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Effects of Photoperiod and Temperature on the Development and Survival of *Eocanthecona furcellata* (Hemiptera: Pentatomidae)

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Abstract: The present study assessed the effects of photoperiod and temperature on the development and survival rate of the predatory insect *Eocanthecona furcellata* under various photothermal gradients in climate-controlled chambers. The eggs of *E. furcellata* did not hatch at 15°C and exhibited the lowest hatching rate at 35°C, with only 28.0% and 13.0% hatching rates under long and short photoperiods, respectively. Moreover, the nymphs hatched under the conditions mentioned above did not survive. Linear regression analysis identified 17.7°C as the developmental threshold temperature for eggs, with an effective accumulated temperature of 62.3 °C. The developmental threshold temperature for nymphs was 17.0°C, with an effective accumulated temperature of 167.3 °C. These findings indicate that temperature exerts a significant impact on the developmental rate of *E. furcellata* during early stages unlike photoperiod. Under both long and short photoperiods, the eggs exhibited high hatching rates, while nymphs demonstrated the highest survival rates at 25°C. Further analysis with the Weibull distribution function showed that the survival rate curves for the insect at the early stages fitted Type I category, indicating that mortality rate increased over time. Thus, we recommend rearing eggs under short photoperiod conditions and nymphs under long photoperiod conditions, maintaining the temperature of the growth chamber at 25°C.

Key words: Developmental duration, effective accumulated temperature, *Eocanthecona furcellata*, insect rearing, survival rate, threshold temperature

Introduction

Sustainable pest control has become a major goal of most national initiatives, promoting green agricultural development and zero growth in pesticide usage, including the European Union, United States of America, Japan, China and Korea (Naranjo and Ellsworth 2009, Yang et al. 2018, Roca-Cusachs et

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al. 2020, Qin et al. 2021, Helepciuc et al. 2022). Predatory insects that serve as crucial biological control agents in agricultural and forestry systems suppress pest populations effectively, which contributes to their development as natural enemy insects, becoming an important part of integrated pest management (Li & Miao 2011; Martin et al. 2013, Zhan et al. 2021). However, the large-scale rearing of natural enemy insects is needed and depends on our understanding of their biology.

Eocanthecona furcellata is a predatory insect from the family Pentatomidae. It is an important natural enemy of various agricultural and forestry pests and is predominantly distributed in tropical and subtropical regions (Lin et al. 1998; Rider and Zheng 2002, Roca-Cusachs et al. 2020; Keerthi et al. 2020). Both the adults and nymphs of *E. furcellata* exhibit strong predatory capabilities (Lin et al. 1998, Yao et al. 2019), preying on over 40 species of pests from orders such as Lepidoptera, Coleoptera, Hymenoptera and Hemiptera (Pan et al. 2020, Vanitha et al. 2018, Lenin and Rajan 2016, Yu et al. 2021). They are known to effectively prey on the larvae of key lepidopteran pests, such as *Spodoptera frugiperda*, *Spodoptera litura*, *Helicoverpa assulta*, *Ostrinia furnacalis* and *Mythimna separata* (Chen et al. 2015, Fan et al. 2019, Liao et al. 2020, Chen et al. 2021, Zhao et al. 2022, You et al. 2023). In addition to the robust predatory ability, *E. furcellata* adversely affects the development and reproduction of its prey (Nuny and Wink 2000, Chen et al. 2014). Lin et al. (1998) reported that *E. furcellata* possesses various beneficial characteristics such as early field occurrence, prolonged activity period, high predation rate, strong mobility and multiple generations per year, making it a promising candidate for mass rearing and a potent biocontrol agent (He et al. 2013, Wen et al. 2017, Fan et al. 2019, Chen et al. 2021).

At present, *E. furcellata* has been artificially raised with artificial diet and a natural or semi-natural diet (Kuang et al. 2022, Del Mundo & Adorada 2024, Yuan et al. 2024). However, the artificial rearing conditions of *E. furcellata* are still being optimised. Understanding the impact of various environmental factors such as light, temperature, humidity and food on its growth, development, reproductive capacity and survival is essential for establishing large-scale rearing protocols for this insect. Zhu (1990) initially determined the developmental durations of various stages of *E. furcellata* under different temperatures. Later & Zhu (2020, 2023) found that low temperature had a significant impact on the growth, development and reproduction of *E. furcellata*. Yao (2019) examined the effects of photoperiod and humidity on its growth and reproduction. Yao et al. (2019) studied the impact of sex ratio on the reproductive capacity and lifespan of adults of *E. furcellata*. He et al. (2013) evaluated the effects of four types of diet on the growth and reproduction of *E. furcellata*, while Chen (2020) assessed the impact of two diets on successive generations. Recently, Yu et al. (2021) investigated the effects of rearing density on the growth and development of *E. furcellata*. These diverse studies have explored the impact of individual factors such as temperature, photoperiod and humidity on the growth, survival and reproduction of *E. furcellata*. These factors collectively influence insect development (Zhang 2011, Li et al. 2024). However, the combined effect of light, temperature and humidity on *E. furcellata* remains unclear.

The present study aimed to elucidate the combined effects of photoperiod and temperature on the development and survival of eggs and nymphs of *E. furcellata*. Our findings provide scientific evidence for the large-scale artificial breeding and field release of *E. furcellata*.

Materials and Methods

Insects

Specimens of *E. furcellata* were collected from a tobacco field in Shilin County, Yunnan Province (longitude 103°41′E, latitude 24°89′N). The collected specimens were reared in cages (45 cm × 45 cm × 45 cm) under laboratory conditions with 25 ± 1°C, 60 -80% relative humidity and a 14L: 10D photoperiod and fed with larvae of *Tenebrio molitor*. After establishing a stable population by continuously rearing for over ten generations, the specimens were used for experiments.

Egg hatching and larval development

The experiments were carried out in a climate chamber (SPX-400; Shanghai Boxun Medical Equipment Factory) with two photoperiod treatments: long photoperiod (15L: 9D) and short photoperiod (9L: 15D), using a constant light intensity of 10,000 lux. The photoperiod was set based on the shortest and longest daylight hours in winter and summer in Shilin County. Five temperature gradients were maintained for each photoperiod treatment: 15.0°C, 20.0°C, 25.0°C, 30.0°C and 35.0°C. Meanwhile, 70% relative humidity was maintained for all treatments, as suggested by Yao's (2019) research on *E. furcellata*.

A total of 100 newly laid eggs from different females were included under each treatment. After hatching, the nymphs were fed healthy mealworm larvae until adulthood. During the entire period, the numbers of hatched eggs, molts for each nymphal instar, emerged adults and deaths at each stage were recorded. The developmental durations of eggs and each nymphal instar were calculated using the collected data and the survival rate curves under various temperature conditions were constructed.

The developmental threshold temperature (°C) and the effective accumulated temperature (K) for eggs of *E. furcellata* and each nymphal instar under long photoperiod were calculated using the temperature gradients (15.0°C, 20.0°C, 25.0°C, 30.0°C and 35.0°C) as follows:

$T = C + KV,$
$\text{Let } V = 1/N$
$\text{Then } N = K/(T - C),$

where T was the treatment temperature and V was the developmental rate at each temperature and N was the developmental duration. Further, the linear regression method was employed to calculate the developmental threshold temperature and the effective accumulated temperature for the egg and each nymphal instar (Zhang 2011).

Weibull distribution function

The Weibull distribution function was used to fit the survival rate curve of each developmental stage (Pinder et al. 1987, Huang et al. 1987):

$$F(t) = 1- \exp [- (t/b)^c], t, c, b > 0,$$

where $F(t)$, b and c were the mortality rate at age t, scale parameter and shape parameter, respectively.

Data analysis

One-way analysis of variance (ANOVA) followed by a Tukey's test was performed to analyse the difference in nymphal developmental duration under different photoperiods and temperatures. Partial correlation analysis was conducted to analyse the effects of photoperiod or temperature on nymphal developmental rate, egg hatching rate and nymphal survival rate. All tests were performed in SPSS 25.0. The differences were considered significant at p = 0.05.

Results

Effects of photoperiod and temperature on the developmental duration of E. furcellata

Under a long photoperiod (15L: 9D), the developmental duration of eggs ranged from 3.6 to 18.0 days, that of nymphal instars – from 12.7 to 46.5 days and that of the overall juvenile period – from 17.7 to 64.4 days. On the other hand, under a short photoperiod (9L: 15D), the developmental duration of eggs ranged from 3.0 to 18.7 days, that of nymphal instars from 15.9 to 44.7 days and that of the overall juvenile period from 21.9 to 63.3 days (Table 1).

Within the 20°C to 30°C temperature range, the developmental rates of eggs and each nymphal instar were positively correlated with temperature (Table 1). Significant differences were detected in the developmental durations of eggs and nymphal instars among the temperature treatments under both photoperiods ($P < 0.0001$). Partial correlation analysis indicated that the developmental rate during the juvenile period was not correlated with photoperiod ($r = 0.086$, $P = 0.891$) but was positively correlated with temperature ($r = 0.944$, $P = 0.016$), suggesting a more significant impact of temperature on the developmental rate of *E. furcellata* than photoperiod during the juvenile period.

Developmental threshold temperature and effective accumulated temperature for various nymphal stages

Further analysis showed that the effective accumulated temperature required for *E. furcellata* to complete its juvenile period (egg stage + nymphal stage) was 233.1 °C ± 1.5 °C, of which 71.8% of the time was accounted for by the nymphal stage (Table 2). We also found that the developmental threshold temperature for eggs was higher than that for nymphs and the effective accumulated temperature required for egg development was greater than that needed for each nymphal instar. The developmental threshold temperatures for different nymphal instars were in the following order: 2nd instar > 4th instar > 3rd instar > 1st instar > 5th instar. The effective accumulated temperatures required for different nymphal instars were in the following order: 5th instar > 2nd instar > 1st instar > 3rd instar > 4th instar.

Effects of photoperiod and temperature on egg hatching rates

Under different temperatures, the hatching rate of eggs of *E. furcellata* ranged from 28.0% to 59.0% under the long photoperiod (15L:9D) and 13.0% to 79.0% under the short photoperiod (9L:15D) (Figure 1). However, the eggs did not hatch at 15°C under either photoperiod. The highest hatching rates were observed at 25°C, with 59.0% under long photoperiod and 79.0% under short photoperiod. Meanwhile, the lowest hatching rates

Table 1. Developmental duration of *Eocanthecona furcellata* under different temperatures and photoperiods.

Treatments		Developmental duration (d)							
Photoperiod	Temperature (°C)	Egg	1st instar	2nd instar	3rd instar	4th instar	5th instar	1st ~ 5th instar	Egg ~ adult
Long photoperiod (15L:9D)	15.0	-	-	-	-	-	-	-	-
	20.0	18.0 ± 0.2a (52)	6.2 ± 0.1a (46)	12.5 ± 0.6a (37)	8.6 ± 0.5a (24)	8.9 ± 1.2a (16)	10.7 ± 1.5a (11)	46.5 ± 2.0a (11)	64.4 ± 7.7a (11)
	25.0	9.3 ± 0.1b (59)	3.2 ± 0.1b (57)	4.5 ± 0.2b (57)	3.2 ± 0.1b (56)	3.3 ± 0.1b (56)	6.4 ± 0.1b (49)	20.3 ± 0.2b (49)	29.5 ± 1.6b (49)
	30.0	5.0 ± 0.0c (56)	2.0 ± 0.0c (51)	2.7 ± 0.1c (48)	2.1 ± 0.1c (46)	2.1 ± 0.0c (46)	3.9 ± 0.1c (36)	12.7 ± 0.1c (36)	17.7 ± 0.6c (36)
	35.0	3.6 ± 0.2d (28)	-	-	-	-	-	-	-
Short photoperiod (9L:15D)	15.0	-	-	-	-	-	-	-	-
	20.0	18.7 ± 0.1a (37)	5.6 ± 0.2a (26)	11.7 ± 1.2a (20)	9.2 ± 1.1a (14)	10.3 ± 0.9a (10)	12.7 ± 1.9a (7)	44.7± 2.7a (7)	63.3 ± 8.0a (7)
	25.0	8.0 ± 0.2b (79)	3.6 ± 0.1b (71)	5.3 ± 0.3b (39)	4.7 ± 0.2b (34)	3.6 ± 0.2b (31)	5.5 ± 0.5b (25)	21.2 ± 0.9b (25)	29.9 ± 5.0b (25)
	30.0	6.5 ± 0.3c (63)	2.6 ± 0.0c (53)	3.1 ± 0.1c (30)	4.5 ± 0.2c (23)	1.9 ± 0.1c (16)	4.1 ± 0.1c (13)	15.9 ± 0.2c (13)	21.9 ± 0.8c (13)
	35.0	3.0 ± 0.3d (13)	-	-	-	-	-	-	-

Note: Sample size is shown in brackets; different letters in the same column indicate significant difference between the respective treatments.

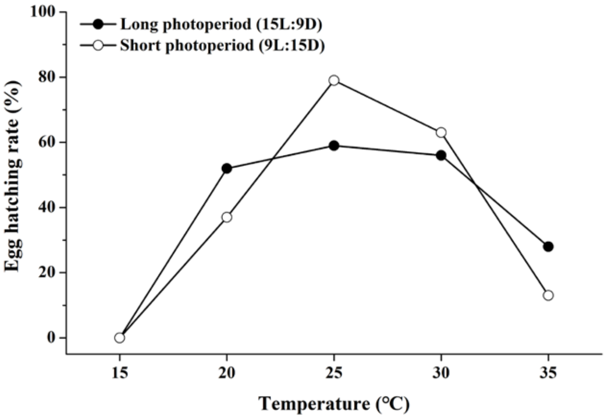

Fig. 1. Hatching rate of eggs of *Eocanthecona furcellata* under different photoperiods and temperatures.

were observed at 35°C, with 28.0% under long photoperiod and 13.0% under short photoperiod. Partial correlation analysis indicated that the hatching rate was not correlated with photoperiod (r = 0.021, P = 0.964) or temperature (r = -0.461, P = 0.298).

Effects of photoperiod and temperature on nymphal survival rate

Under different temperatures, the survival rate of nymphs of *E. furcellata* ranged from 78.2% to 92.0% under the long photoperiod (15L:9D), with 1st instar nymphs having a survival rate of 88.5% to 96.6%, 2nd instar – 80.4% to 100.0%, 3rd instar – 64.9% to 98.3%, 4th instar – 66.7% to 100.0% and 5th instar – 68.8% to 87.5%. Under a short photoperiod (9L:15D), the survival rate of nymphs ranged from 62.8% to 81.4%, with 1st instar nymphs having a survival rate of 70.3% to 89.9%, 2nd instar – 54.9% to 76.9%, 3rd instar – 70.0% to 87.2%, 4th instar – 69.6% to 91.2% and 5th instar – 70.0% to 81.3% (Figure 2A and 2B). Under a long photoperiod, significant differences were found in nymphal survival rates among the different temperatures (F = 10.781, P = 0.002). The highest survival rate was observed at 25°C (87.5% to 100%), significantly higher than that at 20°C (P = 0.002). Under a short photoperiod, no significant differences were found in nymphal survival rates among the different temperatures (F = 0.959, P = 0.411). Here, the highest survival rate was observed at 25°C (54.9% to 91.2%). Partial correlation analysis indicated that nymphal survival rate was positively correlated with photoperiod (r = 0.493, P = 0.007) but not with temperature (r = 0.358, P = 0.056).

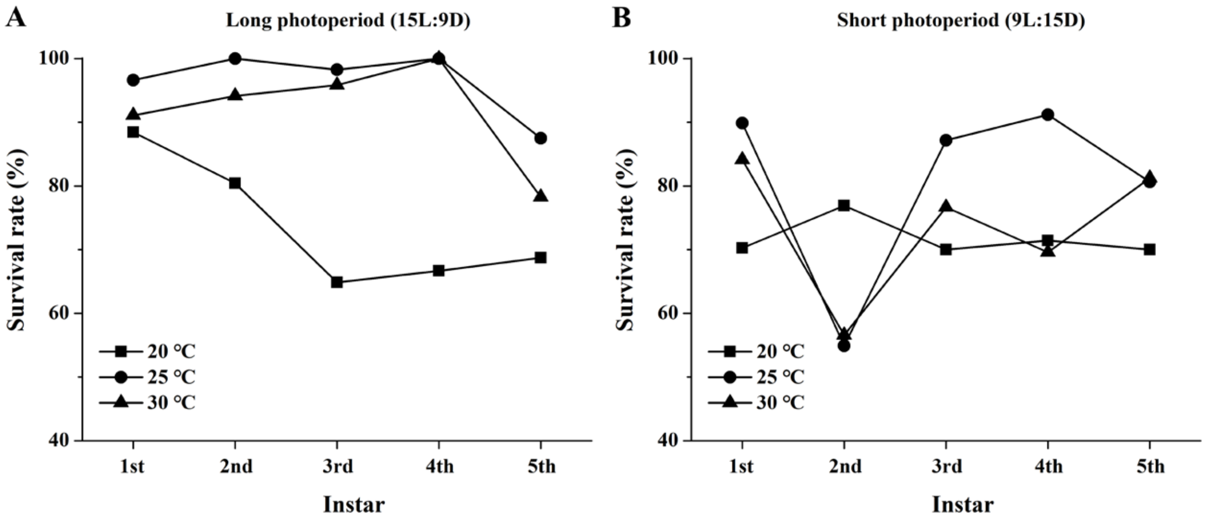

Fig. 2. Effect of temperature on the survival rate of different nymphal instars of *Eocanthecona furcellata* under long photoperiod and short photoperiod.

Table 2. Developmental threshold temperature and effective accumulated temperature of the eggs and nymphs of *Eocanthecona furcellata* under a long photoperiod.

Developmental stage	Developmental threshold temperature (°C)	Effective accumulated temperature (°C)	Forecast formula of developmental duration (N, days)
Egg	17.7 ± 1.0	62.3 ± 1.3	$N=\frac{62.3 \pm 1.3}{T-(17.7 \pm 1.0)}$
1st instar	15.9 ± 0.1	29.1 ± 0.4	$N=\frac{29.1 \pm 0.4}{T-(15.9 \pm 0.1)}$
2nd instar	18.0 ± 0.1	32.3 ± 0.2	$N=\frac{32.3 \pm 0.2}{T-(18.0 \pm 0.1)}$
3rd instar	17.3 ± 0.6	26.8 ± 1.6	$N=\frac{26.8 \pm 1.6}{T-(17.3 \pm 0.6)}$
4th instar	17.6 ± 0.3	25.7 ± 0.8	$N=\frac{25.7 \pm 0.8}{T-(17.6 \pm 0.3)}$
5th instar	15.6 ± 1.0	57.5 ± 5.7	$N=\frac{57.5 \pm 5.7}{T-(15.6 \pm 1.0)}$
1st - 5th instar	17.0 ± 0.0	167.3 ± 0.2	$N=\frac{167.3 \pm 0.2}{T-(17.0 \pm 0.0)}$
Egg – adult	17.1 ± 0.2	233.1 ± 1.5	$N=\frac{233.1 \pm 1.5}{T-(17.1 \pm 0.2)}$

Fitting survival curves for the *E. furcellata* juvenile stages under different photoperiod and temperature treatments

The Weibull function was used to fit the survival rate curves of juveniles of *E. furcellata* at different stages under different photoperiods and temperatures (Table 3; Figure 3A and 3B). The approach displayed good fitting under all photothermal conditions, except for the long photoperiod (15L: 9D) at 25°C. The c-values under all photothermal conditions were greater than 1. This observation indicated that the survival rate curves of juveniles of. *furcellata* belonged to the Type I category, wherein the mortality rate was found to increase with time. Under both long photoperiod (15L:9D) and short photoperiod (9L:15D) conditions, the b-values were the highest at 25°C and the lowest at 20°C, suggesting 25°C as the optimal temperature for developmental survival.

Table 3. Relevant parameters of survival rate curve of the immature stages of *Eocanthecona furcellata* under different photoperiods and temperatures determined by Weibull distribution function.

Photoperiod	Temperature (°C)	Weibull function	Confidence interval	R^2
Long photoperiod (15L:9D)	20.0	S〔t〕= exp〔$(-t/0.268)^{1.956}$〕	「1.319, 2.594」	0.951
	25.0	S〔t〕= exp〔$(-t/0.559)^{22.476}$〕	「14.141, 30.810」	0.690
	30.0	S〔t〕= exp〔$(-t/0.469)^{8.915}$〕	「5.973, 11.858」	0.875
Short photoperiod (9L:15D)	20.0	S〔t〕= exp〔$(-t/0.161)^{1.946}$〕	「1.340, 2.553」	0.998
	25.0	S〔t〕= exp〔$(-t/0.419)^{2.456}$〕	「1.725, 3.186」	0.928
	30.0	S〔t〕= exp〔$(-t/0.275)^{1.844}$〕	「1.280, 2.408」	0.972

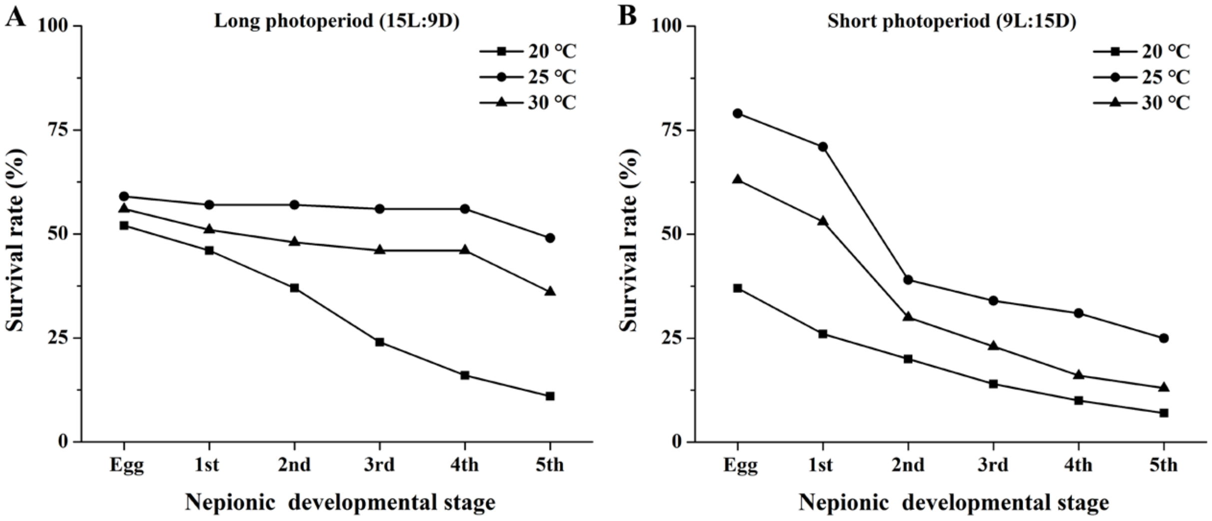

Fig. 3. Survival rate curve of juveniles of *Eocanthecona furcellata* under long photoperiod and short photoperiod at different temperatures.

Discussion

Light and temperature are crucial environmental factors that influence insect growth, development, mating and reproduction. Therefore, these factors are key elements in large-scale rearing (Zhou 2008, Bahsi & Tunc 2008, Pazyuk et al. 2014). The effects of temperature on the development, reproduction and diapause have been reported in *E. furcellata* (Peng et al. 2022, Zhu et al. 2023, Chen et al. 2025). However, the effects of photoperiod and temperature on the development and survival rate of the predatory insect *E. furcellata* remain unclear. The present study explored the mass breeding of the insect through studying the combined effect of light and temperature on the development and survival of eggs and various nymphal instars of *E. furcellata.* Using this approach, we identified the developmental threshold temperature and the effective accumulated temperature for the first time and fitted the survival rate curves under different photoperiods and temperatures. These findings provide a theoretical foundation for the artificial rearing and field augmentation of a significant predatory insect.

Furthermore, we found that within the 20°C to 30°C range, the developmental rate of eggs and nymphal stages accelerated as the temperature increased and the developmental duration significantly shortened. Partial correlation analysis revealed a greater impact of temperature on the developmental duration of the juvenile period of *E. furcellata* than photoperiod, consistent with the findings of Chen et al. (2018) on the tiger butterfly, *Danaus genutia*. Under both long and short photoperiod treatments, eggs did not hatch at 15°C; the highest hatching rates were observed at 25°C, with 59.0% under long and 79.0% under short photoperiod. The lowest hatching rates were observed at 35°C, with only 28.0% under the long photoperiod and 13.0% under the short photoperiod and the nymphs hatched at this temperature did not survive. A previous study on *E. furcellata* has shown that diapause eggs did not occur at 26°C, however, eggs under the 30:28°C (light: Dark) regime entered diapause and more than 80% of eggs of *E. furcellata* diapaused under the 35:33°C (light: Dark) regime (Wen et al. 2017), this report is consistent with our results, which may be related to the strategy to survive adverse high temperatures through diapause (Wen et al. 2017, Saulich et al. 2017). Under the long photoperiod, the highest nymphal survival rate was found at 25°C (96.5%); this rate was significantly higher than at 20°C (P = 0.002). Under the short photoperiod, the highest nymphal survival rate was also at 25°C (80.8%). Partial correlation analysis showed a significant positive correlation between nymphal survival rate and photoperiod (r = 0.493, P = 0.007). Similarly, Chen et al. (2020) studied the combined effect of light and temperature on the survival of the eggs, larvae and pupae of tiger butterflies. They found that a long photoperiod (15L: 9D) was more conducive to larval survival, while a short photoperiod (9L: 15D) favoured egg hatching.

Recently, Zhang et al. (2022) found that, under indoor conditions of 27 °C with 60%-80% relative humidity and a 14L:10D photoperiod, the survival rate of nymphs of *E. furcellata* was 80.2%. Yao (2019) studied the effects of different humidity and photoperiod conditions at a constant temperature of 26°C and found significant effects of photoperiod and humidity on the growth, development and survival of *E. furcellata* during the juvenile period. They observed that the highest nymphal survival rate (72.2%) was under a 16L: 8D photoperiod and 70% relative humidity. Our study found that under a combination of 25°C, 70% relative humidity and a 15L: 9D photoperiod, the nymphal survival rate of *E. furcellata* reached 96.5%, much higher than the previous reports. Thus, the conditions we chose for this experiment were the most suitable conditions for the development and survival of *E. furcellata*. Additionally, we found that the survival rates of 2nd and 5th instar nymphs were lower under all photothermal combinations, consistent with the findings of Lin et al. (1998) and Zhang et al. (2022). The reasons for the low survival rates of the 2nd and 5th instar nymphs remain unclear and future studies are needed for clarification. Moreover, our results suggest that special attention should be paid to the management of 2nd and 5th instar nymphs during rearing.

We also calculated the developmental threshold temperatures and effective accumulated temperatures for various juvenile stages. The developmental threshold temperatures and the effective accumulated temperatures for eggs were 17.7°C and 62.3 °C, respectively, while those for nymphs were 17.0°C and 167.3 °C, respectively. These findings are consistent with our earlier observations and previous reports, indirectly indicating that *E. furcellata* is cold intolerant (Zhu 1990, Lin et al. 1998) and that the insect should be reared at temperatures higher than 17°C. Besides, the effective accumulated temperature required to complete the juvenile period was 233.1 °C. This observation suggests that more than ten generations could be reared annually in the low-altitude areas of tropical and subtropical countries, highlighting the species' substantial potential for mass rearing and utilisation.

Thus, the present study determined the optimal photothermal range for the growth and development of *E. furcellata* during the juvenile period, which provides a basis for further research into the mechanism of how photoperiod and temperature affect the juvenile development of *E. furcellata* and provides a scientific basis for the large-scale rearing of the insect. Based on these findings, we recommend maintaining a short photoperiod for rearing eggs and a long photoperiod for nymphs, both at a temperature of 25°C.

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