Photoelectric Sensing Device for Recording Mosquito Host-Seeking Behavior in the Laboratory

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ABSTRACT   A new automatic device for recording the host-seeking behavior of mosquitoes was designed using photoelectric sensors. The host-seeking rhythms of several species of mosquitoes were recorded under laboratory conditions. Use of CO_2, in addition to heating and black color to activate mosquito flight, proved to be important for evaluation of the present recording device. The diel rhythms of nonblood-fed *Aedes albopictus* (Skuse), *Aedes aegypti* (L.), *Culex quinquefasciatus* Say, *Culex tritaeniorhynchus* Giles and *Anopheles stephensi* Liston females recorded by the device corresponded well with known flight and activity rhythms in field for the same mosquito species. This simple automatic recording device provided accurate information on the flight behaviors of colonized and field collected mosquitoes.

KEY WORDS   *Anopheles*, *Aedes*, *Culex*, host-seeking, photoelectric sensor

Accurate knowledge of the host-seeking flight behavior of mosquitoes is important for controlling and regulating mosquito bites. Many previous studies of mosquito host-seeking behavior have involved field tests relying on landing collections at human or animal baits, or baited traps. The field collection of mosquitoes is one of the most reliable ways of studying mosquito flight behavior. Field studies, however, are labor-intensive, expensive, time-consuming, and sometimes place human volunteers at risk of disease transmission. Certain aspects of mosquito flight behavior can be studied in the laboratory, and those results can be extrapolated to the field to help design more efficient and safe field experiments.

Extensive laboratory studies on mosquito behavior have been reported previously. These studies can be classified into several categories, including: flight behavior (Taylor and Jones 1969, Chiba et al. 1981, Jones 1981), sugar-feeding behavior (Bowen 1992, Yee and Foster 1992), and biting or host-seeking behavior (Ho et al. 1973, Klowden and Lea 1984, Yee and Foster 1992, Takken et al. 2001).

Many laboratory and field studies on mosquito attractants such as heat, color, light, and chemical substances have been reported. Several key factors, including carbon dioxide, 1-octen-3-ol, L-lactic acid, heat ($\approx 35°C$), and black or dark color have been found to be essential for attracting mosquitoes (Takken and Kline 1989, Kline et al. 1991). These observations resulted in the development of a variety of laboratory olfactometers designed to evaluate mosquito flight behavior (Gouck and Schreck 1965, Posey et al. 1998, Takken et al. 2001) and a variety of mosquito traps (Parker et al. 1986, Burkett et al. 1998, Kline 2002) to evaluate mosquito flight behavior in the field. In addition, recording devices were designed to automatically record the flight activity and sugar-feeding behavior of mosquitoes in the laboratory (Chiba et al. 1981, Yee and Foster 1992). Many laboratory studies of mosquito host-seeking behavior, however, have relied on visual or videorecording observations of mosquito flight behavior. The host-seeking and blood-feeding behaviors of mosquitoes in nature are known to be extremely complex. Host-seeking behavior is affected by environmental conditions, mosquito physiological conditions, and host-produced cues. Blood feeding is affected by host physiology and environmental conditions. Simple recording of mosquito flight behavior, therefore, underrepresents the true complexity of mosquito behavior. The introduction of variables such as host cues into flight experiments are needed for a more comprehensive study of mosquito host-seeking behavior. In the current study, we designed an automatic recording device by using photoelectric sensors and report the results of mosquito flight experiments in the laboratory.

Materials and Methods

A General Description of the Automatic Recording Device. The recording device was composed of four photoelectric sensors, an amplifier, a programmable controller unit, a power supply, and monitoring soft-
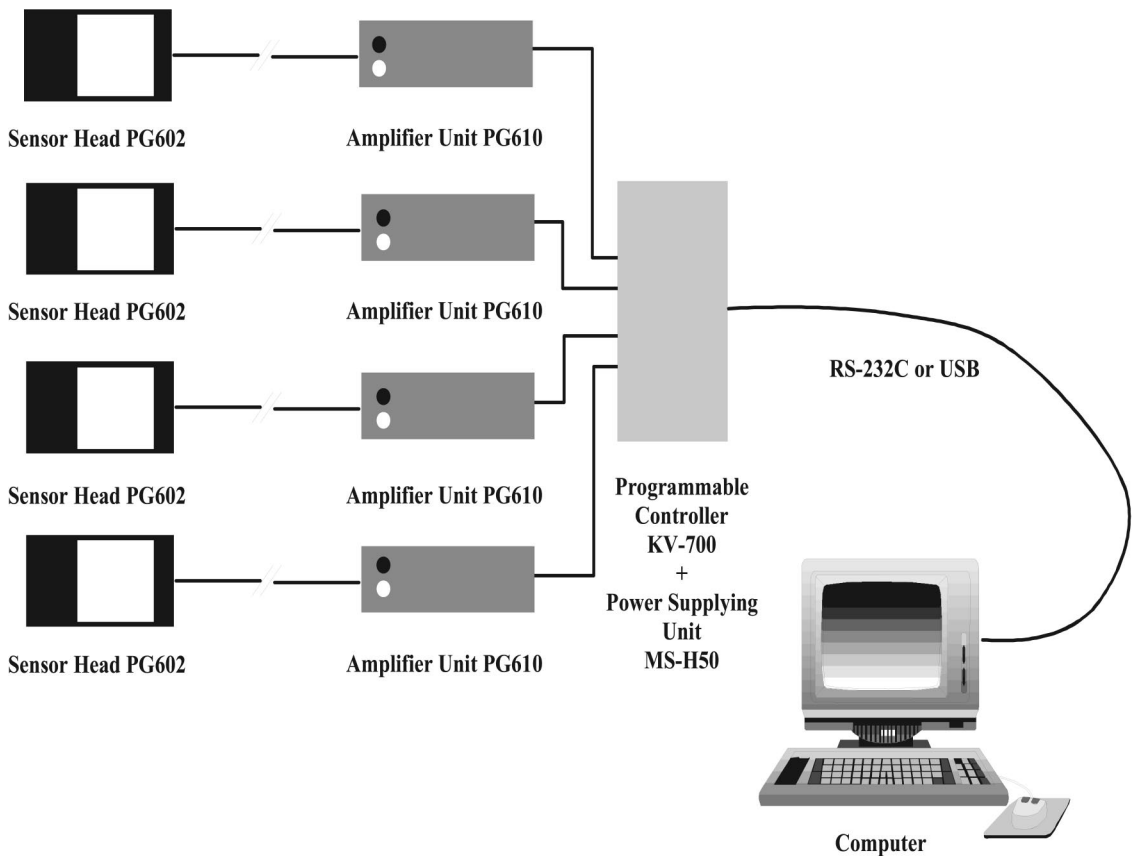
Fig. 1. Schematic view of an automatic recording device to quantify mosquito activity patterns. The device is composed of four photoelectric sensors and amplifiers, a programmable controller unit, a power supplying unit, and monitoring software installed in a computer system. Mosquitoes passing through the sensor windows, composed of an infrared LED with a light-receiving element on both sides, are detected and a signal is transmitted to the computer.

ware (Keyence Corporation, Osaka, Japan) (Fig. 1). The photoelectric sensors had a small sensor head (55 by 30 by 10 mm) with a 21 by 21-mm window. The window was composed of a pair of infrared light emission diode (LED) sensors with light receiving elements on both sides. Small objects (minimum detectable size is 0.5 mm in diameter) passing through the window were detected by the sensors, and a signal was transmitted to the computer through the controller and monitoring software. Nocturnal activity was detected by the same system by using infrared LEDs. The total cost for the recording device was ≈$4,000 (≈$500 for a sensor and ≈$2,000 for a programmable controller and monitoring software).

A water bath unit for the recording device was made from a white Styrofoam box (23 by 30 by 20 cm, 5 cm in thickness) that contained ≈3.5 liter of water (Fig. 2). The temperature of the water was maintained at ≈35°C by a heater with thermo-regulator (Power-thermo ET-30, Kotobuki Kogei Co., Ltd., Osaka, Japan). The box was covered with a white plastic panel that contained the four photoelectric sensors, the bottom sides of which were sealed with black plastic

plates. The top surface of the box, except for the sensors, was covered with a white plastic plate that helped maintain the temperature in the box.

The recording device was placed in a cage (80 by 80 by 80 cm) with white plastic panels that buffered the mosquitoes inside the cage from the external stimulations, such as airstreams, light, human movement, heat, and carbon dioxide (CO_2) (Fig. 3). Carbon dioxide at a flow rate of 500 ml/min was generated by a gas bomb and was released into the cage through a silicone tube, the opening of which was placed ≈10 cm above the sensors. The CO_2 release was regulated (2 min on/13 min off) by a solenoid valve (FSD-0408C, Flon Industry Co., Ltd., Tokyo, Japan) controlled by timer. The air inside the cage was ventilated by an electric fan located in the bottom corner of the cage. The concentration of CO_2 inside the cage was measured ≈1 cm above the sensors by a CO_2 detector (TECH-JAM Co., Ltd., Osaka, Japan). The light intensity in the laboratory was measured with a photo recorder (T&D Corp., Nagano, Japan) before starting the tests.
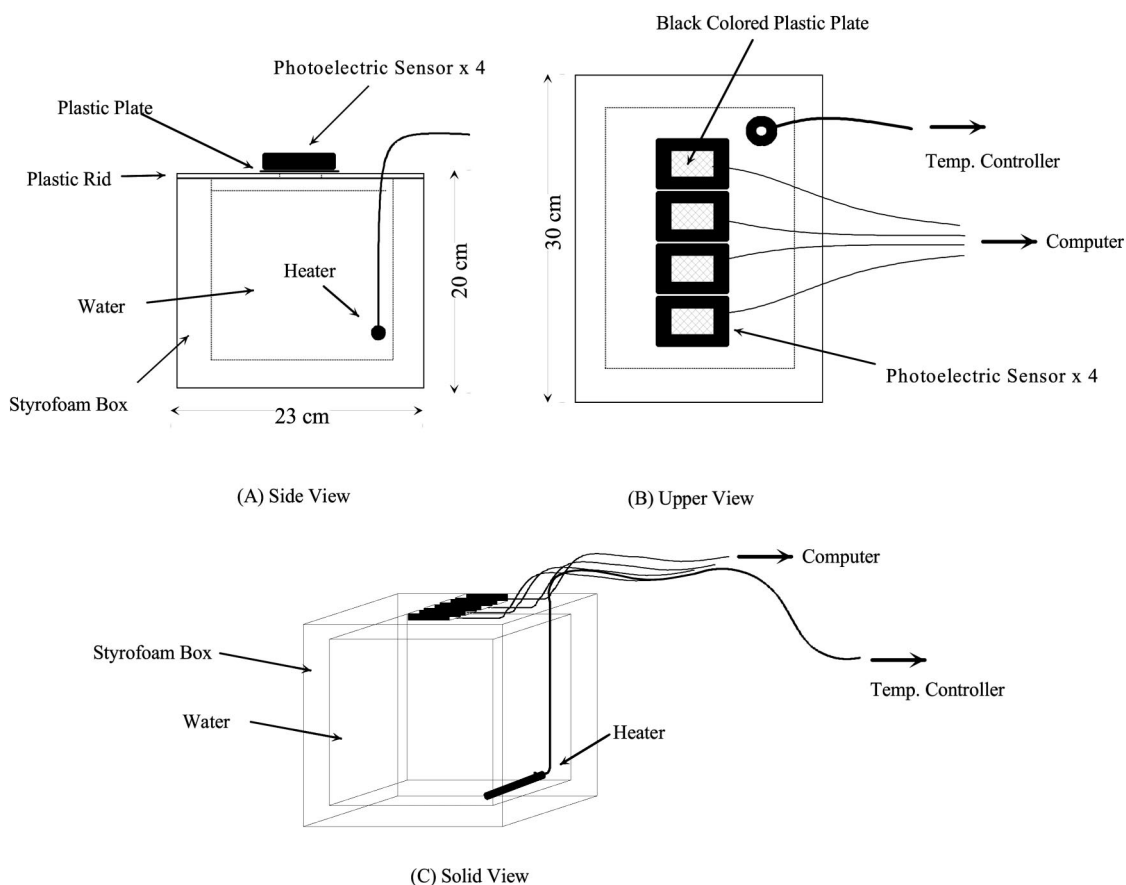
Fig. 2.   Schematic view of the water bath unit of the recording device. A Styrofoam box with 3.5 liter of water kept at 35°C by a heater is covered with a white plastic panel containing four photoelectric sensors, the bottoms of which are covered with black plastic plates. The top surface of the Styrofoam box, except for the sensor plates, is covered with another white plastic plate to concentrate the heat onto the black plates.

Recording Mosquito Flight Patterns in the Presence and Absence of CO_2. Colonized *Aedes albopictus* (Skuse), *Aedes aegypti* (L.), *Culex quinquefasciatus* Say, and *Anopheles stephensi* Liston were used for this part of the study. Both *Aedes* species were collected in Singapore during 2000. *Cx. quinquefasciatus* was collected in Vietnam during 2003. *An. stephensi* was collected in India during the 1950s. Mosquitoes were maintained in the laboratory after collection at 27°C, 70% RH, and a photoperiod of 16:8 (L:D) h. Sixty, ≈10-d-old nonblood-fed females were released into the test cage in the morning between 0700 and 1000 hours. The number of mosquitoes landing on the black plastic plate containing the photoelectric sensors was counted and recorded every 60 min for 72 h. Carbon dioxide was not released during the first 24 h of the experiment and was then released for 2 min every 15 min during the last 48 h of the experiment. Mosquitoes were provided a 1% sugar solution throughout the test. The test was carried out in a room maintained under a photoperiod of 16:8 (L:D) h where the first and last 1 h of the scotophase were in twilight, 25–27°C, and >60% RH regime.

Recording the Flight Behavior of Field-Collected and Colonized *Culex tritaeniorhynchus* Giles. Third and fourth instars of *Cx. tritaeniorhynchus* were collected from a rice paddy field in Isahaya, Nagasaki, Japan, on 14 July 2003. Larvae were reared to adults under the laboratory conditions described above. Sixty, ≈7-d-old nonblood-fed female mosquitoes were released into the test cage at 1000 hours. The number of mosquitoes landing on the black plastic plate containing the photoelectric sensors was recorded as described above. The flight behavior of colonized *Cx. tritaeniorhynchus* (collected from Nishiarita, Japan, in 1998) was recorded in the same manner and compared with the flight behavior of the wild-caught females. Sixty, ≈7-d-old nulliparous females and sixty, ≈14-d-old, parous (in their second gonotrophic cycle) colonized females were used for this test. Three replicates were carried out for each recording for both wild and laboratory colonies.
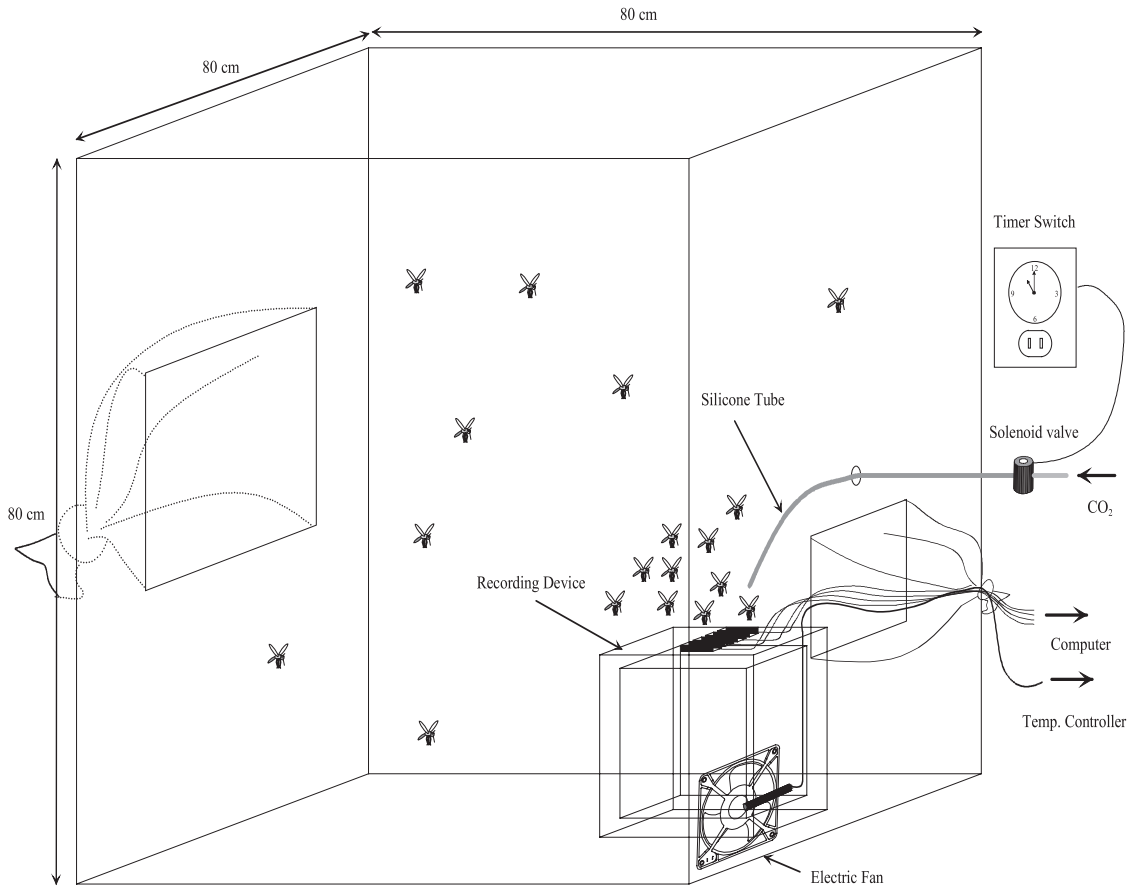
Fig. 3. Schematic view of the mosquito holding cage and recording device. Carbon dioxide was released at the rate of 500 ml/min into the cage through the silicone tube. The release of CO_2 was regulated by solenoid valve controlled by timer. The air inside the cage was ventilated by the electric fan located at the bottom corner of the cage. Mosquitoes inside the cage were provided a 1% sugar solution during the test.

Results and Discussion

The changes in CO_2 concentration in the air ≈ 1 cm above the sensors inside the cage are shown in Fig. 4. The CO_2 concentration rapidly increased to >5,000 ppm (detective limit by the detector) immediately after the release, maintained a high value (>5,000 ppm) for 2–3 min, and recovered to the baseline concentration of $\approx 2,000$ ppm rapidly after closing the solenoid valve. The CO_2 baseline concentration inside the cage was the balance of the release rate of CO_2 and the ventilation capacity with a fan and was slightly higher than that in the laboratory where the CO_2 baseline was 1,600–1,800 ppm.

Figure 5 shows the changes in the intensity of illumination in the laboratory. Intensity of illumination during the photophase, twilight phase, and the scotophase was ≈ 490, 7, and 0 lux, respectively.

Diel changes in mosquito numbers recorded by photoelectric sensors for several laboratory colonies are shown in Fig. 6. There were significant increases in mosquito activity when CO_2 was intermittently released for all species examined in the study (two-way analysis of variance [ANOVA], $P < 0.05$). This indicates that CO_2 provided a strong stimulatory effect for all mosquito species tested. Slight amounts of flight activity were recorded for both *Aedes* species (Fig. 6A and B), even when CO_2 was not released, indicating that the presence of heat and color (the black plastic recording disk) may have stimulated flight activity in these normally diurnal species. The diel activity peaks in *Aedes* mosquitoes, however, were not clearly shown in the absence of CO_2. The stimulating effect of CO_2 was more prominently shown for *Cx. quinquefasciatus* (Fig. 6C) and *An. stephensi* (Fig. 6D) than for the *Aedes* mosquitoes. The difference in the reaction of mosquitoes to CO_2 might be attributable to the difference in the importance of attractant cues, such as heat, color, and CO_2, to each mosquito species, that is to say, *Aedes* mosquitoes might use heat and visual information as attractant cues more than *Culex* and *Anopheles.* Two peaks of *Ae. albopictus* diel activity were observed, one from 1000 to 1200, and the second
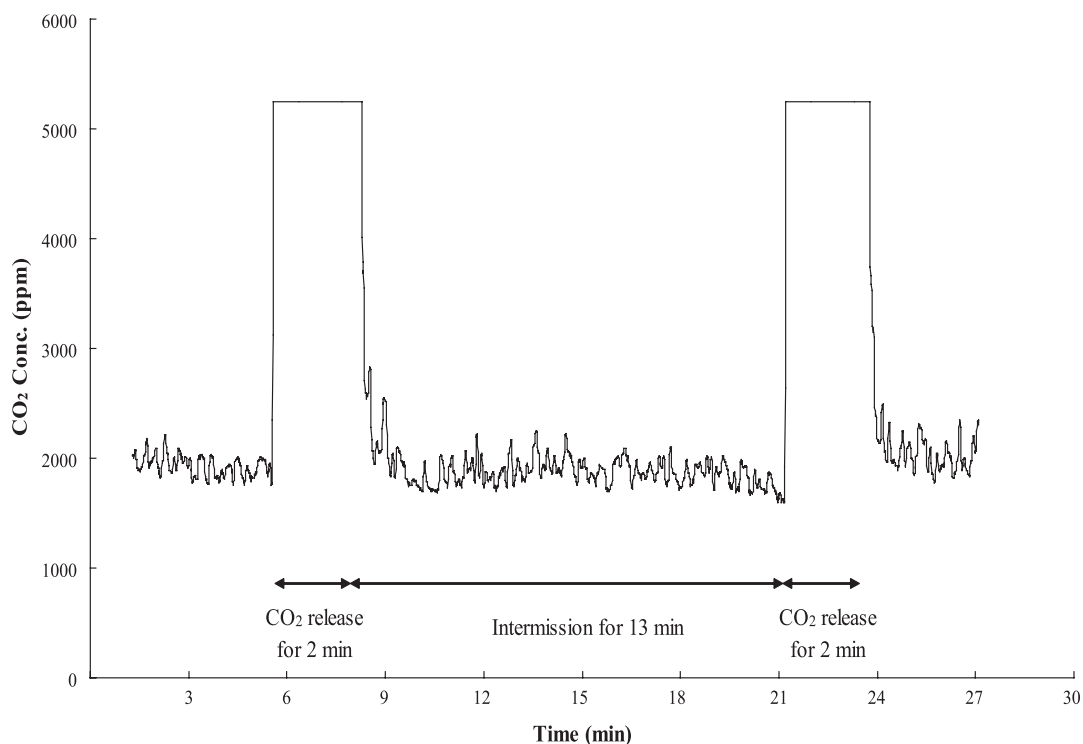
Fig. 4. Changes in the CO_2 concentration inside the test cage. Collection of CO_2 was done from the air ≈1 cm above the sensors.

from 1400 to 1600 hours (Fig. 6A). This corresponds to the diel host-seeking rhythms in field and laboratory for the same species in Singapore reported by Ho et al. (1973). Numerous studies have demonstrated that *Ae. albopictus* rarely blood feed at night and usually exhibited a bimodal diurnal host-seeking rhythm (Hawley 1988). Yee and Foster (1992) reported diel sugar-feeding and host-seeking rhythms in *Ae. albopictus* in laboratory. *Ae. albopictus* host-seeking behavior in their study, however, showed a higher activity throughout the night. Similar observations were reported by Higa et al. (2000). No *Ae. albopictus* night time host-seeking activity was observed in the current study.

Three peaks of *Ae. aegypti* flight behaviors were observed at 0600–0700, 1100–1200 and 1500–1700 hours (Fig. 6B). These results correspond well with field observations for the same species in Trinidad (Chadee and Martinez 2000) where it was observed that landings on human bait were trimodal, with consistent peaks at 0700, 1100, and 1700. A similar trimodal activity pattern was observed for *Ae. aegypti* by Atmosoedjono et al. (1972) in Indonesia and by Corbet and Smith (1974) in Tanzania. Chadee and Martinez (2000) reported an increasing number of *Ae. aegypti* females landing on human hosts during the night in an urban test area, whereas no nocturnal activity was observed in a rural test area. These authors attributed

the above-mentioned difference to the adaptation of insects to electrical lighting in the urban area. Their hypothesis was supported by Taylor and Jones (1969) who reported that both light-on and light-off had phase-setting effects to the flight activity of *Ae. aegypti*, and the total amount of flight activity was correlated with the duration of light in the 24-h period. The above-mentioned theory also might be applicable to the nocturnal activity of *Ae. albopictus.* The night time activity of both *Aedes* species in the current study, however, was very low, suggesting that the dark conditions in our study (Fig. 5) caused no phase-setting or stimulating effects to the insects.

Culex quinquefasciatus and *An. stephensi* both showed typical night time activity patterns in our study. A prominent activity peak was observed for *Cx. quinquefasciatus* at ≈0100–0500 hours, and no daytime activity was recorded (Fig. 6C). Most field studies reported that *Cx. quinquefasciatus* has a broad nocturnal activity pattern with several minor peaks ≈2200–0400 hours (Mahanta et al. 1999, Pipitgool et al. 1998). A similar high and continuous nocturnal activity was observed for *An. stephensi* (Fig. 6D). In contrast to *Cx. quinquefasciatus*, however, multiple nocturnal activity peaks were observed for *An. stephensi.*

Changes in the diel flight activity of field-collected and colonized *Cx. tritaeniorhynchus* are shown in Fig.
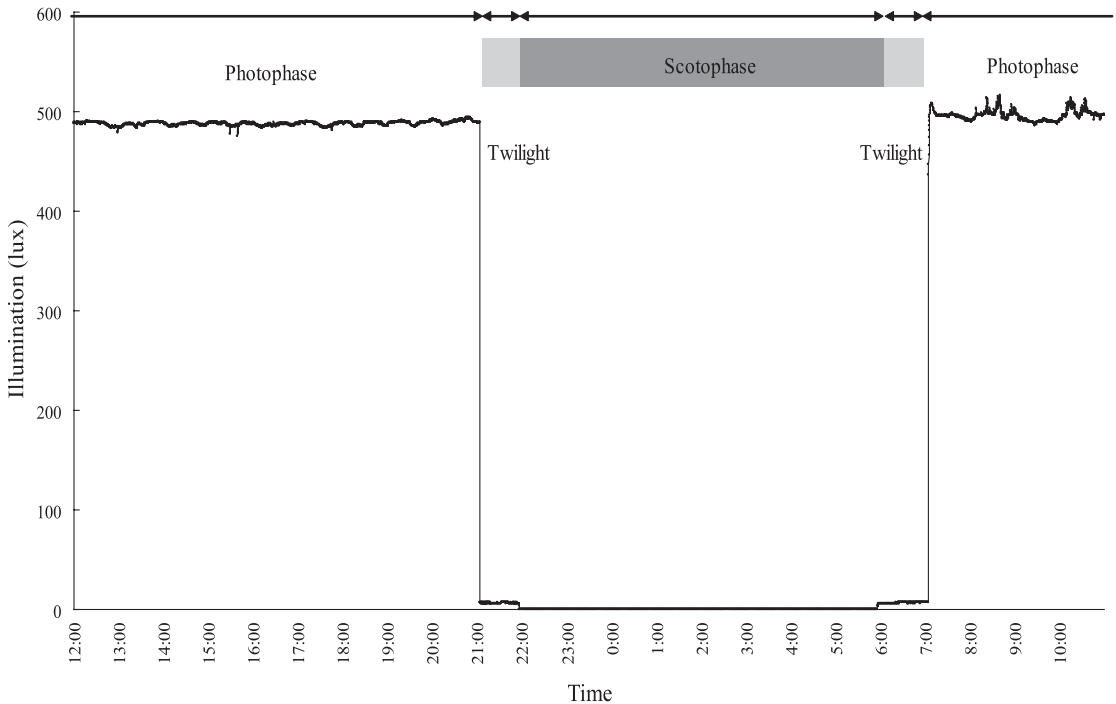
Fig. 5. Changes in the intensity of illumination in the laboratory.

7. A small activity peak at 2200 hours (just after the start of scotophase) and a larger activity peak at 0600–0700 hours (just after start of photophase) were observed in the field-collected mosquitoes (Fig. 7A). The same general bimodal activity pattern was observed for the colonized *Cx. tritaeniorhynchus* females (Fig. 7B and C). The overall activity level, however, was significantly lower for the colonized parous mosquitoes (repeated measures ANOVA, df = 1, P = 0.0176). A similar bimodal activity pattern has been long recognized for *Cx. tritaeniorhynchus* in the field (Wada 1969, Sonoda 1971). Sonoda (1971) reported that the height of each bimodal peak fluctuated regularly according the population trend and the parity rate in the evening peak was lower than that in the morning peak, suggesting that the above-mentioned fluctuation related the age composition of mosquito population. Our results, however, seem to show that *Cx. tritaeniorhynchus* intrinsically has a bimodal host-seeking pattern despite of their age, although the flight activity levels seemed to be different between nulliparous and parous adults.

Heat, dark color, and carbon dioxide have long been demonstrated as effective mosquito attractants (Takken and Kline 1989, Pates et al. 2001, Kline 2002). The addition of attractants, especially carbon dioxide, in the current study greatly enhanced mosquito flight activity. In the absence of a stimulatory substance, background flight activity for nocturnally active species (Fig. 6C and D) was not recorded by our device. Several automatic devices for recording mosquito activity patterns have been developed since the 1960s. Most of these devices record flight. Jones et al. (1967) used a small recording chamber to monitor activity patterns of individual females. Chiba et al. (1981) recorded the circadian flight activity of mosquitoes with an actograph modified by adding a phototransister and far-red beam. Yee and Foster (1992) monitored the sugar-feeding rhythms of mosquitoes with a copper landing platform that completes a circuit during the mosquito feeding. Recording of mosquito host-seeking behavior has been most successful with visual or video observations by using animal or human bait. The automatic recording of mosquito activity patterns are most successful in the absence of a host because, once blood fed, mosquito activity patterns change drastically. The recording device reported in the current study will provide an alternate method to record mosquito activity patterns in the presence of stimulatory effects such as CO_2. The diel activity patterns of non blood fed *Ae. albopictus, Ae. aegypti, Cx. quinquefasciatus, Cx. tritaeniorhynchus,* and *An. stephensi* females recorded by the device compared well with the published diel activities for these species in the field. The device reported here can be used to evaluate the activity patterns of field-collected mosquitoes as well as other hematophagous species that use CO_2, heat, and vision as major cues for orientation to hosts.
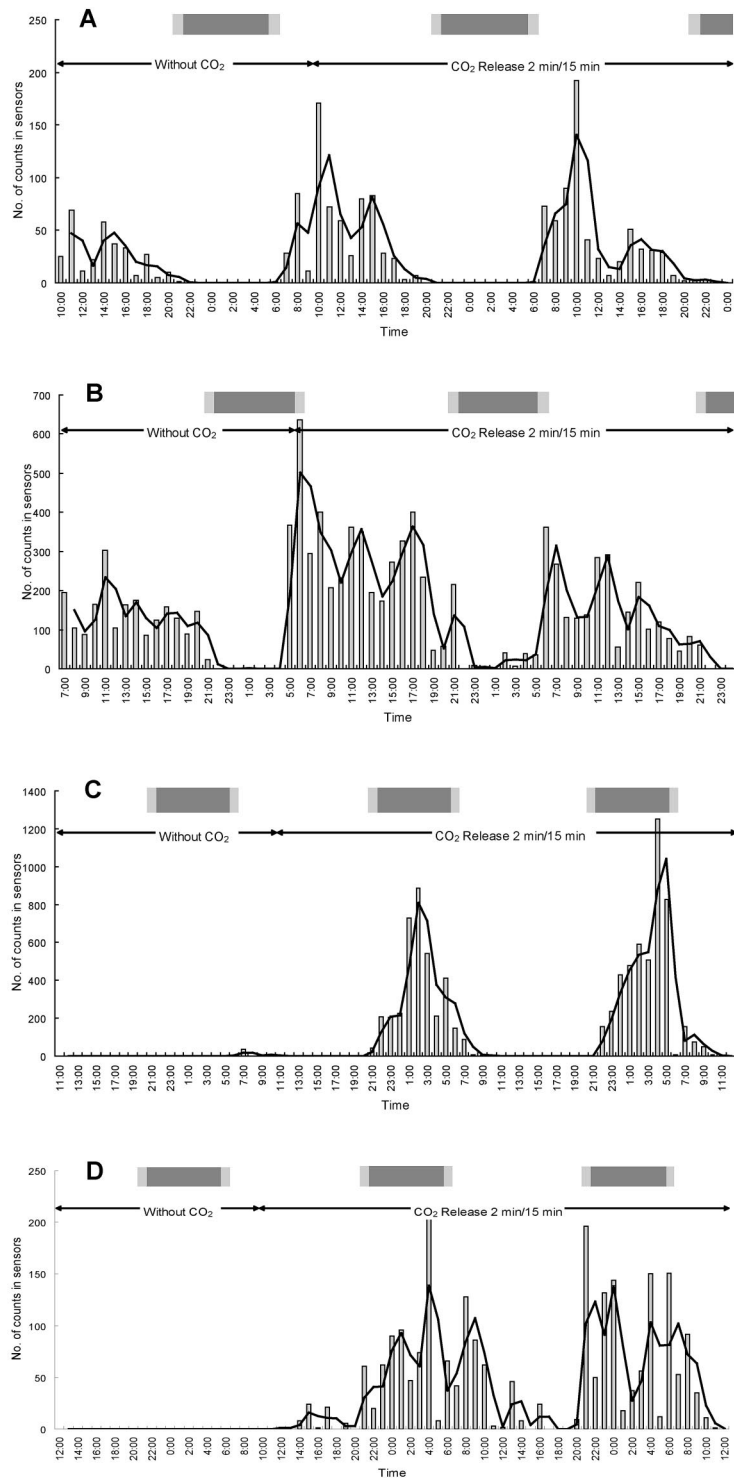
Fig. 6. Activity patterns of female (A) *Ae. albopictus*, (B) *Ae. aegypti*, (C) *Cx. quinquefasciatus*, and (D) *An. stephensi* detected and recorded by the automatic recording device. The solid line indicates a moving average of two successive counts in the sensors. Carbon dioxide was not released during the first 24 h of the test and was then released intermittently (for 2 min at 15-min intervals) during the final 48 h of the test.
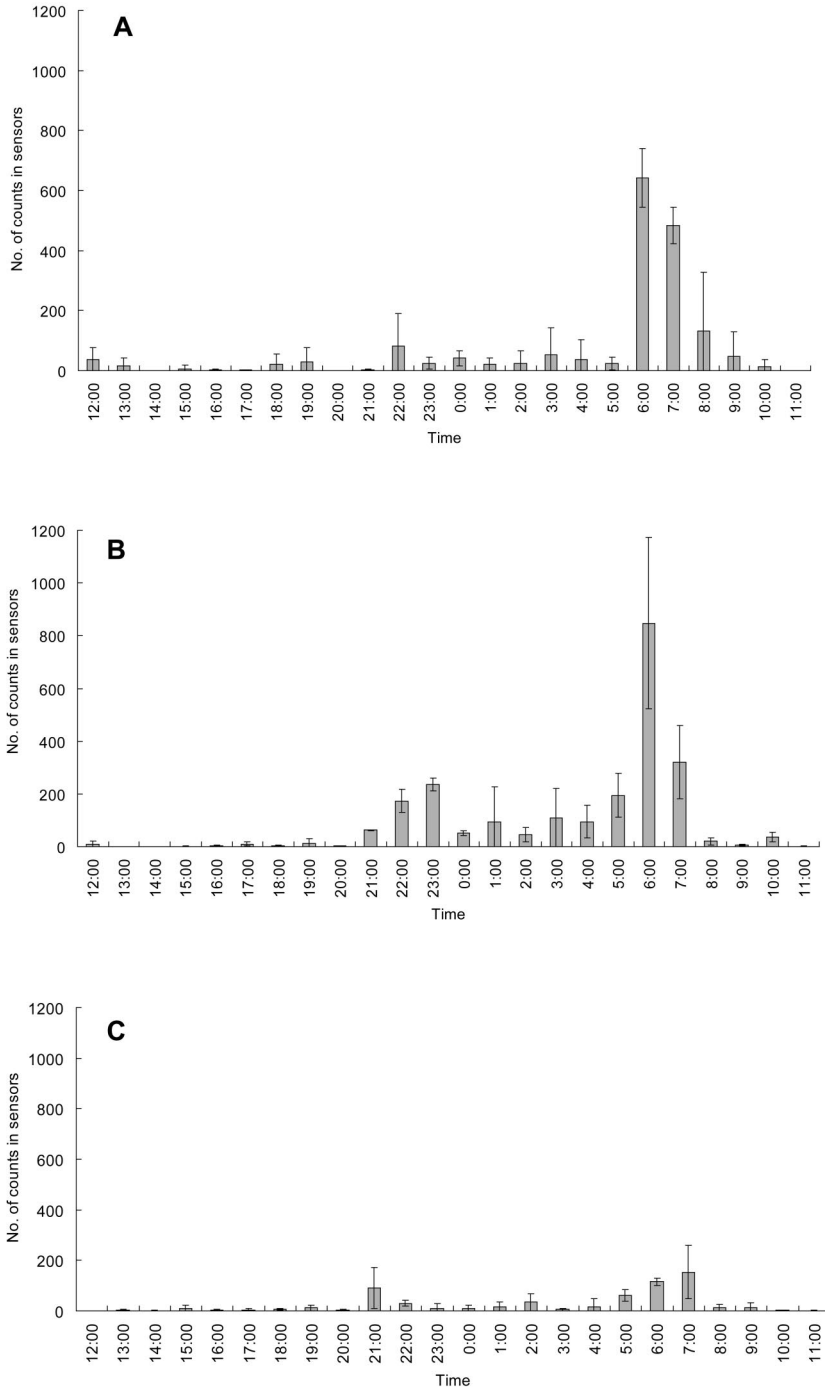
Fig. 7. Activity patterns of (A) field-collected nulliparous *Cx. tritaeniorhynchus* females, (B) laboratory-reared nulliparous *Cx. tritaeniorhynchus* females, and (C) laboratory-reared parous *Cx. tritaeniorhynchus* females detected and recorded by the automatic recording device. Each solid bar indicates the standard deviation. Carbon dioxide was released intermittently (for 2 min at 15-min intervals) throughout the experiment.

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