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ORIGINAL RESEARCH PAPER

Lethal effect of blue light on the developmental stages of the urban mosquito, *Culex pipiens* form *molestus* (Diptera: Culicidae)

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Abstract

We previously reported that short-wavelength visible light (blue light: 400–500 nm) has a lethal effect on various insect species and that the most toxic wavelength to the pupae of the hygiene pest, the mosquito, *Culex pipiens* form *molestus* Forskål (Diptera: Culicidae), is 417 nm. However, previous reports on *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), and *Galerucella grisescens* (Joannis) (Coleoptera: Chrysomelidae) demonstrated that the most harmful wavelengths of blue light differed among different developmental stages. The most toxic wavelengths to the developmental stages of *C. pipiens* f. *molestus*, besides the pupal stage, remain unclear. We investigated the lethal effect of various wavelengths of the blue-light spectrum on the eggs, larvae, and adults of *C. pipiens* f. *molestus* using light-emitting diodes (LEDs). Blue light irradiation had a lethal effect on all life stages tested. Furthermore, our results reaffirmed the results of previous studies, where 417 nm light had a strong effect on all life stages. To our knowledge, this is the first report of an insect species where the most effective wavelength does not vary among developmental stages. In addition, our findings indicate that ~ 420 nm is the most promising wavelength to control *C. pipiens* f. *molestus* populations using blue-light irradiation.

Keywords Hygiene pest · Irradiation · Light-emitting diodes · Mosquito · Short-wavelength visible light

Introduction

Mosquitoes are transporters and transmitters of a variety of diseases and are the most important hygiene pests worldwide. Currently, mosquitoes are mainly controlled by chemical insecticides. However, insecticide resistance has been observed in several mosquito species (Casimiro et al. 2006; Hamdan et al. 2005; Kasai et al. 2007). Therefore, alternative control methods are required.

Culex pipiens form *molestus* Forskål (Diptera, Culicidae) is an urban mosquito that occurs in underground areas (Byrne and Nichols 1999). *Culex pipiens* f. *molestus* is an autogenous and strongly anthropophilic species (Byrne and Nichols 1999; Osório et al. 2014; Vinogradova 2003) that mediates West Nile, Japanese encephalitis, and Rift Valley fever viruses (Tahori et al. 1955; Turell et al. 2006; Zakhia et al. 2018). This species does not diapause and is active throughout year (Nelms et al. 2013; Vinogradova 2003), and can, thus, potentially transmit viruses year-round. Therefore, *C. pipiens* f. *molestus* is one of the most important hygiene mosquito species.

Recently, light-based pest control techniques have attracted attention as alternative methods to insecticides. For example, the phototaxis of insects (i.e., attraction to light sources) has been used in pest control methods, such as light traps and electric insect killers. Nighttime lighting with yellow lamps has also been used to suppress the behaviors of fruit-piercing moths (Meyer-Rochow 1974; Nomura 1966; Nomura et al. 1965). Recently, red light has been shown to inhibit the attraction of thrips to host plants (Katai et al. 2015; Murata et al. 2018a, b). Despite the numerous light-based insect-pest control techniques available, most of these techniques involve the behavioral responses of insects to a light source.

Over the last 5 decades, numerous studies have reported the lethal effects of ultraviolet C (100–280 nm), and B (280–315 nm) on insects (Cohen et al. 1973; Ghanem and Shamma 2007; Gingrich 1975; Okamoto 1989, 1992; Suzuki et al. 2014). Recently, we found that short-wavelength visible light (blue light: 400–500 nm) has lethal effects on *Drosophila melanogaster* Meigen (Diptera: Drosophilidae),

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C. pipiens f. *molestus*, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), and *Galerucella grisescens* (Joannis) (Coleoptera: Chrysomelidae) (Hori et al. 2014; Hori and Suzuki 2017; Shibuya et al. 2018). These studies showed that the most toxic wavelengths differ among the developmental stages as well as among insect species. In a previous study, we revealed that the most toxic wavelength to *C. pipiens* f. *molestus* pupae is ~ 420 nm and that this blue-light wavelength is also lethal to the eggs (Hori et al. 2014). However, the most lethal wavelength to the eggs, and the lethal effects of blue light on larvae and adults remain unclear. Such information is necessary for the development of effective control methods of *C. pipiens* f. *molestus* mosquitoes using blue light.

Here, we investigated the lethal effect of blue light on the eggs, larvae, and adults of *C. pipiens* f. *molestus* to clarify the most toxic wavelengths on the developmental stages of this species of mosquitoes.

Materials and methods

Insects

Culex pipiens f. *molestus* mosquitoes were provided by Earth Chemical Co., Ltd. (Tokyo, Japan) and maintained in our laboratory. Eggs, larvae, and pupae were reared in a plastic container (150 mm diameter × 91 mm height) containing 250 mL of tap water decalcified for 48 h, with a constant supply of fishery feed for trout juveniles (EC 1C, Feed One Co., Ltd., Yokohama, Japan). Adults were maintained in a plastic cage (340 × 250 × 340 mm) containing an Erlenmeyer flask (50 mL), and a plastic cup (30 mm diameter × 35 mm height). Cotton wool soaked with 10% honey solution (50 mL) was placed in the flask (food substitute) and cotton wool soaked with water was placed in the cup (oviposition substrate). Adult females were never fed blood because of the autogenic nature of this species. All stages were maintained in a constant-temperature room at 25 ± 1 °C and ~ 60% relative humidity under a 16 h:8 h (L:D) photoregime.

Light-emitting diode (LED) light radiation

LED lighting units (IS-mini®, ISL-150 × 150 Series; CCS Inc., Kyoto, Japan; light emission surface: 150 × 150 mm; arrangement: 360 LEDs were equally arranged on a panel; LED type: ϕ 3 mm plastic mold) with power supply units (ISC-201–2; CCS Inc., Kyoto, Japan) were used for light radiation. Insects were irradiated with LED light in a multi-room incubator (LH-30CCFL-8CT; Nippon Medical & Chemical Instruments Co., Ltd., Osaka, Japan). The emission spectra and the number of photons (photons•m^{-2}•s^{-1}) were measured using a high-resolution spectrometer (HSU-100S; Asahi Spectra Co., Ltd., Tokyo, Japan; numerical aperture of the fiber: 0.2) in a dark room. The number of photons was adjusted by current control using the power supply unit. During measurements, the distance between the LED lighting unit and the spectrometer sensor was set to be approximately equal to the same distance between the insects and the LED lighting unit in the incubator. Since the mosquitoes were irradiated through a glass plate or glass lid, the same plate or lid was placed between the light source and sensor during measurement. The number of photons was measured five times before and after the experiments. The average values of the number of photons in each experiment are shown in Tables S1–S3. Comparisons of the emission spectra used in the experiments are shown in Fig. S1.

Lethal effect of blue-light irradiation on the egg stage of *C. pipiens* f. *molestus*

Eggs were collected from the stock cultures within 1 h of deposition. Eggs (n = 30) were placed in water (50 mL) in polyethylene-terephthalate ice-cream cups (60 mm diameter × 38 mm height, Risupack, Co., Ltd., Aichi, Japan), which were each covered with a glass plate. Cups were placed in the multi-room incubator (25 ± 1 °C) and irradiated with LED light at wavelengths of 375 (UVA), 406, 417, 427, 438, 452, 467, 493, or 507 nm (blue green) with set values of 7.5×10^{18} photons•m^{-2}•s^{-1}, 10×10^{18} photons•m^{-2}•s^{-1}, or 15×10^{18} photons•m^{-2}•s^{-1} for 48 h. The set values were the same as the tests for the pupae of the mosquito in our previous report (Hori et al. 2014). After irradiation, the cups were maintained under continuous dark (DD) for 72 h (25 ± 1 °C). The number of surviving hatchlings (larvae) was then counted to determine the total mortalities of eggs and hatchlings because of the difficulty in counting unhatched eggs and dead hatchlings. In the control treatment, mosquitoes were maintained under DD (i.e., no irradiation) for 120 h, and the total mortalities were determined using the same method. Ten replications (cups) were performed for each light dose of each wavelength.

Lethal effect of blue-light irradiation on the larval to pupal stages of *C. pipiens* f. *molestus*

Hatchlings were collected from the stock cultures within 12 h of hatching. Hatchlings ($n = 10$) were maintained in water in cups under the same conditions described above. Fishery feed for trout juveniles (EC 1C, Feed One Co., Ltd., Yokohama, Japan) (0.01 g) was supplied in the cups on the first day of irradiation and subsequently, 0.03 g was added every 5 days. The cups, which were each covered with a glass plate, were placed in a multi-room incubator and irradiated with LED light at the same wavelengths described

above, with set values of 5×10^{18} photons•m^{-2}•s^{-1}, 10×10^{18} photons•m^{-2}•s^{-1}, or 15×10^{18} photons•m^{-2}•s^{-1}. In the larval to pupal test, a relatively high lethal effect was obtained at 10×10^{18} photons•m^{-2}•s^{-1}, and therefore, the effect at 5×10^{18} photons•m^{-2}•s^{-1}, but not 7.5×10^{18} photons•m^{-2}•s^{-1}, was investigated. The larvae in the cups were irradiated until they had all died or developed to the adult stage. Similarly, the number of pupated larvae and emerging adults were counted to calculate the larval and pupal mortalities, respectively. In the control treatment, mosquitoes were maintained under DD (no irradiation) until they had all died or developed to the adult stage, after which the total mortalities were determined using the method described previously. Ten replications (cups) were performed for each light dose of each wavelength.

Lethal effect of blue-light irradiation on the adult stage of *C. pipiens* f. *molestus*

Adults were collected from the plastic rearing boxes within 12 h of emergence. Pairs of adults ($n=5$ pairs) were released onto a circular cotton pads (10 g, diameter 90 mm) soaked with 5% honey solution (10 mL) in a glass Petri dish (60 mm diameter × 90 mm height). The Petri dish was placed in the multi-room incubator equipped with the LED lighting unit. The adults were irradiated with different wavelengths of light at 25 ± 1 °C until all of them died. We counted the number of dead adults every day and we replaced the Petri dish containing the honey water every 2 days. Lethal effects of irradiation at the set values of 10 and 15×10^{18} photons•m^{-2}•s^{-1} were compared among the nine wavelengths [375 nm (UVA), 406, 417, 427, 438, 452, 467, 493, and 507 nm (blue green)]. In the adult test, a high lethal effect was not obtained even at 15×10^{18} photons•m^{-2}•s^{-1}, and the effect at 7.5×10^{18} photons•m^{-2}•s^{-1} was, therefore, not investigated. In the control treatment, the longevities of the mosquitoes maintained under DD (no irradiation) were determined. The lethal effect was determined using the reduction rate of longevity (RRL). The RRL was calculated as follows:

$$\mathrm{RRL} = 100(\mathrm{LD} - \mathrm{LI})/\mathrm{LD}$$

where, LD and LI represent the average longevities of nonirradiated and irradiated adults, respectively. Fifty replications (five adult's × ten Petri dishes) were performed for each light dose of each wavelength for each sex.

Statistical analysis

Comparisons of mortalities and RRLs between irradiations and control treatments, and among wavelengths were analyzed using the Steel and Steel–Dwass tests, respectively. The calculations were performed using R version 4.0.2. (R Development Core Team 2020).

Results

Lethal effect of blue-light irradiation on the egg stage of *C. pipiens* f. *molestus*

Irradiation at wavelengths of 375, 417, 427, 438, 467, and 507 nm at 10 or 15×10^{18} photons•m^{-2}•s^{-1} during the egg stage significantly increased the mortalities of eggs and hatchlings (Fig. 1). The strongest lethal effect was observed after irradiation with 417 nm blue light at 10 and 15×10^{18} photons•m^{-2}•s^{-1} (92.0 and 99.7% mortality, respectively). In these photon flux densities, the lethal effect of 417 nm blue light was significantly stronger than that of 375 nm UVA light (34.3 and 56.7% mortality at 10 and 15×10^{18} photons•m^{-2}•s^{-1}, respectively). Although 467 nm blue light also had a strong lethal effect at 15×10^{18} photons•m^{-2}•s^{-1} (92.7% mortality), it did not show the same lethal effect at 10×10^{18} photons•m^{-2}•s^{-1} (11.7% mortality). The lethal effect of 427 nm blue light was relatively strong at 10×10^{18} photons•m^{-2}•s^{-1} (71.3% mortality), and at 15×10^{18} photons•m^{-2}•s^{-1} (75.0% mortality). The 438 nm blue light only had a lethal effect at 15×10^{18} photons•m^{-2}•s^{-1} (36.3% mortality). The other wavelengths of blue light did not have a lethal effect. At 7.5×10^{18} photons•m^{-2}•s^{-1}, only the 375 nm UVA light had a lethal effect on the mosquitoes (54.0% mortality). However, a dose–response relationship was not observed. Although the effect of 507 nm blue–green light at 15×10^{18} photons•m^{-2}•s^{-1} was not investigated because the maximum photon flux density of the unit of this wavelength was low, the 507 nm light did have a lethal effect at 10×10^{18} photons•m^{-2}•s^{-1} (38.0% mortality).

Lethal effect of blue-light irradiation on the larval to pupal stages of *C. pipiens* f. *molestus*

Irradiation with 375 nm UVA light and the 406–467 nm blue light during the larval to pupal stages had significant lethal effects at 10 or 15×10^{18} photons•m^{-2}•s^{-1} (Fig. 2). Irradiation with 417 nm blue light had the strongest lethal effect with 100% larval mortality at 15×10^{18} photons•m^{-2}•s^{-1}, 94.0% total (total of larval and pupal) and 81.0% larval mortality at 10×10^{18} photons•m^{-2}•s^{-1}, and 33.0% total and 19.0% larval mortality at 5×10^{18} photons•m^{-2}•s^{-1}. Irradiation with 427 and 467 nm blue light also resulted in 100% larval mortality at 15×10^{18} photons•m^{-2}•s^{-1}. However, the 467 nm blue light did not have significant lethal effect on larvae at 10×10^{18} photons•m^{-2}•s^{-1} (Fig. S2). Irradiation with 406 nm blue light also exhibited a strong lethal effect at 15×10^{18}

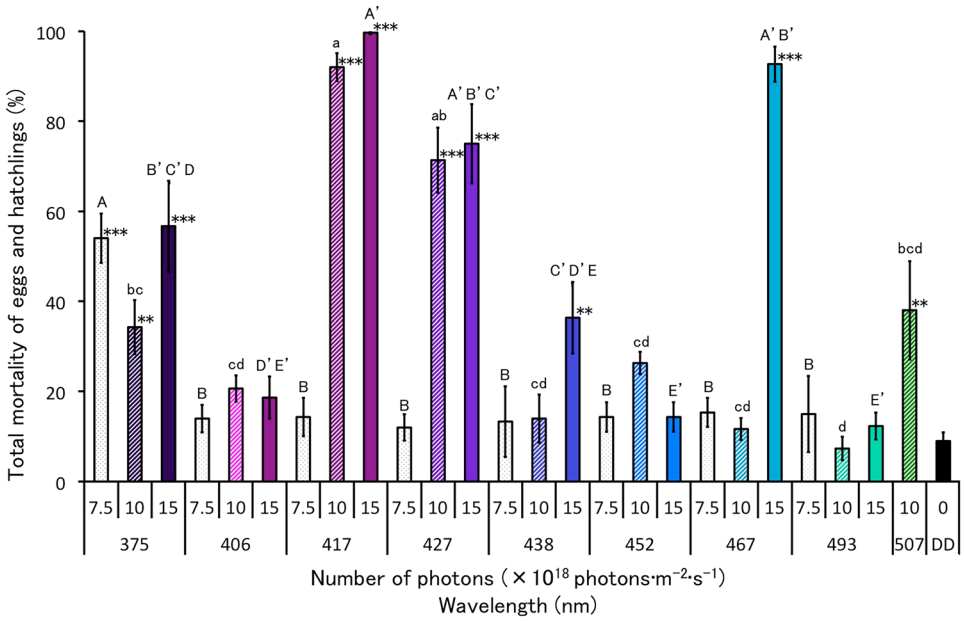

Fig. 1 Mortality of *Culex pipiens* f. *molestus* irradiated with blue light during the egg stage. Data represent the means ± standard errors. Asterisks above the bars indicate significant differences between the treatments (irradiation) and control [dark condition (DD)] (Steel test: $^{**}p < 0.01$, $^{***}p < 0.001$). Bars with same letters are not significantly different (Steel–Dwass test, $p > 0.05$). Ten replications (30 eggs per replicate) were conducted

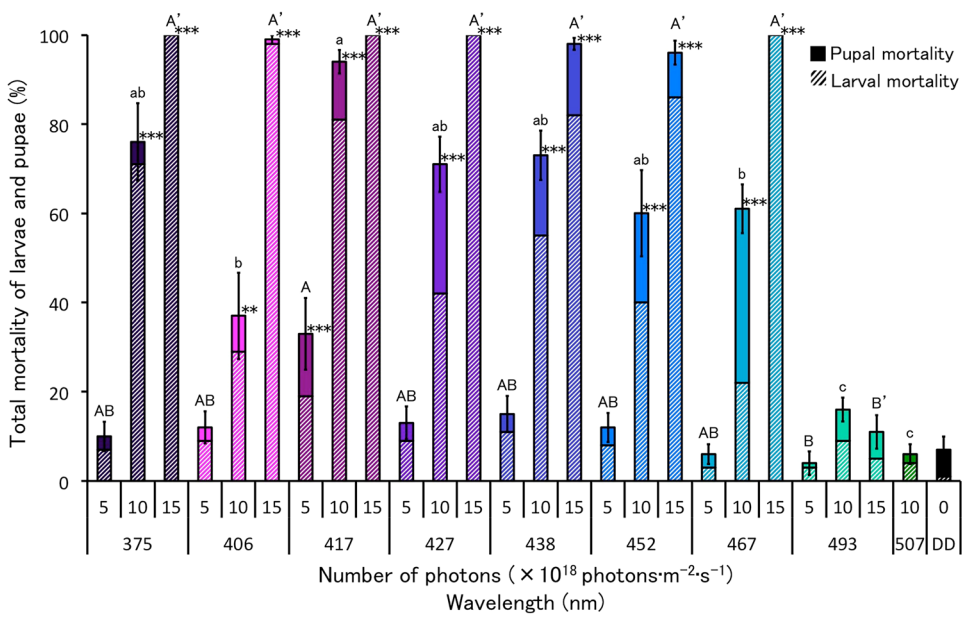

Fig. 2 Mortality of *Culex pipiens* f. *molestus* irradiated with blue light during the larval to pupal stages. Data represent the means ± standard errors. Asterisks above the bars indicate significant differences between the treatments (irradiation) and control [dark condition (DD)] (Steel test: $^{**}p < 0.01$, $^{***}p < 0.001$). Bars with the same letters are not significantly different (Steel–Dwass test, $p > 0.05$). Ten replications (10 larvae per replicate) were conducted

photons•m^{-2}•s^{-1} (98.0% larval mortality), but a mild effect at 10×10^{18} photons•m^{-2}•s^{-1} (29.0% larval mortality). Irradiation with 493 nm blue-light did not have any lethal effect (16.0% and 11.0% total mortality at 10 and 15×10^{18} photons•m^{-2}•s^{-1}, respectively). Although the 375 nm UVA light had a strong lethal effect at 10 and 15×10^{18} photons•m^{-2}•s^{-1} (76.0% total and 100% larval mortality, respectively), it did not have a significant effect at 5×10^{18} photons•m^{-2}•s^{-1} (14.0% total mortality).

Lethal effect of blue-light irradiation on the adult stage of *C. pipiens* f. *molestus*

The longevities of nonirradiated male and female adults were 20.9 and 32.7 days, respectively (Fig. S3). Compared with nonirradiated adults, the longevities of both male and female adults were reduced by irradiation with all tested wavelengths of lights (Steel test, $p < 0.001$) (Fig. 3). The RRLs were similar between males and females. The RRLs

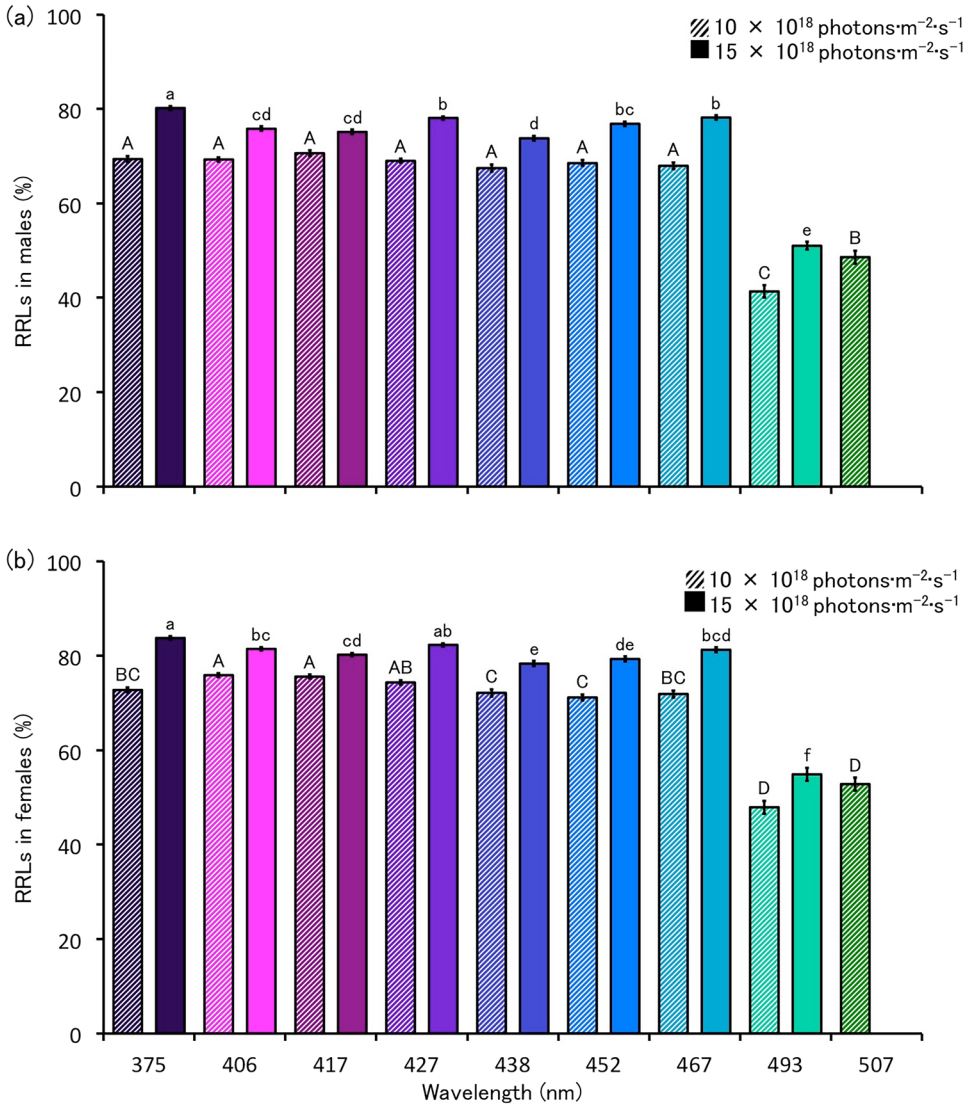

Fig. 3 Reduction rate of longevity (RRL) of *Culex pipiens* f. *molestus* adults irradiated with blue light. RRLs of males (**a**) and females (**b**). Data represent the means ± standard errors. Significant differences were obtained for all wavelengths between the treatments (irradiation) and control (dark condition) (Steel test: $p < 0.001$). Bars with same letters are not significantly different (Steel–Dwass test, $p > 0.05$). Fifty replications (five adult's × ten Petri dishes) were conducted for each light dose of each wavelength for each sex

under the 375–467 nm treatments were higher than those under the 493 and 507 nm treatments. The RRLs of males irradiated with 375 to 467 nm light were 67.9–70.6% and 73.8–80.2% at 10 and 15×10^{18} photons•m^{-2}•s^{-1}, respectively, whereas those at 493 and 507 nm were 41.4–48.6% and 51.0% at 10 and 15×10^{18} photons•m^{-2}•s^{-1}, respectively (Fig. 3a). The RRLs of females irradiated with 375–467 nm light were 71.2–75.9% and 78.3–83.8% at 10 and 15×10^{18} photons•m^{-2}•s^{-1}, respectively, whereas those with 493 and 507 nm were 47.9–52.8% and 54.9% at 10 and 15×10^{18} photons•m^{-2}•s^{-1}, respectively (Fig. 3b).

Discussion

In the present study, we revealed that blue-light irradiation during the egg, larval, and adult stages can kill *C. pipiens* f. *molestus*. Although our study further confirmed our previous findings that blue-light irradiation has a lethal effect on *C. pipiens* f. *molestus* pupae (Hori et al. 2014), we also demonstrated that blue-light irradiation has a lethal effect on all developmental stages of *C. pipiens* f. *molestus*.

The most toxic wavelength of blue light to *C. pipiens* f. *molestus* pupae was previously found to be 417 nm (Hori et al. 2014). In the present study, the 417 nm wavelength was not only confirmed to be the most toxic wavelength to *C. pipiens* f. *molestus* pupae, but also to the eggs and larvae of this species. In *D. melanogaster* and *G. grisescens*, effective lethal wavelengths were shown to differ among insect growth stages (Hori and Suzuki 2017; Shibuya et al. 2018). Therefore, changes in the effective wavelengths of blue light among insect growth stages may be species dependent.

Previously, we indicated that highly toxic wavelengths of blue light are species- and growth stage-specific in insects (Hori et al. 2014; Shibuya et al. 2018). In addition, we showed that reactive oxygen species (ROS) may induce the

lethal effects of blue light on insects (Shibuya et al. 2018). In the primary retinal cells of mice, cell injury by blue-light irradiation was shown to be induced by ROS (Kuse et al. 2014). Therefore, we propose that the highly lethal effects of specific wavelengths of blue light are the result of the ROS generated by the absorption of specific wavelengths of light by the species, and that this absorption potential can vary according to the growth stage-specific photoreceptive parts in insect tissues (Hori et al. 2014; Shibuya et al. 2018). In addition to previous studies, the present study demonstrated that specific wavelengths of blue light can exhibit especially high toxicity effects on the eggs, larval, and pupal stages of *C. pipiens* f. *molestus*. However, in the adult stage, no specific effective wavelength of blue light was identified. It is possible that continuous lighting may influence the flight behavior of adult insects, which may account for the observed reduction in the longevity of *C. pipiens* f. *molestus* adults. However, the RRLs under 375–467 nm light were significantly higher than those under 493 and 507 nm light, and the RRLs increased with increasing photon density. Similar to our findings regarding *C. pipiens* f. *molestus* adults, Shibuya et al. (2018) reported that no specific wavelength of blue light had a lethal effect on the eggs and larvae of *D. melanogaster*. Therefore, the reduction in the longevity of adult mosquitoes is presumably not due to the influence on their flight behavior. The photoreceptive parts that absorb a broad (not specific) range of wavelengths, in addition to the amount of photon energy and transmittance of wavelength, might be involved in the lethal effects on *C. pipiens* f. *molestus* adults, and the eggs and larvae of *D. melanogaster*.

In *C. pipiens* f. *molestus*, the biological implications of different action spectra between the adults and the other stages are unclear. However, the differences in habitats between adult and the other stages may be involved in the different action spectra. Adult mosquitoes live on the ground whereas egg to pupal stages exist near the surface of water. Elucidation of the mechanisms of the lethal effect of blue light is necessary to clarify the biological implications.

Irradiation with 507 nm blue–green light at 10×10^{18} photons•m^{-2}•s^{-1} had a significant lethal effect on the eggs of *C. pipiens* f. *molestus*. The eggs may have photoreceptive tissue that is able to absorb relatively high amounts of light at ~ 507 nm wavelength. It is necessary to investigate the lethal effect of light with wavelengths longer than 507 nm.

The present study demonstrated that light irradiation at ~ 420 nm at 10×10^{18} photons•m^{-2}•s^{-1} or higher was effective in killing the eggs, larvae, and pupae of *C. pipiens* f. *molestus*. *Culex pipiens* f. *molestus* often occurs in water storage containers and septic tanks (Moriya et al. 1967; Noguchi 1962; Noguchi et al. 1965). The eggs of this species form rafts and float on the surface of the water (Harbach et al. 1984; Kassim et al. 2012), and the larvae and pupae also breathe at the water surface using respiratory siphons. Therefore, irradiation of the water surface with ~ 420 nm blue light may be used to kill the eggs, larvae, and pupae of *C. pipiens* f. *molestus* mosquitoes and thus suppress the occurrence of this hygiene pest to reduce associated viral transmissions (e.g., West Nile virus, and Japanese encephalitis virus). Since blue light has been shown to also have a lethal effect on various insect species such as *D. melanogaster*, *T. confusum*, and *G. grisescens* (Hori et al. 2014; Hori and Suzuki 2017), it is possible that blue-light irradiation may be effective in controlling populations of other mosquito species besides *C. pipiens* f. *molestus*. In the near future, we aim to examine the efficacies of ~ 420 nm blue light against *C. pipiens* f. *molestus* under field conditions. The control of mosquitoes using the lethal effect of blue light is a promising, safe and clean technique and is an alternative to the use of pesticides.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13355-021-00737-7.

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