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# Effects of varying photoperiodic regimens on oviposition behavior of *Anopheles subpictus* and *Culex quinquefasciatus*

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## Abstract

**Background** Environmental factors influence the mosquito behavior, particularly the oviposition behavior. Therefore, understanding the response of mosquitoes to their environmental conditions like photoperiod, humidity, rainfall, etc., can lead to more precise predictions of transmission cycles which help in the development of more effective vector control strategies. To understand the importance of photoperiod in determining the oviposition cycle of *Anopheles subpictus* and *Culex quinquefasciatus*, experiments were conducted under different conditions of normal light (LD 12:12), reversed photoperiod (DL 12:12) and continuous light (24 LL) for three consecutive days.

**Results** It was noticed that ovipositional activity was exclusively restricted to the scotophase. When both the mosquito species were exposed to reverse photoperiod, the oviposition activity also reversed but they showed an erratic oviposition behavior when exposed to 24 h light condition.

**Conclusions** Understanding the oviposition behavior of mosquito species may favor to design and develop new vector control strategies.

**Keywords** Photoperiod, Oviposition behavior, *Anopheles subpictus*, *Culex quinquefasciatus*

## Background

The amount of light present throughout a 24-h clock is referred to as photoperiod (Gillot, 2005; Shi et al., 2017). It is commonly known that both natural and artificial light has an impact on organisms (Bradshaw & Holzapfel, 1975; Kollberg et al., 2013). Photoperiod is arguably one of the key abiotic factors that regulates most physiological activities in insects through its effects on ommatidial pigments and photoreceptors in brains and formation of growth hormones (Lopatina et al., 2011), particularly in mosquitoes (Mathias et al., 2006). Bowen et al. (1894) reported that the insect brain can able to detect, receive

and measure the intensity and relative amounts of light and dark (i.e., acting as a circadian clock). It can also act as source of hormonal effector which can able to activate certain physiological and behavioral processes. It can also influence various life processes like survival (Urbaneja et al., 2001), longevity (Chocorosqui & Panizzi, 2003), life span (Lanciani & Anderson, 1993), growth (Leimar, 1996; MacRae, 2010), diapause (MacRae, 2005), ovarian follicular development (Oda & Nuorteva, 1987) and vectorial morphometric indices (Vinogradova & Karpova, 2006).

*Anopheles subpictus* and *Culex quinquefasciatus* are responsible for the spread of important public health diseases. Oviposition behavior is important for mosquito population growth. The mosquito population growth can be reduced by preventing oviposition. Mosquito behavior particularly ovipositional behavior is sensitive to environmental factors. Understanding the response of mosquitoes to one of such factors, i.e., photoperiod, can lead to more precise predictions of transmission cycles which

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help in the development of more effective vector control strategies.

Therefore, current study was designed to understand the influence of different photoperiodic regimens on oviposition behavior of *Anopheles subpictus* and *Culex quinquefasciatus*.

## Methods

### Collection and identification of field-collected mosquitoes

Mosquito species during their different developmental stages were collected from different localities of Ganjam district, Odisha State of India, and mass reared in laboratory after identification as per the identification keys. All the experiments pertaining to the oviposition behavior in response to photoperiod were carried out on F<sub>1</sub> progeny of field-collected mosquitoes of *Anopheles subpictus* and *Culex quinquefasciatus*.

### Oviposition cycle

Five–six-day-old female mosquitoes were offered blood meals. After 48 h, ovitraps half filled with dechlorinated tap water were introduced into cages of 24" × 24" × 24" dimensions containing 10 blood-fed females per cage. The number of eggs/egg rafts collected was checked at 3 h intervals. The ovitraps were replaced, and the number of eggs/egg rafts laid was counted. Observations were made for three days uninterruptedly. The following three different types of photoperiods were tested for both the mosquito species under study as per the protocol by Suleman and Shirin (1981).

- Normal (light day) photoperiod (LD 12: 12)
- Reversed (dark day) photoperiod (DL 12: 12) and
- Continuous light photoperiod (24 LL).

In the insectary, fluorescent tubes were used as light source. During the dark period, observations were made by the help of red light torch, as mosquitoes are supposed to be blind to red light (Beck, 1968).

### Statistical analysis

All experiments were repeated 10 times, and the data obtained were analyzed by using the statistical software package (SPSS) 17.0 version.

## Results

### Effects of varying photoperiodic regimens on oviposition behavior of *Anopheles subpictus*

In order to understand the importance of photoperiod in determining the oviposition cycle, experiments were carried out on *Anopheles subpictus*. The number of eggs laid by *Anopheles subpictus* was observed three-hourly under normal tropical photoperiod (LD 12: 12)

condition for three days uninterrupted. It was clear that ovipositional activity of mosquitoes was restricted entirely to the scotophase, reaching a peak starting at 18.00 h and lessening at the end of the scotophase. During 18–21 h, a maximum number of eggs (38.03%) were laid on the first day observation, and same result was found (27.85%) on second day and (18.88%) third day. A declining trend was noticed immediately following the transition from 15 to 18 h and during 21–24 h (Fig. 1).

Oviposition activity under reversed photoperiod (DL 12: 12) was carried out, and an unimodal oviposition cycle on all the three days between 03 and 24 h was observed. No eggs were laid between 18 and 03 h during the first 24 h. About 23.26% of total number of eggs were laid during 03–06 pm of day one. Similarly, the same time period of day two, the percentage of eggs laid was 19.83, but no eggs were laid during the same time period of observation on day three.

Gravid *Anopheles subpictus* females were allowed to expose to light (24 LL) continuously for three days without any scotophase, and erratic egg-laying behavior was observed during all the three days of observation.

### Effects of varying photoperiodic regimens on oviposition behavior of *Culex quinquefasciatus*

Three-hourly observations on oviposition activity of *Culex quinquefasciatus* were carried out under normal tropical photoperiod (LD 12: 12) for three consecutive days. It was noticed that oviposition activity was restricted almost entirely to the scotophase (18–06 h) and first photophase (06–09 h). Maximum egg rafts were laid, starting at the onset of the scotophase and diminishing toward the end of the scotophase. A large proportion (12.5%) of the egg rafts were laid during the first half of the scotophase (18–21 h). No egg rafts were found during the photophase except in the 06–09 h in first day, and similar pattern of result was also observed in third day, whereas during second-day observation the peak was during 21–00 h only.

Oviposition cycle of *Culex quinquefasciatus* was investigated under various conditions of photoperiods. Oviposition activity under reversed photoperiod (DL 12: 12) was carried out. In the reverse photoperiod (DL 12: 12), it was noticed that there is an unimodal oviposition cycle on all the three days between 03 and 24 h. No egg rafts were laid by the *Culex* mosquitoes between 18 and 03 h during the first 24 h. About 23.26% of total number egg rafts were laid during 03–06 pm on day one. During the same time period of day two, the percentage of eggs rafts laid was 19.83, but no eggs rafts were laid during the same time period of observation on the third day (Fig. 2).

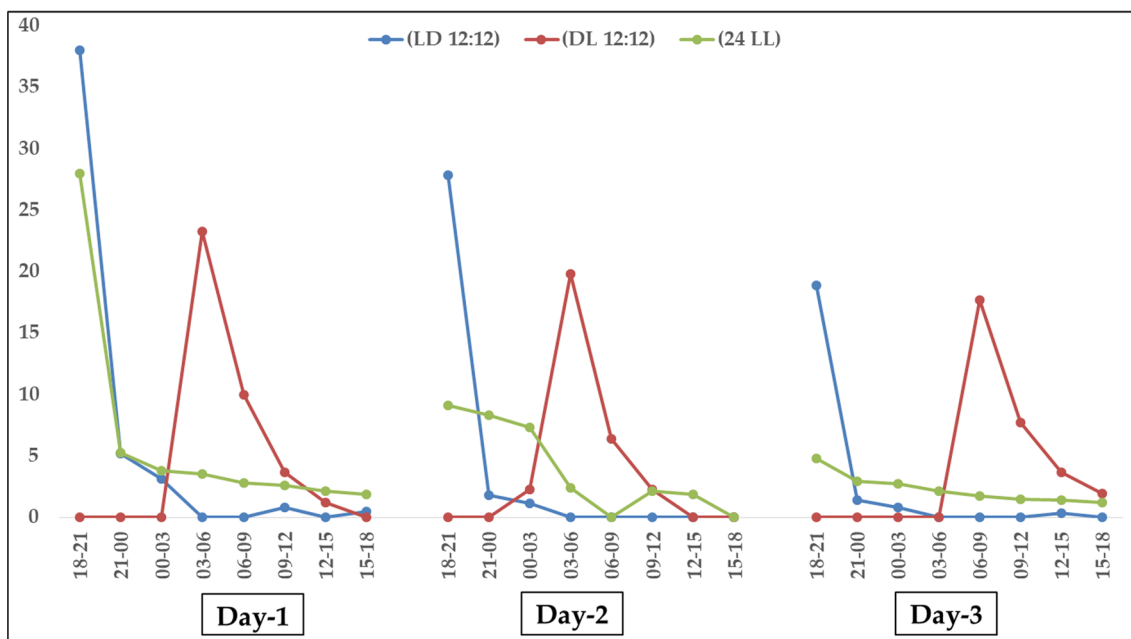


Fig. 1 Oviposition cycle of *Anopheles subpictus* in different photoperiod regimens for three consecutive days

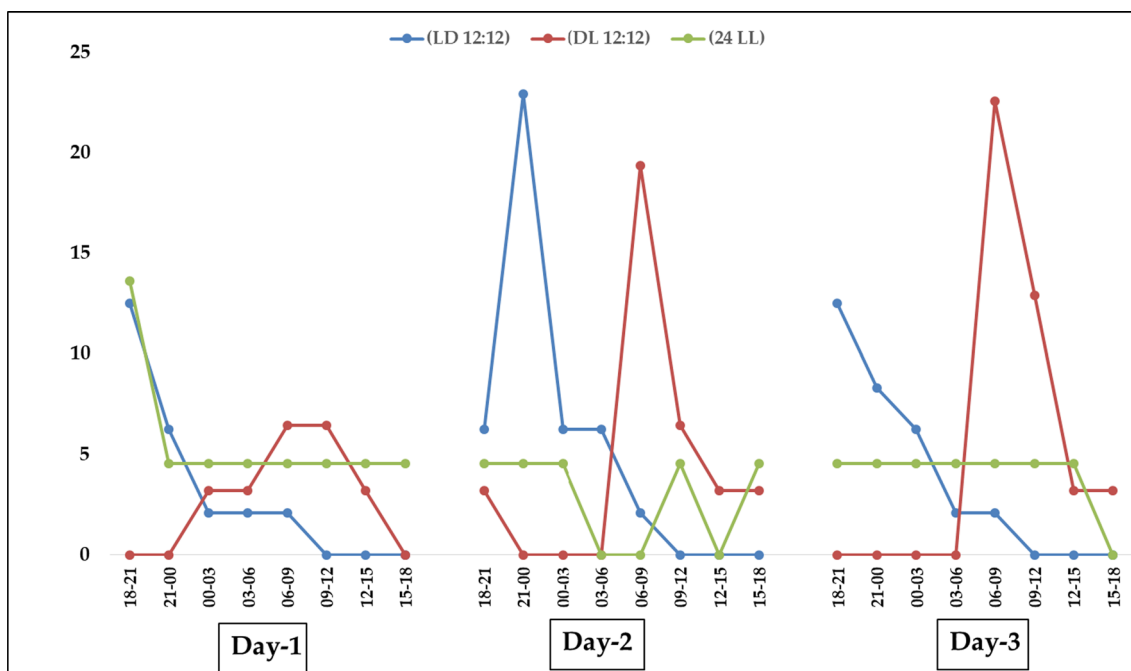


Fig. 2 Oviposition cycle of *Culex quinquefasciatus* in different photoperiod regimens for three consecutive days

Under continuous light (24 LL) condition, oviposition activity was greatly depressed. The egg-laying behavior of *Culex quinquefasciatus* under 24 h lighted condition was erratic in all three days study.

### Discussion

In the present study, experiments were conducted to understand the importance of photoperiod in determining the oviposition time in *Anopheles subpictus* and *Culex quinquefasciatus*. Oviposition cycle of both the mosquito species was studied under different conditions for three

consecutive days. In both mosquito species, oviposition activity was seen to be completely confined to the scotophase during the usual photoperiod, but Panigrahi et al. (2014) showed that a highest number of eggs were laid in the 4th quarter of the light period which subsequently declined thereafter on all the three days of experiments in both *Ae. aegypti* and *Ae. albopictus*. When both the mosquito species were exposed to reverse photoperiod in the present study, the oviposition activity was also reversed but when they were exposed to 24 h light condition, mosquitoes showed an erratic oviposition behavior that may conclude that both of the species were nocturnal and showed circadian rhythm of normal light and dark oviposition behavior. The number of eggs laid by *Anopheles subpictus* was observed three-hourly under normal tropical photoperiod (LD 12:12) condition for three consecutive days. It was noticed that oviposition reached a peak with starting at 18.00 h and decreasing toward the end of the scotophase. The maximum number of eggs (38.03%) laid during 18–21 h of the first day observation, and same result was found on second (27.85%) and third (18.88%) days. An insignificant diminishing activity was found immediately following the shift from 15 to 18 h and during 21–24 h. Similarly, it was observed that oviposition activity was limited almost completely to the scotophase and first photophase (06–09 h) in *Culex quinquefasciatus*. The maximum number of egg rafts was laid, starting at the onset of the scotophase and falling toward the end of the scotophase. During the first half of the scotophase (18–21 h), a large proportion (12.5%) of the egg rafts was laid. No egg rafts were found during the photophase in first day, and the same result was observed on third day, whereas during second-day observation the peak was 21–00 h. There was an unimodal oviposition cycle during the reverse photoperiod (DL 12: 12) on all the three days between 03 and 12 pm in *Anopheles subpictus*.

On day one about 23.26% of total number of eggs were laid during 03–06 pm. The percentage of eggs laid during the same time period of day two was 19.83. Oviposition activity shifted to the daytime hours (i.e., during darkness) and remained cyclical, with 89% of oviposition occurring between 06.00 and 18.00 h in *Culex quinquefasciatus*. During the first day study, the egg-laying behavior was found 00 h–06 h of the scotophase and during the entire photophase except toward the last part (15 h–18 h). Maximum percentage of egg raft laid both during 06 h–09 h and 09 h–12 h of the photophase. During the second day study, the percentage of egg rafts laid was maximum from 06 to 09 h (19.35), which gradually declined toward the last part of the photophase and first part of the scotophase but no egg rafts were laid during 21 h–06 h. However, when the photoperiod was reversed, the oviposition cycle was also reversed in *Aedes aegypti*

(Day, 2016). Under continuous light (24 LL) condition, gravid female mosquitoes were exposed to light continuously for three days without any scotophase. Erratic egg-laying behavior was observed during all the three days of observation that indicated there was a very strong relationship between ovipositional activity of the mosquitoes and the dark periods which clearly indicated that oviposition of mosquito is inhibited by light and stimulated by darkness. The peak time of oviposition of *Culex quinquefasciatus* observed during the present study was similar to the earlier findings of Suleman and Shirin (1981).

## Conclusions

The oviposition activity was found to be reversed when both the mosquito species were allowed to lay eggs in reverse photoperiod conditions. Further, erratic oviposition behavior was noticed when both the mosquitoes were exposed to 24 h light conditions. Understanding the oviposition behavior of mosquito species may favor to design and develop new vector control strategies.

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## Author contributions

SKN executed the experiment and carried out manuscript writing, SNS carried out data analysis and manuscript writing, TSA carried out data analysis and manuscript writing, and TKB designed the experiment and carried out data analysis and manuscript writing. All authors have read and approved the manuscript.

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## Data availability

Data generated during this study are included in this published article.

## Declarations

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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