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Response of *Papilio clytia* & *Euploea core* to Environmental Variables: A Preliminary Study

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ABSTRACT

Butterflies are susceptible to inordinately warm environments which influence their developmental stages. Moderate temperature increases egg production, survival of eggs, and larval length along with many morphological changes in adult butterflies. However, global temperature increase has negative consequences on overall larval growth. Increased temperature is one of the reasons for the vulnerability of larvae to different diseases and several infections. This study was conducted to investigate the effect of environmental variables like temperature, humidity & precipitation on the developmental stages of larvae *Papilio clytia* & *Euploea core* in varying environmental variables. Results have suggested that *Papilio clytia* & *Euploea core* developed faster in warm temperature. Gradual warming, humidity, and precipitation affect the number of developmental days during the pre-adult growth period. These extreme variations disrupt normal metabolic activities and species may be unable to endure harsh environments. More research on the effects of environmental variables is needed to provide insight into the butterflies' response to global climate change for better population management in Tripura.

Keywords Environmental variables, Temperature, Humidity, Precipitation, Larval growth.

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INTRODUCTION

Global warming has been more pronounced in recent years due to continued temperature rises across the globe. It poses a great threat to living organisms including insects (Peter Soroye *et al.* 2020, Skendžić *et al*. 2021). Insects have been considered model organisms for detecting subtle environmental changes that can affect their distribution pattern (Forister *et al.* 2019). The continual rise in temperature is predicted to bring about severe morphological and physiological changes in insects (Spooner *et al.* 2018, Eickermann *et al.* 2023) and several species may be close to extinction (Cahill *et al.* 2013, Román-Palacios and Wiens 2020). Therefore, the concept of climate mitigation demands more in-depth studies and strategies to deal with global environmental changes (Li *et al.*

2023). Vulnerable species need more attention and their establishment in original habitats is a subject of concern.

Most insects are ectotherms i.e. they are unable to regulate body temperature and are more sensitive to climate change. Butterfly populations show higher dominance to temperature than any other environmental variables (Comay *et al.* 2021). Elevated temperatures and extreme weather conditions can increase physiological stress in butterflies impacting their growth, development, and overall well-being (Hill *et al.* 2021, Bonifacino *et al.* 2022). These changes induce variation in pigments which later affects their survival fitness (Tseng *et al.* 2023, Markl *et al.* 2022). Moreover, excessive heat during the egg stage negatively impacts larval growth, body size, and body mass in adults (Klockmann *et al.* 2017). Climate change also advances the reproductive cycles of butterflies (Macgregor *et al.* 2019) leading to population growth. Human activities lead to habitat loss (Newbold *et al.* 2015), which in turn threatens the diversity of larval and nectar host plants, as the abundance of butterfly community depends upon the availability of larval host plants, causing potential harm to the species to find suitable habitats for oviposition, feeding, and overwintering (Fischer *et al.* 2014, Wepprich *et al*. 2019).

Numerous research has already proved that phenology is positively linked to climate change (Ovaskainen *et al.* 2013, Zografou *et al.* 2021, Franzén *et al.* 2022). Phenology serves as a key indicator of abiotic changes. Some shreds of evidence show climate change affects range shifts (Pélissié *et al.* 2022, Rubenstein *et al.* 2023), species interactions (Fontúrbel *et al.* 2021, Wang *et al.* 2022), and composition (Trisurat *et al.* 2023). Mostly specialist butterfly foragers are greatly impacted by minor environmental changes (Hantson and Baz 2013, Swengel 2023). In addition, the availability of suitable oviposition sites, nectar, and mimicry is essential for the successful establishment of butterflies. This paper highlights the environmental pressures contributing to the depletion of butterfly diversity throughout the region. Such information has aided in understanding butterfly responses to ecological change, fostering the development of conservation programs.

MATERIALS AND METHODS

Study area

The study was conducted in the butterfly park of Trishna Wildlife Sanctuary, South Tripura between November 2022- July 2023. Data on oviposition, breeding, and identification of larval host plants were collected through independent surveys of the site. Tripura has a moderate diversity of butterflies and their larval plants are distributed throughout the region.

Rearing of butterflies

Butterfly eggs were collected from the wild and raised from caterpillars to adults on the host plants (*Litsea glutinosa* & *Nerium oleander*). Three experimental groups of *Papilio clytia* & *Euploea core* butterfly larvae were subjected to varying temperatures. The first group was reared in the month of November-December 2022. A second group was raised in March 2023 and the third group in the month of June-July 2023. They were released shortly after the eclosion. Some of the larvae were lost during the study as they did not survive.

Primarily, the GIS data was used to calculate the effect of environmental factors in the mentioned months. Butterfly species were monitored daily to track chrysalis formation and survival rate.

RESULTS AND DISCUSSION

Development days of butterflies

Graphs were plotted for the studied months of both species. It was found that with increasing temperature, the growth of larvae was rapid and vice versa (Fig. 1). The metamorphosis of the *Papilio clytia* and *Euploea core* was faster at higher temperatures. On warmer days, *Papilio clytia* develops in 28 days, and on cooler days, development can take more than 39 days. *Euploea core* takes 21 and 29 days on warmer and cooler days respectively for larval growth. The development period of *Papilio clytia* was observed to be longer than the *Euploea core* during the study.

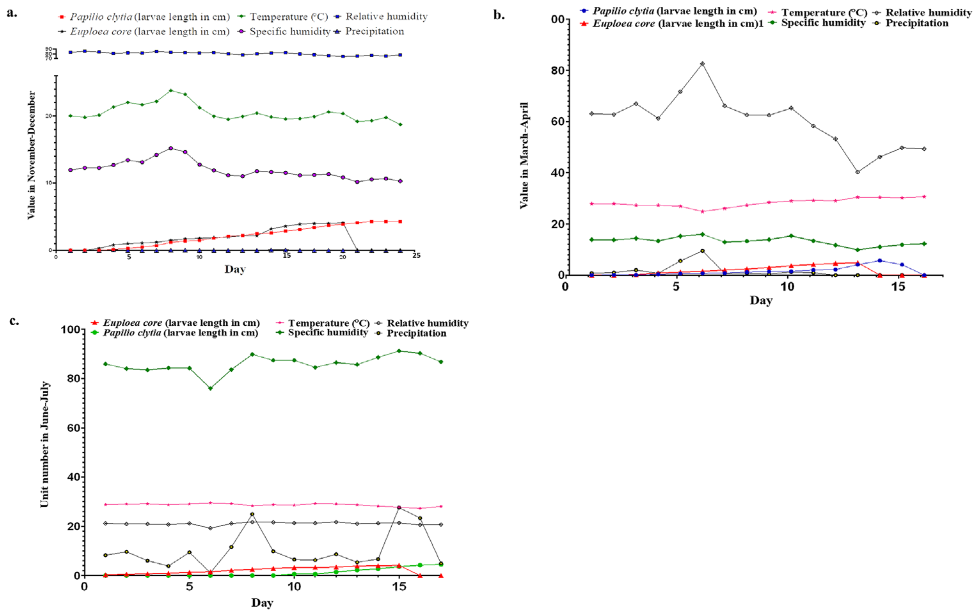

Fig. 1. Shows the development of larvae **(a)** November-December 2022 **(b)** March-April 2023 **(c)** June-July 2023 along with environment variables (Temperature- C, Specific humidity-g/kg, Relative humidity-%, Precipitation- mm/day).

Effect of environmental factors on larval length

Pearson correlation analysis was performed to determine the role of temperature in larval growth development. Previous studies have demonstrated that butterflies are most suitable for uncovering the effect of climate change. The experiment on the larval length showed that *Papilio clytia* & *Euploea core* were found to be positively correlated with relative humidity, specific humidity, precipitation & temperature for November-December 2022 as shown in Figs. 2a – 2b. There was a positive correlation with temperature, relative humidity & specific humidity, while precipitation was non-significant (March-April 2023) as represented in Figs. 3a–3b. Statistical analysis for June-July 2023 was found to be similar with November-December, 2022 (Figs. 4a –4b). The value $p<0.05$ is regarded as statistically significant.

The findings of the present study underline the role played by environmental factors in the larval development of butterflies and highlight the substan-

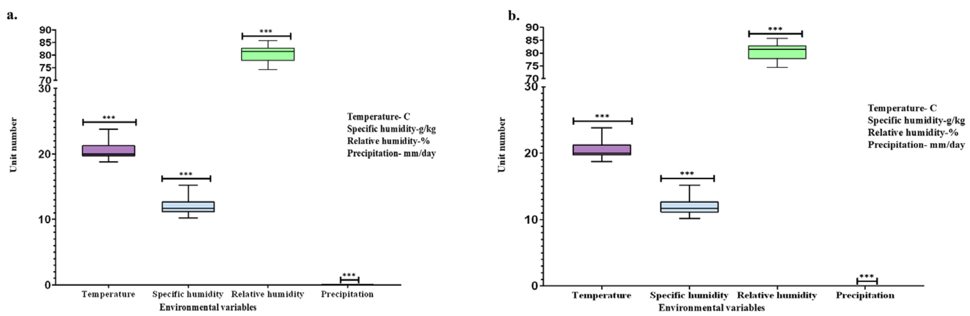

Fig. 2. Box Plot showing the relationship between larval length and environmental factors for November-December, 2022. **(a)** *Euploea core* **(b)** *Papilio clytia.*

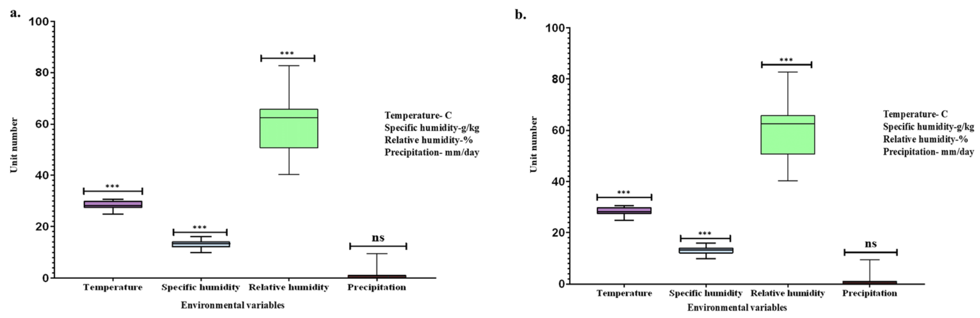

Fig. 3. Box Plot showing the relationship between larval length and environmental factors for March-April 2023. **a.** *Euploea core* **b.** *Papilio clytia.*

tial effect of relatively high temperatures on butterfly species. All three groups were able to survive during the study period which showed their adaptability to the environmental changes. Butterflies as ectotherms develop faster at high temperatures (Angilletta 2009, Barton *et al.* 2014). It was observed that those larvae experiencing a temperature of about 28°C take approximately 15 days to go through all five instars compared to those raised at lower temperatures. There seems to be a positive relationship with temperatures and larval length between 22˚C and 28°C. However, an increase in mortality rate was seen above 28°C.

Larvae exposed to warm conditions developed at a faster rate because of increased metabolism (Solensky and Larkin 2003). While, the metabolic functions slow down at lower temperatures (Knapp and Řeřicha 2020, Dubiner *et al.* 2023). The metabolic enzymes either become inactive or degraded at different temperatures (Roberts and Williams 2022). Although *Euploea core* was found to survive in sweltering temperatures, many larvae showed higher mortality during the study.

Temperature is crucial in determining larval growth (Saraf and Vijaykumar 2021). It was observed that higher temperature (March-April & June-July), enhances the larval length as compared to lower temperature (November-December). Temperature affects oviposition rates, mortality, fecundity, wing size, wing pattern, of the species (Semsar-Kazerouni *et al.* 2022, Cui *et al.* 2018). The experimental temperature treatment on monarch larvae showed a significant positive relation between temperature and larval development time (Solensky and Larkin 2003). Similar results were observed during our study. However, a significant rise in temperature was found to have a detrimental effect on survival & reproduction (Klockmann *et al.* 2017). This may be the reason for the vulnerability of the larva to diseases and several infections in the group reared during June-July.

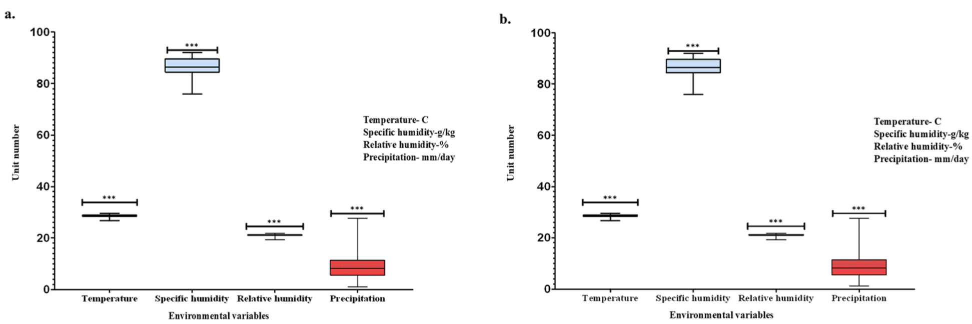

Fig. 4. Box Plot showing the relationship between larval length and environmental factors for June-July 2023. **(a)** *Euploea core* **(b)** *Papilio clytia.*

Further studies on the effect of humidity in butterflies recorded the lowest number of individuals in highly varied humid conditions (Gupta *et al.* 2019, Mahata *et al.* 2023). The high fluctuation in humidity levels during June- July led to an increased rate of mortality rate in our study. Several studies were carried out to correlate environmental factors like temperature, rainfall, and relative humidity and their effects on Lepidopteran diversity and abundance (Woestmann and Saastamoinen 2016, Maurer *et al.* 2018, Unnikrishnan and Sekarappa 2024). These environmental variables result from man-made activities which contribute to the rate of mortality, and abnormal growth in different stages of development (Halsch *et al*. 2021).

Rainfall is another important factor associated with the diversity of butterflies (Gibbs *et al.* 2011, Checa *et al.* 2019, Rowe *et al.* 2023). Results showed that the growth of larvae was influenced by precipitation except for March & April 2023 where it was found to be negatively associated. South Tripura receives the maximum rainfall during the wet season from June to September and is nearly absent in other months.

The environmental variables are responsible for the phenology of the butterflies which affect their life cycle and timing of host plant events. Phenology can alter food availability, adult lifespan, and population dynamics (Keller and Shea 2021, Edwards *et al.* 2024). The early emergence of adults can mismatch with the flowering period implying short flight seasons and negatively affecting their populations (Forrest 2015, Renner and Zohner 2018). Thus, the growth and development of butterflies require an optimal environmental condition to maintain their healthy diversity.

The study on the selected butterfly species is limited, thus comprehensive data on their growth pattern, and habitat could not be compiled. An extensive study can help to address the environmental stress that can impact their life cycle. Moreover, changes in larval metabolic growth, wing pattern, and wing size are yet to be investigated. The study highlighted that temperature, humidity, and precipitation affect larval development. It was also observed that anthropogenic activities lead to abnormal warming which causes a fluctuating behavior in the diversity of butterflies. This study ascertained that a temperature of 25˚C-28˚C was the optimum required by the species. Considering the fact that butterflies adapt to different environmental conditions, it is essential to examine the long-term effects on the species. Butterflies are key pollinating indicators, and their population reflects the overall health of the ecosystem. Therefore, the conservation of the species would help sustain ecological balance.

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