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Thermal Stress Responses in Antilochus coquebertii: Behavioral and Developmental Plasticity Under Short Cold Exposure

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Abstract

The pyrrhocorid bug *Antilochus coquebertii* F. (Hemiptera: Pyrrhocoridae) is a specialist predator of members of the genus *Dysdercus*. Climate extremes, especially temperature extremes, are becoming more frequent and intense, affecting insect populations. However, *A. coquebertii* responses to these changes are not well understood. In the present study, the developmental biology of *A. coquebertii* was examined in a growth chamber at six different temperatures between 0°C and 12°C for 3 h. The effects of all these temperatures were compared with the effects at 25°C (control). After cold exposure, the insects were transferred to controlled laboratory conditions. According to the result of cold shock treatment, the developmental time of all the immature stages (except eggs) of *A. coquebertii* increased significantly as the temperature decreased from 12°C. While maximum survival rate was recorded at 25°C with respect to other lower temperatures examined. Longevity of male was recorded maximum at 25°C (117 days) as compared female which was maximum at 6°C (78 days). Likewise, female reproductive traits including fecundity, egg hatching percentage, and egg-laying frequency, all were negatively impacted at temperatures below 9°C. Short-term low-temperature stress significantly impacted the development, survival, and reproduction of *A. coquebertii*. Temperatures below 9°C prolonged immature stages and reduced female reproductive performance. These findings highlight the species' vulnerability to cold and its limited adaptability to extreme climate variations.

1. Introduction

Pakistan's most significant cash crop is cotton or *Gossypium hirsutum* L. (Malvaceae), with a 2.4% share in agriculture and 0.6% share in the country's gross domestic product. In recent years, adverse weather and pest infestations have significantly impacted the cotton crop (Anonymous, 2021). In Pakistan, *Dysdercus koenigii* F. (Hemiptera: Pyrrhocoridae), commonly known as the red cotton bug, is a major insect pest (Naqqash et al., 2014). The nymphs and adults of *D. koenigii* cause damage by feeding on mature cotton bolls. The damage is visible when feeding starts inside the boll, ultimately staining the lint (Shah, 2014). In Punjab, it causes 16–25% yield loss in the cotton crop (Jaleel et al., 2014). Different control methods, such as chemical, botanical, and biological methods, have been used to control *D. koenigii* populations (Hussain et al., 2021). Among these, biological control is one of the best alternatives because of its safety and non-toxic effects on the environment and human health (Grønvold et al., 1996).

Antilochus coquebertii F. (Hemiptera: Pyrrhocoridae) is an important predator that feeds voraciously on all stages of *D. koenigii* (Sahayaraj and Fernandez, 2017, Shafiq et al., 2020). It is widely distributed in cotton-growing areas of Asia (Azher et al., 2019, Kohno, 2003, Sahayaraj and Fernandez, 2021, Sarmad et al., 2020b). The population of *D. koenigii* is effectively controlled by *A. coquebertii* because of its longer lifespan and the predatory potential of both nymphs and adults (Sahayaraj and Fernandez, 2021, Sarmad et al., 2020b). Therefore, it can be successfully used in integrated pest management as a biological control agent for the management of *D. koenigii*.

Various abiotic factors, such as temperature, humidity, and rainfall affect the population of insects in the field, with temperature being one of the most important factors responsible for biological changes in arthropods (Arif et al., 2006). Every insect performs best within its specific temperature regime, which also determines its developmental biology (Liu and Tsai, 2000, Sarmad et al., 2020a). Currently, Research is primarily focused on the effects of constant temperature on insect populations (Muluvhahothe et al., 2024, Zamani et al., 2006). However, insects experience periodic fluctuations in nature. Short-term temperature variations can have lasting effects on insects development and reproduction (Huang et al., 2020). Different studies have shown significant differences in the biological parameters of insects under constant and changing environmental conditions (Huang et al., 2020, Jeffs and Leather, 2014, Sarmad et al., 2020a). The developmental duration and number of immature instars fluctuate with temperature (Aguilon and Velasco, 2015). Increases or decreases in temperature cause insects to develop at slower rates than at the optimum temperature (Sampaio et al., 2021). The metabolic profiles of insects (amino acids, sugars, and polyols) are also affected by decreases in temperature (Lalouette et al., 2007). As the temperature decreases, predators exhibit a decrease in predation rate (Parajulee et al., 2006). In the case of parasitoids, the parasitism rate increases as temperature decreases (Prasad et al., 2002). A sudden decrease in temperature causes the highest mortality among insects, unlike gradual temperature falls (Chen et al., 1987).

The developmental biology of predators is adversely affected by temperature fluctuations, particularly low temperature (Dubey and Mishra, 2016). According to Sarmad et al., (2020a) and Li YanHong et al. (2014), sudden temperature drops have been documented in cotton-growing areas, which could have an impact on insect populations and the activity of biocontrol agents. As the temperature does not remain constant in the natural environment, the primary goal of this study was to ascertain the impact of brief low-temperature fluctuations on the life history parameters of *A. coquebertii*. Furthermore, we aimed to determine whether *A. coquebertii* can withstand temperature fluctuations in the field.

2. Material and methods

2.1. Collection and rearing of insects

Laboratory-reared populations of *A. coquebertii* and *D. koenigii* were initially collected from cotton fields of the Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Punjab, Pakistan. Populations of both insect species were reared in plastic jars (26 × 12 × 12 cm) with a 3 cm layer of moistened sterilized soil at the bottom to create a natural environment for oviposition under controlled laboratory conditions (26°C ± 2°C, 65% ± 5% relative humidity [RH]). Water-soaked cotton and cotton seeds were used as the food sources for *D. koenigii.* Meanwhile, *A. coquebertii* was fed *D. koenigii* and water-soaked cotton. Muslin cloth was used to cover the jars to allow aeration (Saeed et al., 2018).

2.2. Effect of low temperature on the survival of immatures

Freshly laid eggs and newly molted nymphs (< 12 h old) of *A. coquebertii* were taken from the laboratory-reared culture and then shifted into glass petri dishes (diameter, 9 cm; depth, 1.5 cm) covered with

muslin cloth. After that, they were exposed to five different temperatures i.e., 12°C, 9°C, 6°C, 3°C, and 0°C, in a versatile environmental growth chamber (MLR-352H, Japan) for 3 h (Sarmad et al., 2020a). After exposure to specific selected temperatures, the exposed samples were placed under controlled laboratory conditions (26°C ± 2°C, 65% ± 5% RH) to check the survival rate and developmental time. Each specific temperature was considered a separate treatment replicated thirty times. The *A. coquebertii* were provided water-soaked cotton and nymphs of *D. koenigii* as food sources on a daily basis. The survival rate (which was determined based on visible movement, or responded to stimuli. Death was confirmed if there was no movement, response to external stimuli, or feeding activity) and developmental time (was determined by observing exuviae after molting) of the eggs and nymphal instars of *A. coquebertii* were observed every 24 h (Sarmad et al., 2020a).

2.3. Effect of low temperature on adult longevity and reproduction

Newly emerged adults both male and female (< 12 h old) of *A. coquebertii* taken from the laboratory-reared culture were paired and then transferred into glass petri dishes for cold temperature exposure (same temperatures as in Section 2.2) in a growth chamber for 3 h. After exposure to certain temperatures, the exposed paired adults were placed in small plastic jars (diameter, 5.5 cm; height, 6 cm) under laboratory conditions. The jar contained a 1-cm-thick layer of moist sterilized soil at its bottom. Each treatment was repeated thirty times, and data on adult longevity and reproduction were recorded daily.

2.4. Statistical analysis

Results of different sexes (male and female) were compared using pairwise *t*-tests, and the remaining data were analyzed by comparing the means using one-way ANOVA followed by the least significant difference (LSD) test at P = 0.05, using Statistical Analysis System (SAS) (Anonymous, 2021).

3. Results

3.1. Effect of low temperature on developmental time and survival rate of eggs

The statistically non-significant (P = *0.013*) difference was observed in the developmental time of eggs, tested on all the temperatures, except 0°C (Table 1). However, the survival rate of the eggs decreased substantially (P = *0.04*) with decreasing temperature. Maximum survival rate of (83%) was recorded at 25°C compared to those at 12°C, 9°C, 6°C, and 3°C, and 0°C (66.67%, 66.67%, 58.33%, and 50%, respectively. While, it was recorded minimum at 0°C (25%, Table 2).

Table 1
Influence of short-term cold temperature on developmental time of eggs and nymphal instars

Temperature (°C)	Developmental time (days ± S.E)†					
	Eggs	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar
25 (Control)	7.00 ± 0.00b,B	2.34 ± 0.19c,E	4.08 ± 0.08c,D	6.08 ± 0.29c,C	6.17 ± 0.21c,C	9.75 ± 0.48c,A
12	7.00 ± 0.00b,B	2.50 ± 0.07bc,D	5.71 ± 0.22bc,C	6.17 ± 0.21c,BC	6.34 ± 0.24c,BC	11.83 ± 0.70bc,A
9	7.00 ± 0.00b,B	2.75 ± 0.14abc,D	5.83 ± 0.35b,C	6.88 ± 0.41bc,BC	6.92 ± 0.29c,BC	12.75 ± 0.64b,A
6	7.00 ± 0.00b,B	2.79 ± 0.12abc,C	6.42 ± 0.25ab,B	6.83 ± 0.26bc,B	7.17 ± 0.47bc,B	13.00 ± 0.43b,A
3	7.25 ± 0.25ab,C	3.00 ± 0.41ab,D	6.38 ± 0.43ab,C	7.63 ± 0.38b,BC	9.83 ± 1.71ab,B	13.67 ± 0.59ab,A
0	7.50 ± 0.29a,C	3.25 ± 0.25a,D	7.83 ± 1.14a,C	8.75 ± 0.48a,BC	11.00 ± 1.08a,B	15.63 ± 0.92a,A

†Mean ± S.E. Different lower-case letter within each column shows the significant difference in the mean developmental time of each stage at different temperature and different upper-case letters within each row shows significant difference in the mean developmental time of different stages at same temperature using ANOVA: LSD at level P = *0.05*.

Table 2
Influence of short-term cold temperature on survival rate of eggs and nymphal instars

Temperature (°C)	Survival rate (% ± S.E)					
	Eggs	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar
25 (Control)	83.33 ± 9.62a,B	91.67 ± 8.33a,AB	100.00 ± 0.00a,A	100.00 ± 0.00a,A	100.00 ± 0.00a,A	100.00 ± 0.00a,A
12	66.67 ± 13.61a,B	83.33 ± 9.62ab,AB	91.67 ± 8.33ab,A	100.00 ± 0.00a,A	100.00 ± 0.00a,A	100.00 ± 0.00a,A
9	66.67 ± 19.25a,A	66.67 ± 13.61abc,A	75.00 ± 8.33abc,A	83.33 ± 9.62ab,A	100.00 ± 0.00a,A	100.00 ± 0.00a,A
6	58.33 ± 15.96ab,A	58.33 ± 15.96bcd,A	66.67 ± 13.61abc,A	75.000 ± 8.33b,A	83.33 ± 9.62ab,A	91.67 ± 8.33a,A
3	50.00 ± 9.62ab,AB	41.67 ± 8.33cd,B	58.33 ± 8.33bc,AB	66.67 ± 13.61b,AB	75.00 ± 8.33b,A	66.67 ± 13.61b,AB
0	25.00 ± 15.96b,B	33.33 ± 0.00d,AB	50.00 ± 16.67c,AB	33.33 ± 0.00c,AB	41.67 ± 8.3'3c,AB	58.33 ± 8.33b,A

Different lower-case letter within each column shows the significant difference in the mean survival rate of each stage at different temperature and different upper-case letters within each row shows significant difference in the mean survival rate of different stages at same temperature using ANOVA: LSD at level P = *0.05*. Survival rate of eggs is the ratio between total number of eggs to number of eggs hatched and survival rate of nymphs is the ratio between total nymphs to survived nymphs at each treatment

3.2. Effect of low temperature on the developmental time and survival rate of nymphs

The developmental time of immature *A. coquebertii* was significantly (P = *0.04*), affected by various low temperature stresses. The 1st instar nymphs of *A. coquebertii* showed the maximum developmental time at 0°C, followed by those at 6°C, 9°C, and 12°C. Minimum developmental time was observed at 25°C (Table 1). The 1st nymphal instar showed notably (P = *0.07*) maximum survival rate at 25°C compared to those at 12°C, 9°C, 3°C, and 0°C (Table 2).

The 2nd instar nymph of *A. coquebertii* took a substantially (P = *0.01*) longer time for development at 0°C and 3°C (7.83 and 6.42 days, respectively) than at 12°C and 25°C (5.71 and 4.08 days, respectively; Table 1). The highest survival rates (100% and 91.67%) were recorded at 25°C and 12°C, respectively, which were significantly (P = *0.05*) higher than those at 3°C and 0°C (58.33% and 50.00%, respectively; Table 2).

The 3rd nymphal instar took a significantly (P = *0.00*) longer time to develop into the 4th instar at 0°C, 3°C, and 9°C (8.75, 7.63, and 6.88 days, respectively) than at 12°C and 25°C (6.17 and 6.08 days, respectively; Table 1). However, the 3rd nymphal instar showed notably (F_5 = 10.22, P = *0.00*) higher

survival rates at 25°C and 12°C (100%) than at 6°C, 3°C, and 0°C (75.00%, 66.67%, and 33.33%, respectively; Table 2).

The 4th nymphal instar showed maximum developmental time at 0°C (11.00 days) as compared to 12°C (6.43 days) and 25°C (6.17 days; Table 1) (F_5 = 4.75, P = *0.001*). However, there was no significant difference in the survival rates of the 4th instar nymphs at 25°C, 12°C, and 9°C (100%) compared to those at 3°C and 0°C (75 and 41%, respectively; Table 2).

The developmental time of the 5th instar nymphs was significantly (F_5 = 7.81, P = *0.001*) higher at 0°C and 3°C (15.63 and 13.67 days, respectively) than at 9°C, 12°C, and 25°C (12.75, 11.83, and 9.75 days, respectively; Table 1). However, 100% survival was observed at 25°C, 12°C, and 9°C (F_5 = 7.12, P = *0.001*), unlike the survival rates at 3°C and 0°C (66.67% and 58.33%, respectively; Table 2).

3.3. Effect of cold temperature on adult longevity

The male adults of *A. coquebertii* showed maximum longevity at 25°C (117.33 days) and minimum longevity at 0°C (18.67 days) (F_5 = 7.68, P = *0.00*). In female adults, the maximum longevity was observed at 25°C (55.00 days), while the minimum was recorded at 0°C (11.00 days) (F_5 = 6.86, P = *0.00*: Table 3).

Table 3
Influence of short-term cold temperature on longevity of adults

Temperature (°C)	**Longevity (d ± S.E)** [†]	
	Male	**Female**
25 (Control)	117.33 ± 2.50 a, A	55.00 ± 1.45 ab,B
12	114.00 ± 5.46 a, A	54.00 ± 2.32 ab, B
9	94.00 ± 2.98 ab, A	49.00 ± 2.34 b, B
6	84.67 ± 3.43 ab, A	78.33 ± 4.98 a,A
3	61.67 ± 4.40 b, A	48.00 ± 3.43 b,A
0	18.67 ± 6.50 c, A	11.00 ± 4.56 c,A

[†]Mean ± S.E. Different lower-case letter within each column shows the significant difference in the mean longevity from other of same sex at different temperature using ANOVA: LSD at level P = 0.05 and different upper-case letters within each row shows significant difference in the mean longevity of both male and female at same temperature using t-test at level P = *0.05*.

There was a significantly different relationship between both sexes of *A. coquebertii* adults at 25°C (F_1 = 8.11, P = *0.01*), 12°C (F_1 =4.11, P = *0.04*), and 9°C (F_1 = 4.41, P = *0.04*), whereas non-significant differences were observed at 6°C (F_1 =0.44, P = *0.01*), 3°C (F_1 =0.57, P = *0.62*), and 0°C (F_1 = 1.15, P = *0.37*: Table 3).

3.4. Effect of cold temperature on reproductive traits of A. coquebertii

The pre-oviposition period was notably (F_5 =0.33, P = *0.88*) higher at 12°C, 6°C, and 9°C (10.67, 9.67, and 8.33 days, respectively) than that at 25°C and 0°C (7.67 and 5.33 days, respectively). Furthermore, the oviposition period was significantly (F_5 =7.52, P = *0.25*) higher at 6°C (61.67 days) and 25°C (45.67 days) than at 0°C (0.33 days). Meanwhile, the maximum post-oviposition period (10.67 days) was recorded at 3°C compared to that at 25°C (2.00 days; Fig. 1).

The egg-laying capacity of females significantly decreased with a dip in short-term cold temperature stress. The highest numbers of eggs laid by females (80.17, 68.82, and 66.67 eggs) were observed at 25°C, 12°C, and 9°C, respectively, which were much higher than those at 3°C and 0°C (30.89 and 5.00 eggs, respectively; Fig. 2). Significantly, the maximum oviposition frequencies of females were observed at 25°C, 12°C, and 9°C (11.67, 9.33, and 8.67 times, respectively), which were substantially higher than those at 3°C and 0°C (1.67 and 0.33 times, respectively; Fig. 3).

Similar findings were observed in the hatching percentage of eggs at 25°C, 12°C, and 9°C (91.36%, 90.89%, and 81.09%, respectively) ($F_{5,}$ = 6.39, P = *0.00*), whereas a significant difference was observed at 6°C, 3°C, and 0°C (59.76%, 31.68%, and 0.00%, respectively; Fig. 4).

4. Discussion

Thermal shocks, negatively affect arthropod populations (Jeffs and Leather, 2014). Among these, cold temperatures have a significant effect on the biological parameters of insects (Sarmad et al., 2020a). Our results revealed that temperature had no discernible effect on egg incubation duration. However, a significant reduction in survival rate was observed with a decrease in temperature. In terms of incubation period, the findings of Huang et al. (2014) contradicted our results, i.e., the incubation period of *Proprioseiopsis asetus* (Mesostigmata: Phytoseiidae) eggs was affected by a reduction in temperature, whereas the survival rate results were in accordance with our results. Similar observations related to the survival rate of eggs have also been reported in *Wollastoniella rotunda* (Hemiptera: Anthocoridae), showing the lowest survival rate at 15°C compared to higher temperatures (Shima and Hirose, 2002).

Our study demonstrated that a decrease in temperature (from the optimum temperature range) significantly increased the developmental duration of all the nymphal instars of *A. coquebertii*. These results corroborate the finding that different temperatures affect the developmental biology of *Aleurodicus dugesii* (Hemiperta: Aleyrodidae), as all the immature stages of *A. dugesii* develop faster as the temperature increase from 20°C, whereas the maximum developmental time occurs at 15°C (Schoeller and Redak, 2018).

According to the findings of the present study, a sudden decrease in temperature adversely affected the survival rate of all immature stages of *A. coquebertii.* The lowest survival rate was recorded at 0°C and

3°C and the highest, at 25°C. Findings parallel to our results were reported by Zhou et al. (2017) in *Axinoscymnus apioides* (Coleoptera: Coccinellidae), in which the maximum survival rate of all larval instars was recorded at 29°C and minimum at 17°C.

The shortest lifespan of both males and females was recorded at 0°C. These results conflict with the findings in *Phenacoccus madeirensi* (Hemiptera: Pseudococcidae), in which the lifespan of both males and females at 15°C was higher than those at 20°C and 25°C (Chong et al., 2003). Findings comparable to our results have been recorded for *D. koenigii* (Sarmad et al., 2020a). While the female of *A. coquebertii* showed the longest longevity at 6°C as compared to male that showed longest at 25°C. This variation may be attributed to gender-specific physiological and metabolic responses. This Sexual dimorphism in thermal tolerance has been reported in various insect species, with females often displaying slower metabolic rates and greater stress resilience under suboptimal conditions compared to males (Sinclair et al., 2003). Our results showed that the oviposition period of *A. coquebertii* females reduced significantly with a decrease in temperature, in contrast to the findings of Cao et al. (2018) in *Thrips hawaiiensis* (Thysanoptera: Thripidae). Similar to the oviposition period, the egg-laying capacity of females also declined significantly with a sudden decrease in temperature, which is comparable to the findings of Kim et al. (2008) in *Pseudococcus cryptus* (Hemiptera: Pseudococcidae). They showed the highest fecundity of *P. cryptus* to occur at 28°C rather than at 20°C and 16°C. The results of the current study demonstrated that the highest number of eggs hatched at 25°C, declining with a further fall in temperature, and the lowest hatching percentage was recorded at 3°C and 0°C. These results are consistent with findings in *Encarsia guadeloupae* (Hymenoptera: Aphelinidae) Mota et al. (2008). This study indicates that extreme cold shock negatively affects the population dynamics of *A. coquebertii*. These findings may be helpful in determining the population dynamics of *A. coquebertii* after sudden temperature drops under natural field conditions. This information will help us adopt better management strategies against *D. koenigii* under changing environmental conditions. Additional research is required to explore the functional analysis of the genes responsible for altering the critical biological traits of *A. coquebertii*.

Conclusion

Short-term exposure to low temperatures had a significant impact on the development, survival, and reproduction of *Antilochus coquebertii*. As temperatures decreased, the developmental period of immature stages increased, and survival rates declined. The reproductive performance of females was particularly affected, indicating that colder conditions can impair population growth. The study highlights that temperatures below 9°C are unsuitable for the successful development and reproduction of *A. coquebertii*, suggesting that extreme temperature fluctuations due to climate change could pose challenges to its survival and predatory efficiency. These findings provide valuable insights into the insect's thermal tolerance and its potential adaptability to changing environmental conditions.

Declarations

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Authorship contribution statement

A.M., S.M.Z. and W.J. conceived and designed the study, curated the data, carried out the investigation, and supervised the work. S.M.Z. and W.J. administered the project. A.M., M.S., M.U.A., M.Z.K., S.M.Z., R.Az., R.Ak., A.T., H.M.S. and W.J. performed the formal analysis, developed the methodology, validated the results, and prepared the figures. M.S., M.U.A., M.Z.K., S.M.Z., R.Az., R.Ak., A.T., H.M.S. and W.J. provided resources. All authors wrote the original draft of the manuscript and reviewed and edited it. All authors reviewed the final manuscript.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of competing interest

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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Figures

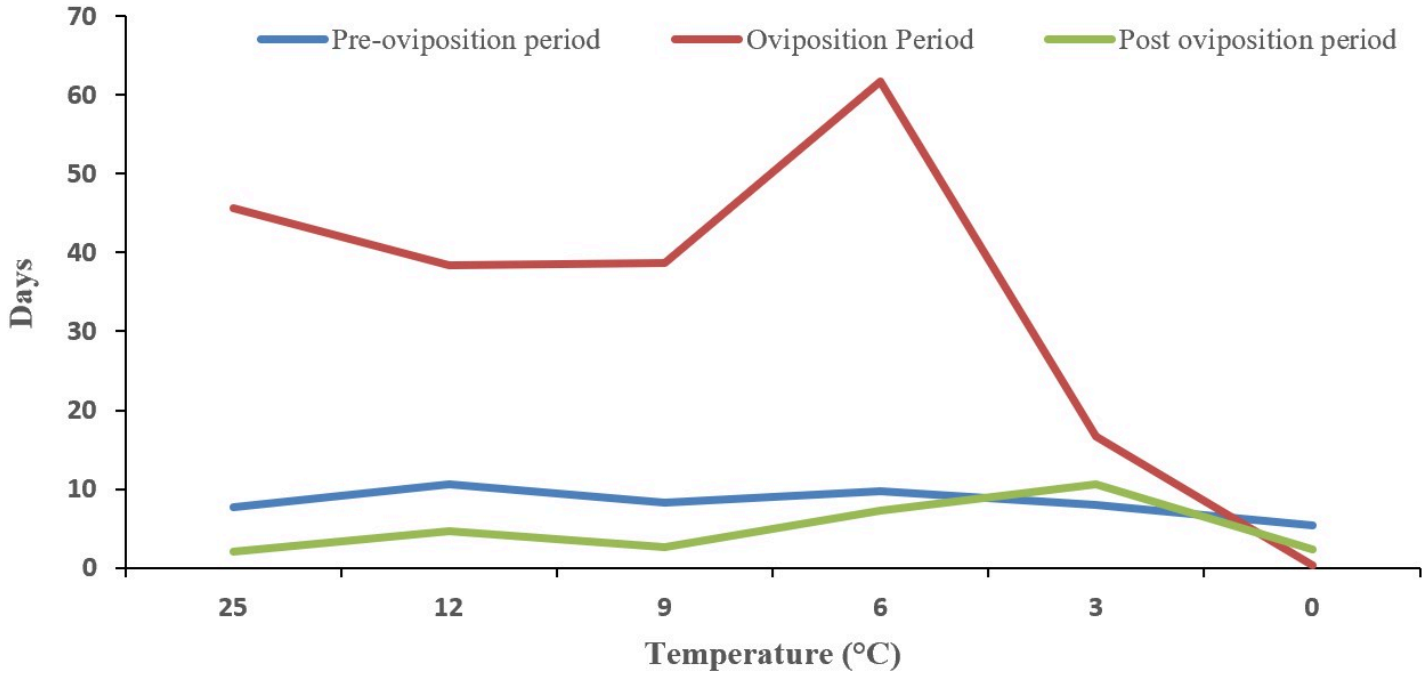


Figure 1

Short-term cold temperature effect on the pre-oviposition, oviposition, and post-oviposition periods.

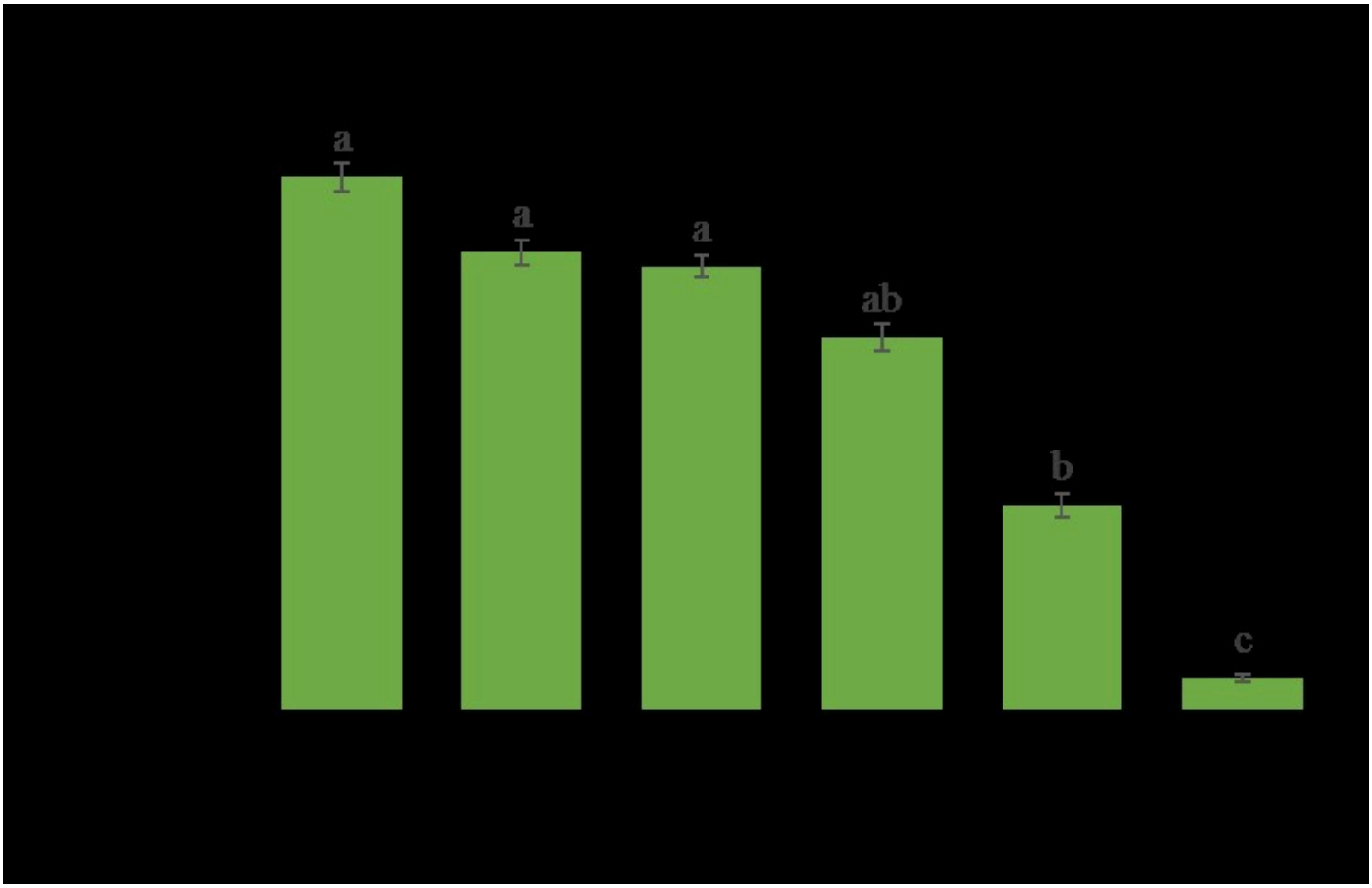


Figure 2

Short-term cold temperature effect on the egg-laying capacity of females. Different small letters among column shows significant difference among six different temperatures using ANOVA LSD at level P= 0.05.

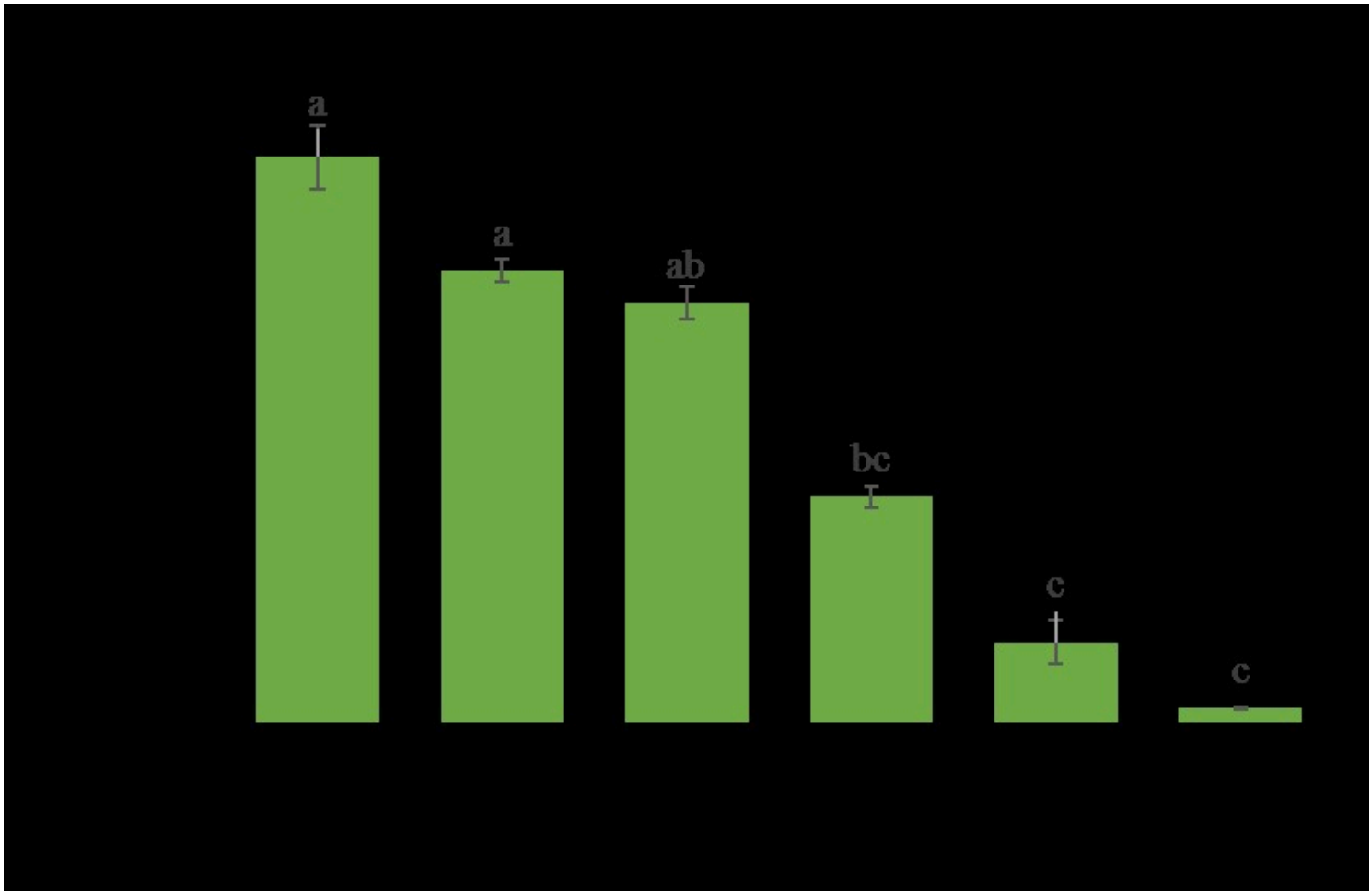


Figure 3

Short-term cold temperature effect on the egg-laying frequency of females. Different small letters among column shows significant difference among six different temperatures using ANOVA LSD at level P= 0.05.

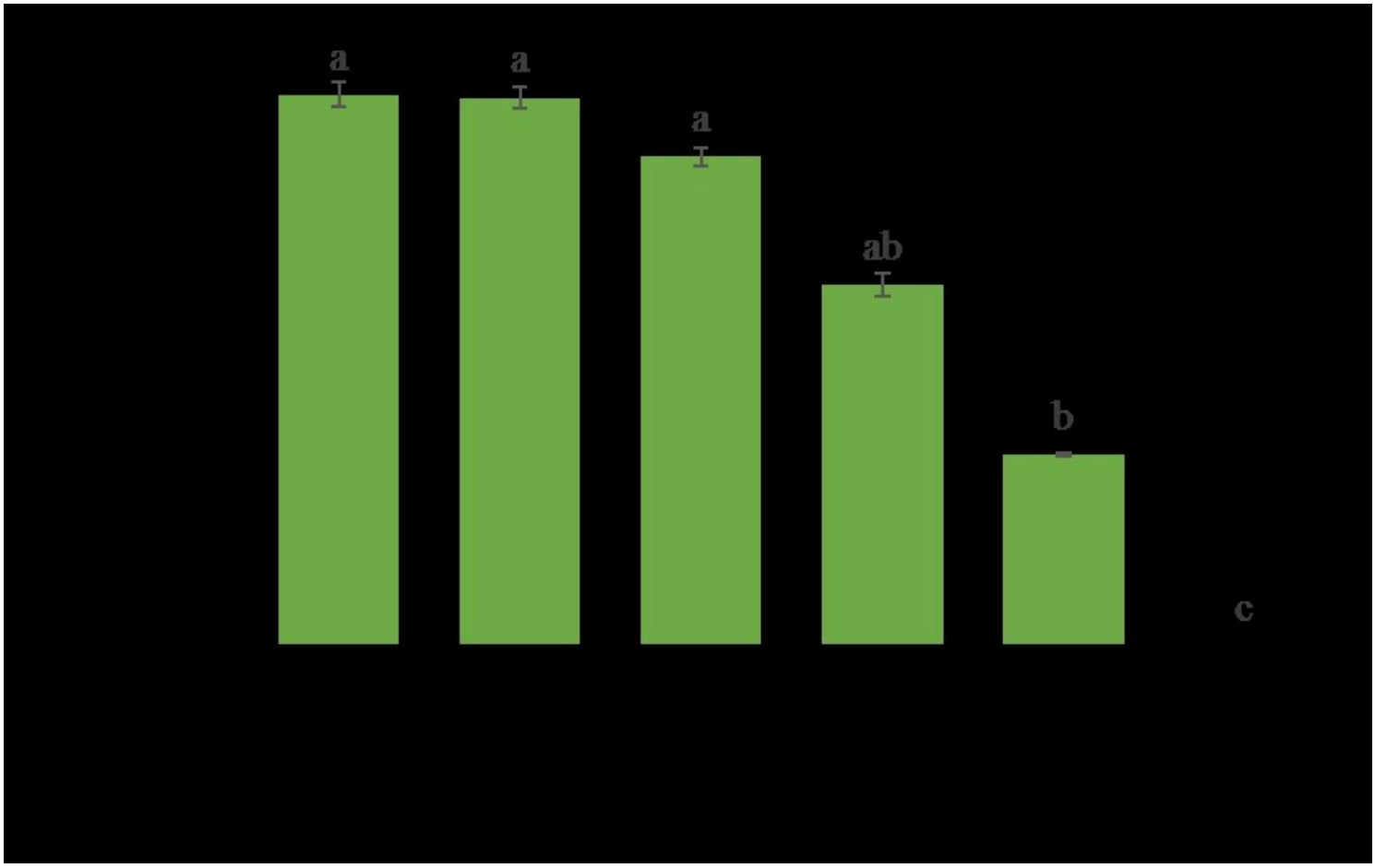


Figure 4

Short-term cold temperature effect on the hatching percentage of eggs. Different small letters among column shows significant difference among six different temperatures using ANOVA LSD at level P= 0.05