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EFFECTS OF PHOTOPERIODS ON THE DEVELOPMENT OF *Diplonychus rusticus* (Fabricius, 1871) (Hemiptera: Belostomatidae): CONNECTIONS TO LIGHT POLLUTION

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ABSTRACT

Light pollution caused by artificial light at night has significant impacts on biodiversity, particularly on insects that are strongly attracted to light, by disrupting their physiology and ecological interactions. The aquatic bug, *Diplonychus rusticus* is an important invertebrate predator in most freshwater habitats of Southeast Asia, particularly in lentic systems such as wetlands and ponds. The species is also known for its high potential in controlling mosquito vectors of transmitted diseases in urban areas, where light pollution has become increasingly prevalent. While the species is not attracted to the light, we aim to examine whether light pollution influences the development, survival and fertility of *D. rusticus* by exposing them to different photoperiods. Our study revealed that the development time from the first instar to the adult stage was positively correlated with the daily duration of light exposure, whereas the body size (length and width) of all life stages was not affected by photoperiods. In addition, the survival rate and fertility of adult bugs are considerably decreased under prolonged lighting conditions. These results have provided insights into the impacts of light pollution on the aquatic insects that are not attracted to the light showing a necessary strategy for future conservation efforts for insects in urban environments.

Keywords: Aquatic bugs, conservation, environmental stress, life cycle, urbanization effects.

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INTRODUCTION

Light is one of the main environmental factors that influence many aspects of animal life (Ruchin, 2020). Particularly, natural photoperiods provide organisms with signals to migration (Davies et al., 2014), navigation (Horváth et al., 2009; Meyer & Sullivan, 2013) or have influences on species' distribution (Meyer & Sullivan, 2013). However, humans have dramatically altered natural light regimes across much of the globe through the addition of artificial light at night (ALAN) such as streetlights, advertisement boards, and vehicles' lights. These light sources interfere with the natural patterns of light and darkness (Davies et al., 2014; Falchi et al., 2019; Parkinson et al., 2020).

ALAN has been shown to cause negative consequences in animals and plants at all biological organization levels, from molecular to community and ecosystem levels (Gaston et al., 2013; Gaston et al., 2015; Sanders et al., 2021). Particularly, the alteration of the light-dark cycle has a significant influence on circadian functioning and the development of organisms (Brüning et al., 2011). In addition, the increase in ALAN is considered a major threat to biodiversity (Hölker et al., 2010), with profound effects on many insect species (Owens et al., 2020). Insects are disappearing globally at a high speed (Desouhant et al., 2019), and ALAN has been shown to be associated with declines in both their populations and diversity (Kalinkat et al., 2021). ALAN can affect insect movement (Dacke et al., 2013), modify foraging behavior (Van Langevelde et al., 2017), or disrupt the biological clocks of insects, like those of vertebrates (Smolensky et al., 2015). ALAN can also inhibit or alter melatonin synthesis, leading to changes in the molting and metamorphosis process, and potentially resulting in developmental defects (Durrant et al., 2015; Richter et al., 2000). However, the current understanding of the effects of ALAN in freshwater ecosystem is still insufficient (Desouhant et al., 2019), particularly the impact of artificial light pollution on the freshwater aquatic insects in the tropical regions where an overwhelming majority of the Earth's biodiversity has received little attention and rarely been investigated.

Previous research showed the effects of ALAN on the developmental time and the fertility of insects. Negative consequences of ALAN on growth duration of larvae, reproduction and survival of adults were investigated in the diurnal fly, *Drosophila melanogaster* Meigen, 1830 (McLay et al., 2017). ALAN modifies the environmental cue used by caterpillars of *Mamestra brassicae* Linnaeus, 1758 (Noctuidae) to initiate pupal diapause, resulting in suppressing diapause initiation and reducing larval developmental time (Van Geffen et al., 2014). In the research of Thakurdas et al. (2009), high intensity lighting at night reduced the eclosion rate of pupae and caused arrhythmic eclosion events in the tropical fruit fly *Drosophila jambulina* Parshad & Paika, 1965. On the contrary, green light illumination at night positively affected all different developmental stages except the egg stage and the longevity of the oriental armyworm *Mythimna separata* Walker, 1865 (Kim et al., 2020).

The water bug species *Diplonychus rusticus* Fabricius, 1871, belonging to the giant water bug family Belostomatidae (Insecta: Hemiptera), is commonly found in various types of freshwater bodies in tropical and subtropical regions (Ohba, 2019). The potential of *D. rusticus* (herein also referred to as "water bugs") in controlling mosquito vectors as a biological agent has been documented in several previous studies (Das & Maity, 2023; Duong et al., 2021; Rajan, 2015). However, the urban environment has many negative impacts on insects and evaluations of the effects of light pollution on *D. rusticus* have yet to be reported. Light pollution, particularly caused by overexposure to artificial lighting, can alter the growth, development, and interactions of the bugs with the environment as well as their predatory effectiveness (Parkinson et al., 2020).

MATERIALS AND METHODS

Field collection of *Diplonychus rusticus*

Adult individuals of *D. rusticus* were collected from a natural pond in a rural area of Dai Dong Commune, Thach That District, Ha Noi City, Viet Nam (21°05’24.7”N, 105°34’08.0”E) that was not affected by ALAN. All live specimens were kept in plastic containers during field trips and then transferred to and maintained in the laboratory of the Department of Applied Zoology, VNU University of Science, Ha Noi.

Laboratory rearing condition

All water bugs were reared with dechlorinated tap water in a rearing system at laboratory condition with water temperature of 22–24 °C, pH ≈ 6.5. For manipulating photoperiods, LED lights (24 W, 120l m/W efficiency, white light output at 6500K, CRI > 80 color rendering index) were used for rearing containers. This LED light was chosen because it does not generate heat, ensuring that it does not influence experimental results. In each rearing container, we also added a transparent nylon float to provide perching surfaces for the bugs, reducing their stress while ensuring that no shading occurred, which could otherwise influence the experiment on the effects of light.

The 3rd and 4th instars of the *Aedes* mosquito were used as prey in all experiments. Mosquito larvae were supplied by the Faculty of Entomology, National Institute of Malariology, Parasitology and Entomology, Ha Noi.

Developmental test: the effect of photoperiods on the development of *Diplonychus rusticus*

In this experiment, we set up a rearing system with modifications from that of Duong et al. (2021), with five different lighting conditions set to check the effects of photoperiods on the development of *D. rusticus* (Fig. 1).

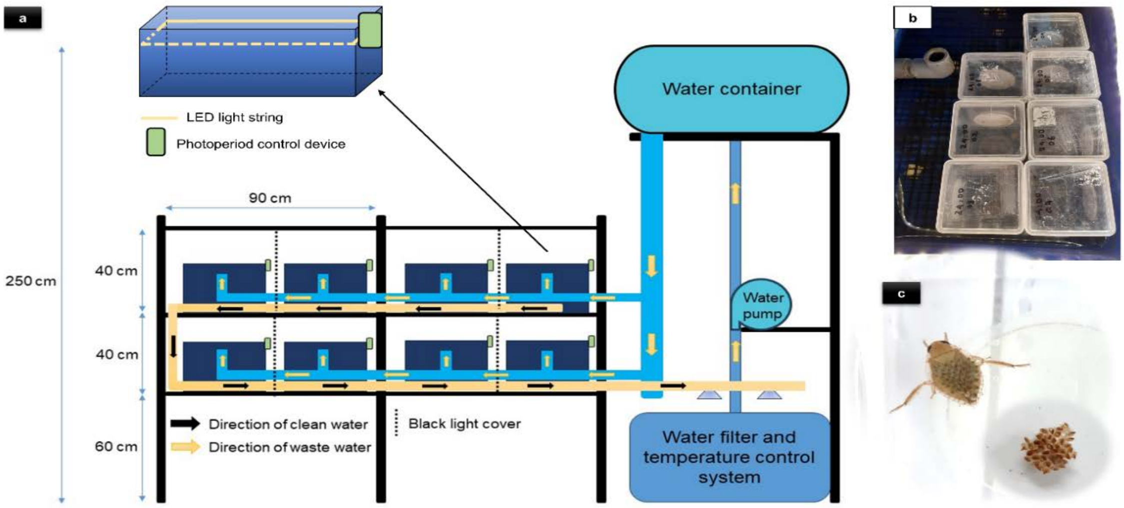

Figure 1. Rearing system modified from Duong et al. (2021) and water bug *Diplonychus rusticus*. a. Rearing system design, b. Rearing containers, c. A carry-egg male *Diplonychus rusticus* and egg batch

Males and females of water bugs collected from the field were randomly paired for breeding in the laboratory. Forty F1 individuals hatched from one pair of parents (F0) were separated into five groups categorized by different lighting conditions, namely Group 1 (00L:24D), Group 2 (06L:18D), Group 3 (12L:12D), Group 4 (18L:06D), and Group 5 (24L:00D) (Fig. 1b). Bugs were reared separately in plastic containers (9.8 × 6.7 × 6.7 cm) located in rearing system (Fig. 2). Each of them was subjected to a diet of three *Aedes* larvae per day for the first instar, four larvae for the second instar, and five larvae for the later development stages (based on Duong et al., 2021).

In this experiment, photoperiods with longer lighting time were used to simulate the effect of light pollution on bugs, while photoperiods with longer dark period than light period showed the importance of light on the life cycle of this species and photoperiods of 12L:12D to represent the typical lighting conditions found in tropical areas.

For each development stage, the number of days between two molts and the body size (length and width) of *D. rusticus* after each molting were recorded. For measurement of body size, the bugs were temporarily transferred into a white tray containing a damp thick tissue paper for photographing. All photos were taken with a digital camera (Nikon z6ii, Nikon Corporation, Tokyo, Japan) which was placed perpendicular to the tray and at about 30 cm away from the tray, and a ruler with a precision of 1 mm was accompanied in the tray for scale. The photos were then analyzed in Photoshop (Adobe, 2020) to calculate the body length and width of the bugs (Fig. 2).

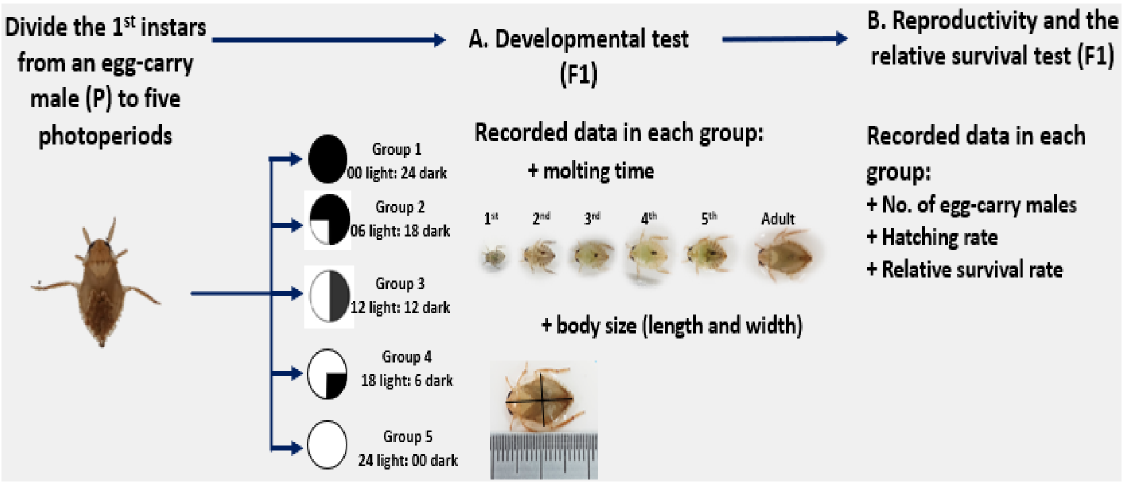

Figure 2. Research scheme for evaluating the effects of photoperiods on development, reproduction and survival rate of *Diplonychus rusticus* under laboratory conditions

Evaluating the reproductivity and the relative survival rate of *Diplonychus rusticus*

All five groups in the previous experiment were continuously reared in the same lighting conditions and were used to evaluate their reproductivity and the relative survival rate (Fig. 2). First, we examined their sex, in which the male can be separated from the female by having apically truncate genital operculum and a pair of small tufts of setae, while the female has rather pointed genital operculum and without tufts of setae (Millanes & Javier, 2019). As a result, the number of males in Groups 1, 2, 3, 4, and 5 was 3, 3, 3, 2, and 3, respectively, forming the same number of pairs in each group. After that, we reared each pair in different plastic containers (9.8 × 6.7 × 6.7 cm) for mating. When males were observed carrying eggs on their back, the females were removed into new containers. The number of eggs on each male was counted, and the egg-carrying males were checked daily to evaluate the hatching success rate, which was determined by the number of eggs that successfully hatched out of the total number of eggs on each male's back.

For measuring the relative survival rate, we continued to rear the adults in each lighting group separately. Because all individuals of Group 1 died at day 138 since hatching, the relative survival rate of the remaining groups was calculated based on the number of live individuals at that day.

Data analysis

To evaluate the significant difference between developmental time or body size (length and width) of nymphs and adults after molting in different photoperiods, a two-way ANOVA test was used. All analyses were conducted using the R program (RStudio Team, 2020).

RESULTS

Effect of photoperiods on development time and body size

The results showed a significant difference of development time of *D. rusticus* rearing different lighting conditions (ANOVA two-ways, F = 65.10, df = 1, p < 0.001, Fig. 3).

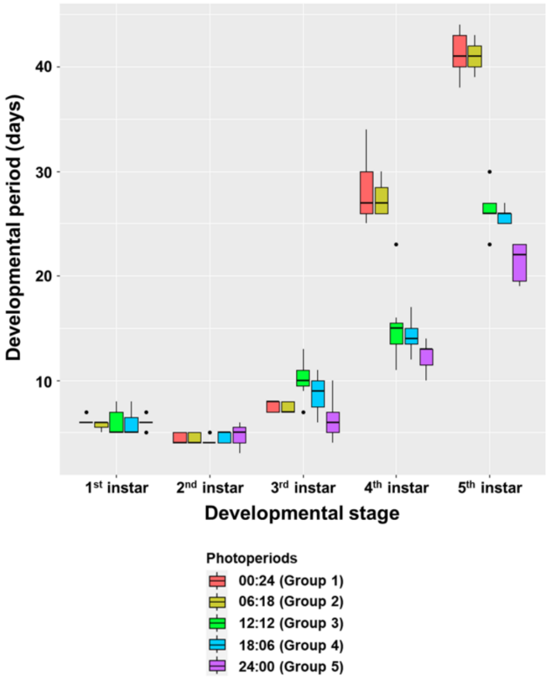

Figure 3. Duration of instar development (days) in five different light:dark photoperiods

Specifically, the development time (the number of days from first instar to adult stage) of *D. rusticus* in Group 5, under the photoperiod of 24L:00D, was the shortest among the five groups, averaging 50.57 ± 1.90 days. This was followed by Group 4 (18L:06D) and Group 3 (12L:12D), with average development times of 59.14 ± 2.27 days and 62.00 ± 5.74 days, respectively. In contrast, water bugs reared in Group 2 (06L:18D) and Group 1 (00L:24D) had the longest average development times, at 86.00 ± 1.29 days and 87.71 ± 1.80 days, respectively. The difference was most pronounced in the development time from the fourth to the fifth instars.

Regarding body size, there was a significant increase after each molting time in any group. However, there were no significant differences in the average size of both body length and body width when comparing between Groups at any developmental stage (ANOVA two-ways, F < 1, df = 1, p > 0.05).

Reproduction and relative survival rate of *Diplonychus rusticus*

The study results also showed a distinct difference in the reproductive success of *D. rusticus* among the five experimental groups under varying photoperiod conditions. Notably, no egg-carrying males were observed in Group 1 (00L:24D), indicating no successful reproduction (Table 1).

In Group 2 (06L:18D), only one male was observed carrying eggs, but the egg-hatching rate was 100%. Group 3 (12L:12D) showed the highest mating success, with all three males carrying eggs and an egg-hatching rate of 99.12%. In Group 4 (18L:06D), the egg-hatching rate was slightly lower than in Group 3 (92.67%). Notably, in Group 5 (24L:00D), three out of four males were observed carrying eggs, but these males discarded the egg batches shortly after the females laid them.

All individuals in Group 1 (00L:24D) were the first to die, with the last individual died on day 138 since hatching. Consequently, the survival rates for the other groups were calculated on day 138. The survival rate was

100% in both Groups 2 and 3 (all seven individuals survived), while it slightly declined to 85.7% in Group 4, which suffered longer lighting conditions of 18 hours of light and 6 hours of dark. Noteworthy, a considerable decrease to 57.1% under the fully-lit condition in Group 5 (24L:00D, Table 1).

Table 1. Reproduction and survival rate of *Diplonychus rusticus* in different photoperiod conditions. N/A: Not available

Groups (Light:Dark)	No. of egg-carry male	Hatching rate (%)	Survival rate (%)
Group 1 (00:24)	N/A	N/A	0%
Group 2 (06:18)	1	100%	100%
Group 3 (12:12)	3	99.12%	100%
Group 4 (18:06)	2	92.67%	85.7%
Group 5 (24:00)	3	30.11%	57.1%

DISCUSSION

Our experiment results showed the significant influences of light regimes on several aspects of *D. rusticus*, such as development time, reproduction, and survival rate under laboratory conditions indicating the negative impacts of ALAN on aquatic insect species, which are not even attracted to light sources. Particularly, the development time of the water bugs significantly decreased with increasing lighting duration (Fig. 3). Notably, the developmental time of Group 5 (50.57 ± 1.90 days) was reduced by almost 50% compared to that of Group 1 (87.71 ± 1.80 days). This result suggests that light pollution, in the form of longer photoperiod, can shorten the developmental time of D. *rusticus*, suggesting longer light hours could accelerate development, while shorter periods may lead to slower growth or even diapause. This phenomenon has also been found in the findings of Cymborowski & Giebułtowicz (1976), which also proved that the photoperiods shortened the developmental time of moth larvae, *Ephestia kuehniella* Zeller, 1879, but contrasts with the results of Kryspin et al. (1974), who evaluated illumination conditions' effects on wax moth *Galleria mellonella* Linnaeus, 1758. It is possible that light pollution triggers different physiological responses in different insects, leading to varying changes in their life cycles when exposed to prolonged lightning.

Additionally, our experiment was the first to investigate the influence of different photoperiods on the size of *D. rusticus* nymphs at each instar stage. There were no statistically significant differences between the experimental groups, indicating that the change of development time due to differing light conditions did not affect the size of this insect, at least within a generation. The size to which an individual and its body parts grow is affected by both genetic and environmental factors that operate through complex molecular and physiological mechanisms (Nijhout, 2003). *D. rusticus* may have a fixed growth trajectory dictated by its genetics; therefore, changes in photoperiod possibly do not alter body size in any life stage of the species. Furthermore, some insects typically grow in response to the nutritional availability (Koyama & Mirth, 2018) or other environmental interactions (Wonglersak et al., 2020) rather than light cues alone. In the current study, all individuals originated from the same parents to ensure a consistent genetic background. Additionally, all groups were provided with identical diets and living conditions, with the only variable being the photoperiod. Therefore, any observed differences among the groups can reasonably be attributed to the effects of photoperiod. This controlled approach strengthens the validity of the conclusions drawn regarding the influence of light exposure on the studied traits.

Noteworthy, we successfully evidenced the importance of light to the development, reproduction and survival rate of an aquatic insect. First, in the environment of the absence of light, all individuals died soon after reaching the adult stage (Group 1), highlighting the importance of light as an indispensable factor in the survival, development, and reproduction of the water bug. Further, although the water bug reared under longer lighting durations (Group 4: 18L:06D and Group 5: 24L:00D) had shorter development times, it was not entirely beneficial, as the reproduction rate and survival rate were significantly reduced in these groups. As a result, shorter development time seems to be an evolutionary trade-off in this study, given the reduced survival and reproduction in adult *D. rusticus* caused by prolonged lighting exposure. While faster development could lead to earlier reproduction, it may also compromise the reproductive quality of different groups in the current study with fewer eggs or lower-quality offspring. The reduction in adult survival and fecundity due to ALAN is similar to the phenomenon observed in the fruit fly *D. melanogaster* (McLay et al., 2017). However, there was no effect of ALAN on the duration of the juvenile phase in the fruit fly, like the shortened development times in the nymphs of the water bug. Additionally, insects may not have enough time to adapt to environmental changes (longer lighting time in our study) because of increased metabolic stress caused by accelerated growth (Yadav & Sharma, 2014). Consequently, light pollution may be a key factor in the disappearance or loss of biomass among many insect species. We propose that ALAN could potentially have long-term negative impacts on the sustainable development of populations of predatory aquatic insects. Our results provide a foundation for modeling water bug species' responses to light pollution and contribute to understanding the potential consequences of ALAN for non-strictly nocturnal insects.

While the laboratory results show a clear photoperiod effect under otherwise constant conditions, natural populations are subject to variation in temperature, prey density, water chemistry, vegetation, and interspecific interactions that may modulate or mask this effect. Future work should test whether the laboratory photoperiod effects persist under natural complexity using replicated semi-natural mesocosms and field enclosures that manipulate perceived day/night regimes while monitoring temperature and water chemistry combined with prey density and the presence of aquatic plants.

CONCLUSION

In conclusion, our study demonstrated that the duration of light exposure was positively correlated with the developmental pace of the water bugs, but photoperiods did not influence body size across experimental groups at any development stage. However, prolonged lighting significantly diminished the survival and reproductive success of the species. These findings also suggest that ALAN potentially reduce their reproductive success and overall fitness of *D. rusticus* in the ecosystem. Future research should explore the effects of light pollution on *D. rusticus* in environments with aquatic plants, offering potential strategies to protect aquatic insects from the adverse effects of light pollution. Moreover, to obtain results that are representative of the species over the long term, it is essential to observe individuals from different parents with diverse genetic backgrounds across multiple generations. Additionally, considering the responses of *Aedes* mosquito larvae to ALAN effects could help us better understand changes in predator-prey interactions between water bugs and mosquito larvae under ALAN.

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