

Effects of temperature on the phenology and fitness traits of *Papilio polytes* (Lepidoptera: Papilionidae) in Dhaka, Bangladesh

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Abstract

Butterflies are very sensitive to temperature changes and are considered important bio-indicators. In the present study, an attempt was made to determine the effects of temperatures (32 °C, 29 °C and 20 °C) on the phenology, fitness traits, feeding, growth and development of *Papilio polytes* (Lepidoptera: Papilionidae) under laboratory conditions (16L : 8D; RH 78 ± 2) from May 2023 to February 2024. Different larval instars (n = 20 at 20 °C, n = 15 at 29 °C, and n = 15 at 32 °C) were used to evaluate the effects of the selected temperatures. The life cycle was the longest (45.86 ± 1.45 days) at 20 °C. The results reveal temperature-dependent effects on the survival rate of larvae. The mortality rate was significantly higher at 20 °C ($F = 46.801$; $df = 2$; $P = 0.0000083$). Egg hatchability (n = 13 at 20 °C, n = 15 at 29 °C and n = 27 at 32 °C) significantly varied across the three rearing temperatures ($\chi^2 = 10.575$; $df = 2$; $P = 0.0064$). The relative hatchability of eggs was the highest at 32 °C (n = 27) and the lowest at 20 °C (n = 13). Food consumption did not vary significantly across the three rearing temperatures ($H = 4.488$; $df = 2$; $P = 0.1060$), and faecal production of larvae was not significantly affected either ($F = 0.896$; $df = 2$; $P = 0.414$). The results indicate better performance of *P. polytes* under warmer temperatures.

Introduction

Climate change is considered a major anthropogenic disturbance to natural ecosystems, causing a rise in extreme temperature events (Diffenbaugh et al., 2005; Sala et al., 2000; Thomas et al., 2004; IPCC, 2014). Temperature is one of the most important ecological factors for the survival of ectothermic species (Parmesan, 2006; Overgaard et al., 2008). Butterflies are important bio-indicators for monitoring population changes in the context of global warming (Kremen, 1992; Wood & Gillman, 1998; Schulze et al., 2010; Singh & Sondhi, 2016). They are highly sensitive to temperature, changes in microclimate and habitat (Erhardt, 1985; Sakuratani & Fujiyama, 1991; Shobana et al., 2012). Climate change may potentially impact the life history traits and population dynamics of butterflies (Braby

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et al., 2014; Whitworth et al., 2018). The phenology of butterflies is affected in response to regional temperature variations (Walther et al., 2002; Parmesan & Yohe, 2003; Diamond et al., 2014; Dennis et al., 2016).

The butterflies belonging to the family Papilionidae represent a stunning assemblage and are globally distributed. They are ecologically significant pollinators and maintain the health of forest ecosystems (Murphy & Weiss, 1988; Pollard & Yates, 1993; Spitzer et al., 1997; Rajeswari & Jeyabalan, 2017). Though *Papilio polytes* is highly host-specific in the selection of larval food plants (Nakayama et al., 2003), this butterfly maintains a wide range of host plants during its life cycle. This species is considered a major pest of commercially important citrus fruit plants (Bashar, 2014; Peggie et al., 2022; Rao, 2015; Igarashi & Fukuda, 2000; Murakami et al., 2002; Corbet & Pendlebury, 2020).

The ongoing climate change and increasing temperature variability pose serious threats to many butterfly species having lower thermal tolerance (New et al., 1995; Piyaphongkul et al., 2012; Kingsolver et al., 2015). The survival rate of a butterfly is dependent on its ability to adapt to extreme temperatures (Weiss et al., 1988; Anderson et al., 2012). The egg size of the butterfly changes with temperature, leading to increased mortality rates (Fisher et al., 2003). The growth, development, and phenology of *P. polytes* is also dependent on its association with its host plants (Shobana et al., 2012; Peggie et al., 2022; Igarashi & Fukuda, 2000; Revathy & Mathew, 2014). Butterflies often exhibit plastic responses to differences in ambient temperatures (Islam et al., 2017; Jaafar et al., 2013; Schirmer & Gawryszewski, 2026). Elevated temperature is lethal for the survival, larval development, and egg hatchability of some swallowtail butterflies (Huang et al., 2024). Adult butterflies of *Vanessa cardui* that experienced the higher ambient temperature of 28 °C were larger than those experiencing lower temperature (24 °C) during the developmental stage (Mesler & Mabry, 2024). The development of *Papilio demoleus* L. and *Papilio polytes* L. was significantly affected by temperatures of 23 °C, 28 °C and 32 °C in India (Kumar et al., 2024). The pronounced effects of a similar range of rearing temperatures (20 °C, 27 °C and 32 °C) on the biology of *P. polytes* were also reported in Iran (Atapour & Osouli, 2017). The population of *P. polytes* at the immature stages varied in dry and wet seasons in Malaysia. Previous studies also revealed the effects of temperatures 25.5 °C to 32.1 °C on the hatchability rate of *P. Polytes* (Jaafar et al., 2013; Suwarno et al., 2010). Effects of temperature on the egg hatchability and larval development of *P. Polytes* have also been studied in Japan (Nakayama et al., 2003; Murakami et al., 2002). The biology and mortality rates of different developmental stages of this butterfly at 29 °C were studied under laboratory conditions (Islam et al., 2017). Very limited research has been carried out on *P. Polytes* in Bangladesh. Although the previous studies focused on the effects of rearing temperatures on the phenology of *P. Polytes*, no data exists regarding the effects of different seasonal temperature ranges (low, medium, and high) on the phenology, fitness traits, and larval development of this butterfly in the context of Bangladesh. Under the changing climatic conditions, it is important to determine the favorable range of temperatures for the

development of butterflies. Information is required to understand how this butterfly may adjust to the changing seasonal and specific regional temperature regime in an urban setting. Moreover, successful and effective conservation of this butterfly depends on a sound knowledge of its interactions with different temperature ranges. Therefore, the objectives of the present study were to evaluate the effects of temperature ranges (low representing winter, medium representing autumn and high representing summer) on the phenology, fitness traits, growth, development, food consumption and faecal production of *P. polytes* larvae. This research aims to understand the impacts of temperature on an urban butterfly (*P. polytes*) to assist effective conservation and biodiversity management of this butterfly.

Materials and methods

Study area and study period

The study was conducted in the Entomology Research Laboratory, Department of Zoology, University of Dhaka from March 2023 to February 2024. The samples of *Papilio polytes* (eggs, larvae and pupae) were collected once a week during the study period along with the host plant (*Glycosmis pentaphylla*) from the Germplasm Center of the Zoological Garden (GPC), University of Dhaka (latitude 23.726899° N, longitude 90.401542° E).

Sample collection and preparation

The butterfly samples were identified according to their external morphology and taxonomic keys (Rao, 2015), and the host plant was identified (Ahmed et al., 2008). Collected adults were preserved for wing venation and morphometric identification (Jaafar et al., 2013; Bashar, 2013). Observation of adult females and their egg-laying activities was conducted regularly (Kumar et al., 2024; Islam et al., 2019; Jermy & Szentesi, 1978). The eggs were attached to the host plant leaves; collected immediately after oviposition (within one day) by cutting the stems, and the cut ends were wrapped with soaked cotton (Fig. 1a, b). Water was sprayed on the eggs to prevent desiccation (Bashar, 2013; Arju et al., 2015a; Arju et al., 2015b; Miah et al., 2015). Collected larvae with host plant stems (Fig. 1c, d, e, f) were also wrapped with soaked cotton and immediately transferred into a 500 mL beaker containing water to prevent desiccation. Temperature and humidity at the GPC were recorded regularly using a Clock and Hygro-thermometer (Brannan, England) during the study period.

Preparation of samples and Temperature selection for rearing

During pre-treatment experiments, four rearing temperatures (20 °C, 23 °C, 30 °C and 35 °C) were selected based on previous studies and the overall seasonal temperature variations in Dhaka city. Five larvae of different instars (1st to 5th) were observed at each temperature. The results revealed no notable differences in the larval survival rate at 20 °C and 23 °C; hence, we selected 20 °C as a lower temperature during winter. Similar results

were found in the case of the higher temperature regime (30 °C and 35 °C). However, most of the treated larvae and supplied foods (host plants) were slightly desiccated at 35 °C. Hence, we selected 32 °C as the higher temperature during summer. We selected 29 °C as an intermediate temperature range of autumn in Dhaka. All selected rearing temperatures were related and justified with the recorded seasonal temperature in the study area (GPC) during the study period. The experimental work was executed sequentially: treatment at 32 °C, followed by 29 °C and then 20 °C. The replicates were increased in number gradually depending on the availability of samples (larvae) in nature. Seasonal variations in larval abundance were found during summer, winter, and autumn. The mortality rate was comparatively higher at 20 °C than in the other two treatments. The replicate numbers were adjusted with this rearing temperature for successful completion of phenology and temperature-dependent experiments.

The collected eggs of *P. polytes* were placed in Petri dishes with some water-soaked cotton, and the incubation period was observed under a portable binocular stereo microscope (Euromex EduBlue series) (Arju *et al.*, 2010). The larvae were kept in a special three-layered butterfly rearing cage (39.62 cm × 35 cm × 30 cm). A specialized round rearing box (15.5 cm × 7 cm) was placed inside the rearing cage. The rearing box was perforated with some pores (4-5) at the bottom so that the stems of the host plants were attached properly and connected with water to keep the stems alive. Adequate amounts of young shoots of *Glycosmis pentaphylla* were supplied regularly at 24-hour intervals as larval food. Four larval rearing boxes were prepared repeatedly for the fourth, third, second, and first instar larvae individually. Two emergence rearing boxes (52.5 cm × 45 cm × 90 cm) made of mosquito net and iron rods with dry stems of the host plants placed inside at different angles were used for pupation (Alam *et al.*, 2017). All samples were kept in a climate chamber (BIOBASE) under a 16L:8D photoperiod and at 78 ± 2 % RH at temperatures of 32 °C, 29 °C, and 20 °C. Larvae of different instars were used as sample replicates ($n = 20$, $n = 15$, and $n = 15$) for rearing at 20 °C, 29 °C, and 32 °C, respectively.

Effects of temperature on the phenology of P. polytes

The developmental duration (days) of each stage was recorded individually for each larval instar (1st to 5th), pre-pupal and pupal stages. To observe the phenology and survival rate, each rearing box contained two to three larvae of the same instar in order to avoid a lack of information in case of unusual larval death. The larvae were observed regularly at 8-hour intervals to supply food and to observe molting. Fresh leaves of the host plant were supplied at 2-day intervals for the 1st instar and at 1-day intervals for the 2nd to 5th instar. The weight of the supplied leaves was measured before and after feeding by using a digital balance (KERN ADB 220-4), and old foliage was removed regularly (Koda & Nakamura, 2010; Koda & Nakamura, 2012). In addition, water was sprayed on the supplied food of larvae to avoid moisture loss. Damp sponges were kept in the rearing box to prevent desiccation.

Effects of temperature on the egg hatchability of P. polytes

To investigate the effects of temperature on egg hatchability, the number of newly hatched larvae from collected eggs was counted at 24-hour intervals across all replicates (Islam et al., 2017; Geister et al., 2009). Egg production peaks particularly in July to October and is lower in December to April during the study period. Replicate numbers of eggs ($n = 13$, $n = 15$ and $n = 27$) for the experiment at the three rearing temperatures (20 °C, 29 °C, 32 °C) were justified on the basis of availability of eggs and presence of natural predators of eggs during winter, autumn and summer in nature.

Effects of temperature on the developmental period of P. polytes

Another similar set of butterfly rearing experiments was conducted to evaluate the effects of rearing temperatures on fitness traits and larval development. The larval period of an instar was recorded from the time of first larval appearance to the molt of the next instar (Peggie et al., 2022; Zalucki et al., 2002). The number of surviving larvae was counted across the three rearing temperatures. When larvae became sluggish, brownish, remained motionless, and ceased feeding were considered dead. The mortality of larval stages was recorded twice (morning and evening) daily.

Effects of temperature on the larval development and metabolism of P. polytes

First instar larvae were used as replicates ($n = 15$), and an equal number of replicates were used for rearing at 20 °C, 29 °C, and 32 °C. A total of 15 replicates were prepared separately for the three rearing temperatures to observe larval body weight, length, daily food consumption, and amount of excreted faeces at 24-hour intervals (Fig. 1c, d, e, f). A single larva was considered a replicate and reared individually in a box. Larval rearing boxes were prepared for individual larvae. Each larva and its rearing box were kept in a rearing cage. We prepared five rearing cages for each temperature treatment and conducted experiments sequentially. Immediately after collection, the leaves were supplied to the larvae. The weight of the supplied fresh leaves was measured before and after feeding to measure food consumption. The treated larvae were provided with 20 g of fresh and young leaves of the host plant regularly. The excreted faeces were dry and granular. The amount of collected faeces and body weight were recorded using a digital balance (KERN ADB 220-4). After supplying the young leaves, a small amount of water was sprayed on them to prevent desiccation and maintain turgidity (Arju et al., 2015a; Alam et al., 2017). Larval body length was measured using a millimeter scale.

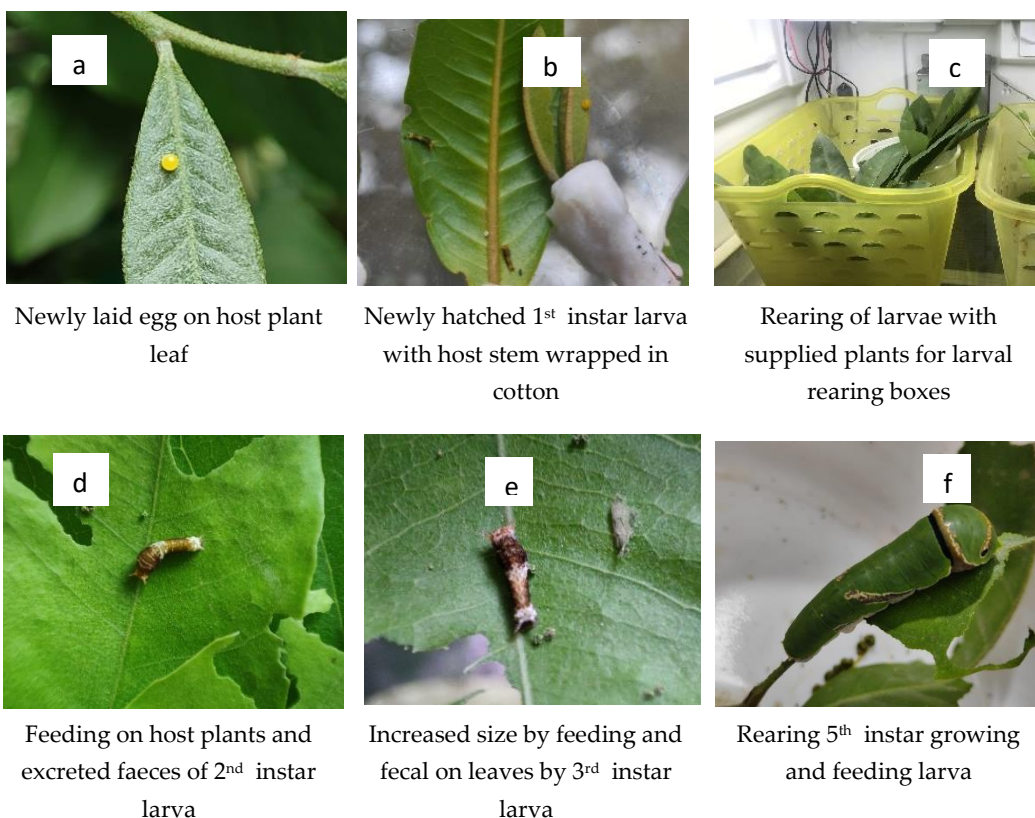


Fig. 1. Rearing of *P. polytes* with host plant *G. pentaphylla* at three temperatures (20 °C, 29 °C and 32 °C) under laboratory conditions.

Statistical Analysis

The mean developmental period of the egg, larval and pupal stages at each temperature was computed, and the standard error was determined. Mean values for mortality, duration of development (days), body weight, length, and amount of food consumption and faeces for larval replicates at different rearing temperatures were calculated individually. Differences in egg hatchability across the rearing temperatures were analyzed using a Chi-square test. ANOVA assumptions were checked before selecting the final test. Residual normality was assessed using the Shapiro-Wilk test, and variance homogeneity was assessed using Levene's test and the Brown-Forsythe test. Differences in mortality were analyzed using ANOVA to evaluate the effects of rearing temperature as a fixed factor. To find the effects of temperature on larval food consumption, the Kruskal-Wallis Test was used as residual normality did not meet the ANOVA test. To find the relationship between body weight and food consumption, Spearman's rank correlation was selected. All statistical analyses were performed in R (Version 4.3.2), Minitab (Version 21.4)

and Microsoft Excel. Descriptive and inferential analyses were conducted to evaluate differences among variables (temperature), with statistical significance set as P-value < 0.05.

Results and discussion

The results revealed that the phenology of *P. polytes* consists of four developmental stages (egg, larva, pre-pupa and pupa) across all temperatures.

Effects of temperature on the phenology of P. polytes

The total developmental period was the longest at 20 °C, lasting approximately 45.86 ± 1.45 days, and it gradually shortened to 28.38 ± 2.68 days at 32 °C and days at 29 °C. At 20°C, 1st, 2nd, 3rd, 4th and 5th instar larval stages and pupa lasted about 3.33 ± 0.67 days, 3.12 ± 0.25 days, 3.75 ± 0.95 days, 4.5 ± 1.1 days, 6.33 ± 1.5 days and 16.75 ± 3.06 days respectively. The incubation period decreased at 32 °C compared to 20°C. Pupal development was notably shorter at 32 °C and 29 °C compared to that at 20 °C (Fig. 2).

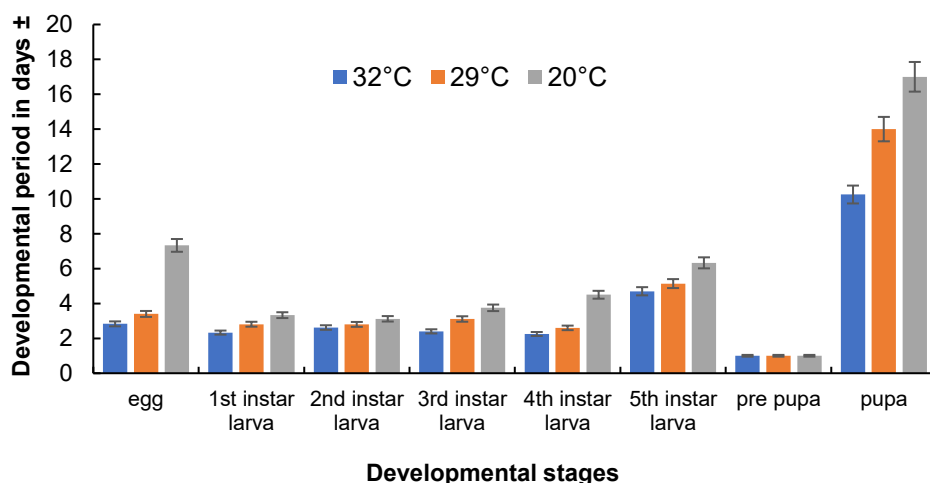


Fig. 2. Effects of temperatures 20 °C, 29 °C and 32 °C on the developmental period of *P. polytes* under laboratory conditions.

The incubation period of *P. polytes* was 3.54 ± 0.45 days, similar to the findings of a previous study stating deviation in incubation period of about 3-5 days (Islam et al., 2017; Garrad et al., 2015). A similar finding revealed that the pre-pupal stage was longer at 20 °C, compared to 25 °C and 30 °C (Koda & Nakamura, 2012). Higher temperatures increase the metabolic rate of the butterfly, causing them to utilize more energy. Increased metabolic rate, consequently, increases the developmental rate, thereby shortening their developmental period (Kingsolver et al., 2015). Incubation period and larval period increased notably as the temperature decreased. If the larval stage is prolonged, greater food resources are required (Garrad et al., 2015). Temperature influences survival,

development, metabolic rates and growth rates, which may pose substantial challenges for individual survival and reproduction (Kennelly *et al.*, 2017; Clarke, 2003). Another study suggested that in *Plutella xylostella*, the rearing temperatures 10°C to 30 °C influenced the growth and developmental rates (Xing *et al.*, 2025). In butterflies, a small temperature rise is likely to increase their metabolic rate. However, excessively high temperatures may exert adverse effects on larval development (Klockmann *et al.*, 2016; Klockmann *et al.*, 2017). In our study, the higher temperature regime led to a shorter developmental period than the lower temperature. When larvae are reared at higher temperatures, increased food consumption and higher growth rate are observed. Results indicate that temperature heavily influences the overall time required for development and are consistent with previous studies that a lower range of temperatures prolonged the developmental period. In *P. demoleus*, the higher temperature (32 °C) decreased the larval stage duration from 16.49 to 11.18 days, and in *P. polytes*, from 14.88 days to 13.15 days, indicating their temperature sensitivity (Kumar *et al.*, 2024; Islam *et al.*, 2019). Temperature actually influences the genetic expression of a particular gene, life history traits, and phenology of butterflies (Wilson & Fox, 2021; Yu *et al.*, 2023).

Effects of temperature on the survival rate of P. polytes

The larval survival rate was significantly lower at 20 °C, where the peak mortality rate was observed at both the initial and pupal stages compared to the other stages. At 20 °C, the mortality rate increases as this temperature is below the optimum required temperature for the normal growth, development, and metamorphosis of *P. polytes*. In temperate areas, this butterfly usually does not face very low temperatures since this species is adapted to a higher temperature regime (Atapour, 2024). However, at 29 °C and 32 °C, the survival rate was higher except for the 5th instar larvae and pupal stage (Fig. 3). Overall, the mortality rate varies significantly across different stages ($F = 46.801$; $df = 2$; $P = 0.0000083$) at different temperatures. This indicates better performance of *P. polytes* at high temperatures in a city.

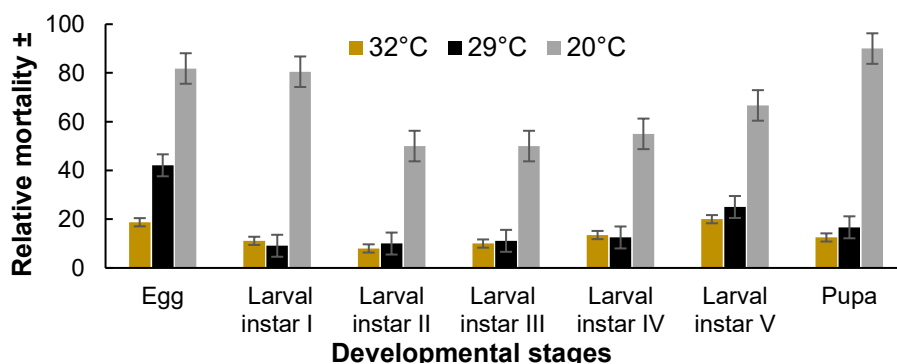


Fig. 3. Effects of temperatures 20 °C, 29 °C and 32 °C on the survivability of *P. polytes* in different developmental stages under laboratory conditions.

This result shows consistency with the finding that the mortality rate of *P. polytes* at immature stages was 8.0% (eggs), followed by 9.33% at the 4th larval stage and 7.69% at the pupal stage (Islam et al., 2017). Temperature has effects on the survival rate of butterflies. The highest mortality was recorded at the 5th instar larval stage (Suwarno et al., 2010). The egg mortality was about $84.61\% \pm 3.90$ at 20 °C, similar to *Heliconius erato demophon*, where the egg survival was higher (49%) at 30 °C and three times lower at 26 °C (Huang et al., 2024). Seasonal low temperature is one of the most critical factors in the life cycle of butterflies that regulates its survival rate (Atapour, 2024).

Effects of temperature on the egg hatchability of P. polytes

The lowest egg hatchability rate was $18.18\% \pm 0.094$ at 20 °C. Some of the pupae remained dormant, and no adults emerged from the pupal cases after 20 days at this low temperature. These pupae were considered dead. However, at 32 °C, hatchability and pupation rate were higher, measuring $81.21\% \pm 0.023$ and 25%, respectively (Fig. 4). Egg hatchability significantly varied across the three rearing temperatures ($\chi^2 = 10.575$; $df = 2$; $P = 0.0064$). In *Lampides boeticus* (Lycaenidae), 50% of the eggs hatched at 29 °C and the mean egg incubation period was 4.00 ± 0.17 days (Miah et al., 2015). There was a significant difference in the hatching rate, and the effects of rearing temperature extended into the subsequent larval and pupal stages. High temperature had strong adverse effects on egg-hatching success (Piyaphongkul et al., 2012; Kingsolver et al., 2015; Klockmann et al., 2017).

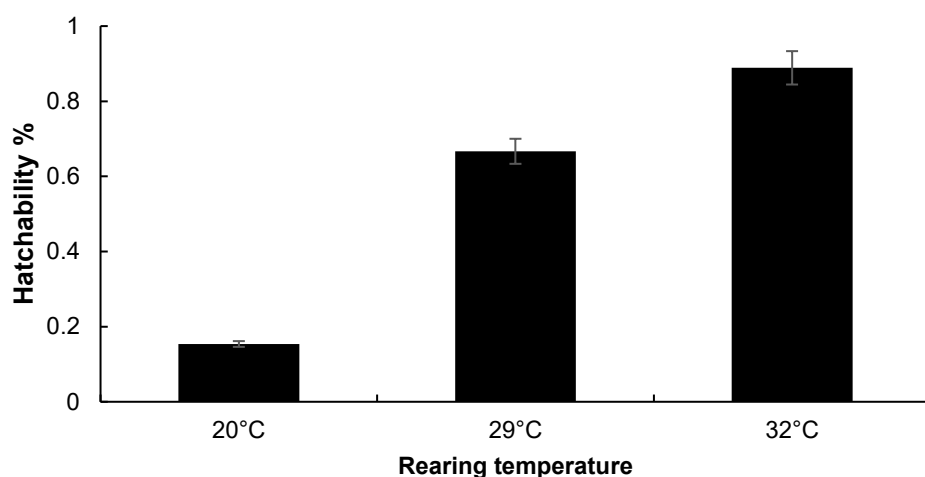


Fig. 4. Effects of temperatures 20 °C, 29 °C and 32 °C on the hatchability of eggs of *P. polytes* under laboratory conditions.

However, some of the previous studies reported that high temperature sometimes could exert adverse effects on egg survival (*Shijimiaeoides divinus barine*: Lycaenidae). Egg hatchability was higher (88.0%) at 20 °C and declined sharply when exposed to higher temperatures (about 30.0% at 30 °C) and no eggs hatched at 35 °C, indicating a lower

temperature preference. A similar finding was also reported in *Lycaeides argyrognomon*, where the duration of incubation decreased significantly as temperature increased up to 30 °C (Koda & Nakamura, 2012). The incubation period became shortened (2.82 days) in *P. demoleus* at higher temperatures like 23 °C (Kumar *et al.*, 2024). Temperature influences egg survival and egg laying behaviour of females. Adults laid smaller eggs at higher temperatures and larger eggs at lower temperatures (Fox *et al.*, 1997; Steigenga & Fischer, 2009). In another study, it was reported that egg survival of *Heliconius erato demophon* was lower at 26 °C than at the elevated temperature range of 36 °C (Huang *et al.*, 2024).

Effects of temperature on the larval body weight and length of P. polytes

The mean weight of the 1st instar larvae to 3rd instar did not vary across rearing temperatures. The larvae started to gain weight rapidly from the 3rd instar, and this rate of increase was substantially higher at 29 °C and 32 °C. The body weights for the 4th instars were noticeably lower (0.25 ± 0.12 g) at 20 °C than at 32 °C (1.46 ± 0.15 g). However, despite some variations in larval weight, it increased when reared at higher temperatures ($F = 1.790$; $df = 2$; $P = 0.035$). (Fig. 5).

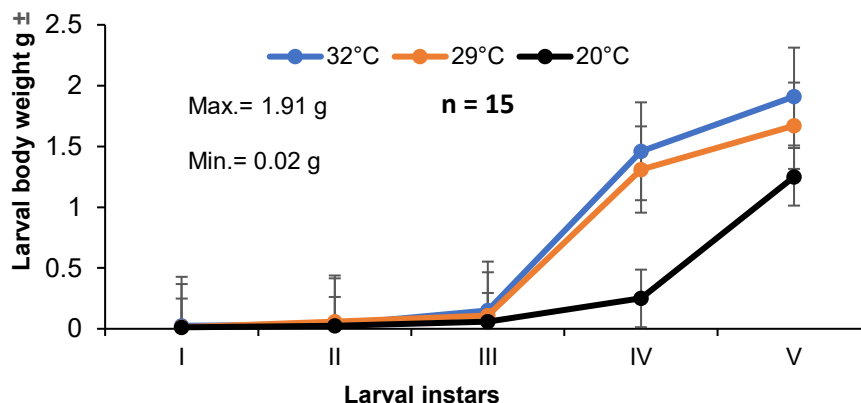


Fig. 5. Effects of temperatures 20 °C, 29 °C and 32 °C on the body weight (g) of *P. polytes*.

The length of the larvae across different instars varied during their developmental periods. Larval length varied across the high and low rearing temperatures, larvae being significantly longer at 32 °C than at 29 °C and 20 °C ($F = 3.301$; $df = 2$; $P = 0.0459$). The lengths of the 1st and 5th instars were 5.9 ± 0.88 mm and 32.7 ± 1.25 mm, respectively, at 32 °C (Fig. 5). On day 13, the length of the fifth instar larva was 42 ± 2.25 mm at 32 °C, 30 ± 1.5 mm at 29 °C, and 19.25 ± 0.65 mm at 20 °C. However, at the beginning of larval development, there was no notable difference in the length till day 7 (Fig. 6).

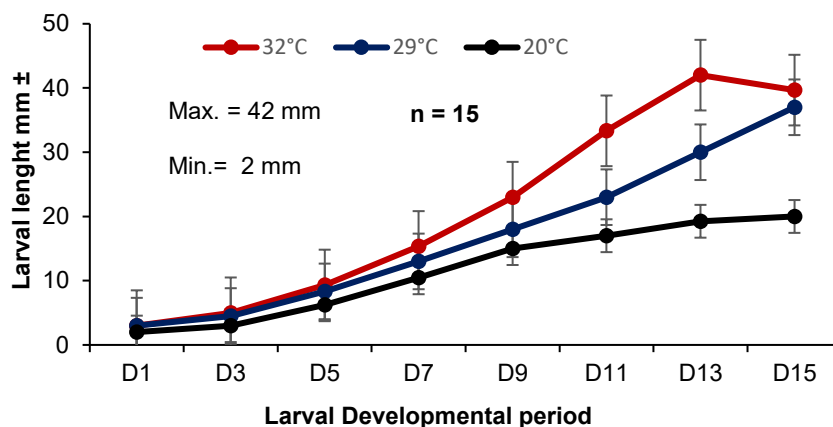


Fig. 6. Effects of temperatures 20 °C, 29 °C and 32 °C on the body length (mm) during the larval developmental period of *P. polytes*.

A similar finding was also reported in *P. demoleus*, where the length of the 1st instar larva was 5.23 ± 0.529 , and the length of the 5th instar larva was 41.23 ± 2.03 (Rao, 2015). The size of the larvae depends on the availability of food sources and types of host plants of the butterfly. There was a distinct change in color and size in the larvae during the course of development from the 4th to 5th instar (Jaafar et al., 2013; Koda & Nakamura, 2012).

Effects of temperature on the food consumption of P. polytes

The amount of leaf consumption increases along with larval development. Food consumption did not significantly vary across the three rearing temperatures ($H = 4.488$; $df = 2$; $P = 0.1060$). Larvae reared at 32 °C and 29 °C consumed the maximum amount of food from day 9 to day 13. The average food consumption was 4.64 ± 0.19 g at 32 °C, 2.780 ± 0.12 g at 29 °C and 1.70 ± 0.07 g at 20 °C. The maximum food consumption was recorded in the 5th instar (Fig. 7). This finding shows similarity with the larvae of *Eurema hecabe* (Pieridae), as its 5th instar larvae consumed the maximum amount of food (2.12 g), while the 1st instar larvae consumed the minimum amount of food (0.10 g). Another butterfly, *Catopsilia crocale* (Pieridae), also consumed a maximum amount of leaf at the final larval instar and the minimum was at the 1st instar when reared at 24 °C. The feeding nature and amount of food consumption gradually increased throughout the successive development of larval stages (Arju et al., 2015a; Arju et al., 2015b; Miah et al., 2015). After reaching their maximum body weight on day 15, the 5th instar larvae gradually decreased their leaf consumption until reaching the pre-pupal stage. The final stage of the larva needs to consume massive food reserves to survive the upcoming non-feeding stage. This consumed energy acts as a fuel for the improvement of adult morphology, fitness and flight performance, which is related

to their resource allocations, which facilitates their ability to adjust to environmental variations (Boggs & Freeman, 2005).

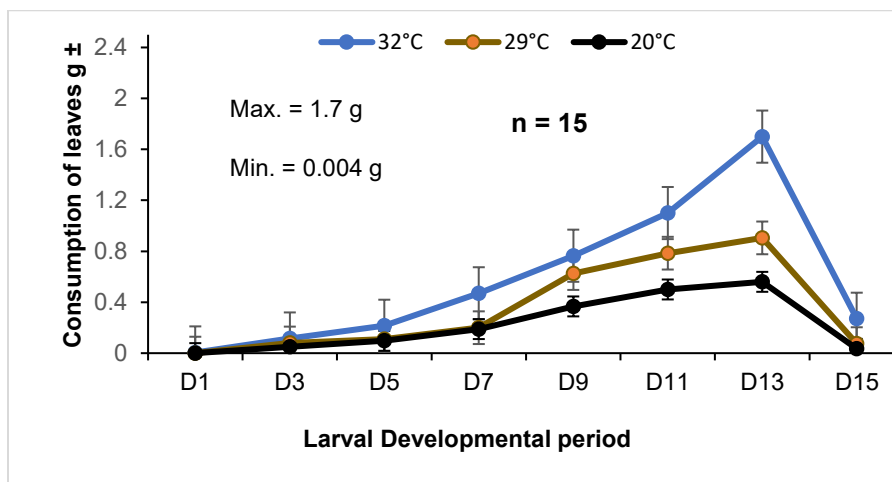


Fig. 7. Effects of temperatures 20 °C, 29 °C and 32 °C on the food consumption of *P. polytes* during the developmental stages.

The larvae of *P. demoleus* also ceased feeding at the end of 5th instar during development and the periods varied due to nutritional differences in host plants, temperature and host plant quality interacting and influencing development (Islam et al., 2019). Larval body weight increases with higher food consumption and there was a significant positive relationship between body weight and food consumption ($r = 0.936$, $P < 0.001$).

Effects of temperature on the excreted faeces production of P. polytes

The larvae excreted a large quantity of granular, greenish faeces during their 3rd to 5th larval instar developmental period. Faecal production did not vary across the three rearing temperatures during the early developmental period (7 days) but gradually increased after 9 days along with the increased amount of food consumption. The maximum mean faeces were 7.94 ± 0.132 g and 6.398 ± 0.113 g at 29 °C and 32 °C respectively. The minimum mean faeces was 4.33 ± 0.088 g, produced by larvae when reared at 20 °C (Fig 8). Temperature had no significant effect on faecal production during their developmental period ($F = 0.896$; $df = 2$; $P = 0.414$).

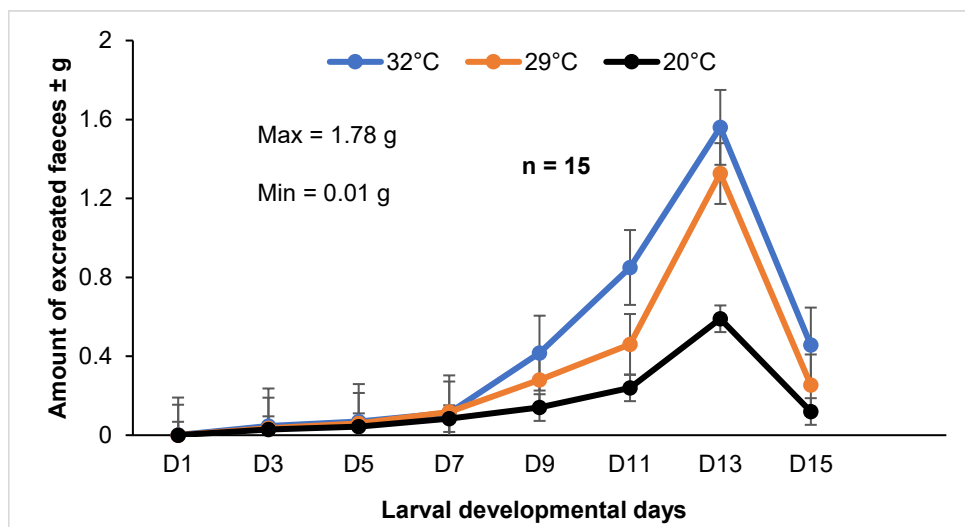


Fig. 8. The effects of temperatures 20 °C, 29 °C and 32 °C on faeces production during the developmental period of *P. polytes*.

Our present result is supported by the previous study with *Danaus chrysippus*, whose faecal amount was higher at a high temperature of 32 °C than at 27 °C and also required a higher temperature range for growth and development (Mathavan & Pandian, 1975). In another butterfly (*C. crocale*) the amount of faeces increased alongside developmental stages from the 1st instar (0.04 ± 0.001 g) to the final instar larva (0.347 ± 0.1 g) reported by (Arju et al., 2015a; Arju et al., 2015b). In *Pieris rapae*, higher temperatures were found to accelerate development and enhance metabolism, thereby speeding up growth and development (Jones et al., 1982; Angilletta et al., 2003). Temperatures in tropical regions drive the physiological and adaptive thermal responses of *P. polytes* (Arju et al., 2010). Insects are largely dependent on their environment for their thermoregulation, and they are particularly vulnerable to changing temperatures (Ashe-Jepson et al., 2023).

Conclusions

The present study demonstrates that temperatures ranging between 29 °C and 32 °C are favorable rather than lower temperatures (20 °C) for the survival, egg hatchability, development, and increase in fitness of *P. polytes*, highlighting its preference for tropical thermal environments. Temperatures of 29 °C and 32 °C improved fitness traits, whereas temperatures lower than the optimum (20 °C) led to higher mortality and slower development. These findings contribute valuable insights into the phenological responses of this butterfly and provide important baseline data for developing effective conservation strategies. Despite the findings of our study, there were some limitations. Due to a smaller number of replicates, a narrow temperature range, and the use of field-collected larvae from different seasons across sequential temperature treatments, seasonal variation may have

introduced potential confounding effects on thermal tolerance. Future studies might resolve these limitations by focusing on broader temperature ranges and using a higher number of replicates in order to get proper insights into the thermal biology and thermal responses to higher temperatures of this butterfly.

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Conflict of interest

The authors declare that they have no conflict of interest.

Authors' Contribution

Parven Nousheen: Conceptualization, validation, investigation, review, editing and funding acquisition; **Refath Tasnin:** Writing original draft, data collection, data visualization and funding acquisition; **Kashfia Bintey Rabbi:** Writing original draft, review and editing; **Takia Raisa:** Review and editing; **Muntahina Islam Payel:** Review and references compilation; **Amdadul Hoque Ratul:** Data visualization and analysis; **Mursida Begum:** Validation and review; **Md. Abul Bashar:** Conceptualization and objective selection.

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