 10 hr dark condition. 'Short' means 10 hr light and 14 hr dark condition. Combination of day length (e.g., Long–Long) indicates the day-length condition of eggs before and during the observation.

with an obligate diapause commencing before conditions become unfavorable, or genotypes that produce both diapausing and non-diapausing phenotypes with or without modification of diapause frequency by environmental cues (Seger and Brockmann 1987, Hopper 1999). Diapause theory suggests that in environments with unpredictable lengths of favorable seasons, the proportion of individuals diapausing should increase during the favorable season as the likelihood of completing another generation declines. This leads to that a gradual increase in the proportion diapausing as the season advances, suggesting risk-spreading as long as the variation in diapause frequency is not genetic (Seger and Brockmann 1987, Hopper 1999).

Egg-hatching in *Aedes* mosquitoes is regarded as bet-hedging, since emergence is often staggered even if environmental conditions are favorable and development is prepared (Evans and Dennehy 2005). Since a bet-hedging strategy implies an evolutionary tradeoff between risk aversion and early reproduction, many organisms may be affected by competing selective pressures for both immediate and delayed hatch (Khatchikian et al. 2010). Selection for risk-spreading might explain variation in frequency of diapauses in species with facultative diapause (Walker 1980, Bradford and Roff 1993, Groeters 1994, Fontes et al. 1995). Assuming a model that considers genetic variation in the duration of egg dormancy in populations occupying larval habitats that occasionally become entirely unsuitable, the model shows that a more catastrophic environment will favor the late-hatching allele, presenting the possibility for a decline in the intrinsic rate of increase (Denlinger and Armbruster 2014) with an increase in environmental uncertainty (Livdahl 1979). In *Aedes triseriatus*, both low precipitation and high variability in precipitation directly increase the delaying pattern, which is an adaptive bet-hedging strategy that allows the species to manage desiccation risks (Khatchikian et al. 2010).

As precipitation is low during winter in Hanoi, it is reasonable to conclude that delaying egg hatching under short day would develop as risk aversion for low and unpredictable precipitation in winter. Since season and precipitation are closely related in Hanoi, the delaying pattern would be influenced by day length. The sharpness of the photoperiodic response will be greater the larger the standard deviation from the mean maturation date of a particular generation, which may explain why mosquitoes, which usually have short generation times, have diapausing fractions which increase only gradually as the season advances (Cohen 1970). Insects that were under greater pressure from natural selection for timing of the induction of the overwintering exhibit a steeper curve through the critical photoperiod (Lees 1968). The strains of *Ae. albopictus* from northern Asia and North America showed higher overwintering survival rates than the strains from tropical Asia, Hawaii, and Brazil in field experiments (Hawley et al. 1989). Our results show that hatchability gradually changed with day-length, suggesting that natural selection for overwintering is not as strong as in temperate zone *Ae. albopictus*.

Our study also showed that *Ae. albopictus* eggs hatched at higher rates as the period before or after immersion in water was prolonged, suggesting that hatching is dependent on the energy content of the eggs themselves. Diapausing insects with low energy reserves have higher mortality during diapause than those with enough energy reserves (Hahn and Denlinger 2007). Metabolism is proportional to temperature in diapausing insects (Irwin and Lee 2003). The low temperatures during winter greatly favor conservation of energy reserves to maintain high survival (Irwin and Lee 2000). Though diapause is not uncommon phenomenon among tropical insects, metabolic depression is still important in diapausing insects living here (Denlinger 1986). When winter temperatures are mild, as in Hanoi, it may be hard for eggs to maintain low metabolic levels, even if they enter diapause.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

Acknowledgments

K. Tanaka (Ecological Laboratory, General Education, Miyagi Gakuin Women's College) provided stimulating discussion on many topics. We appreciate N. Minakawa (Department of Vector Ecology and Environment, Institute of Tropical Medicine, Nagasaki University) for various suggestions. We are very grateful to the Public Health Center staff in Bat Trang commune, Hanoi, for support of mosquito collection in the field. L.F.C. was funded by Nagasaki University (Program for Nurturing Global Leaders in Tropical and Emerging Communicable Diseases). This work was supported by the Center for Infectious Disease Research in Asia and Africa, Vietnam Research Station, Nagasaki University, and the Program of Japan Initiative for Global Research Network on Infectious Diseases (J-GRID), MEXT, Japan.

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Received 26 February 2015; accepted 25 July 2015.