



## Life history and larval performance of the dark grass blue butterfly, *Zizeeria karsandra* (moore, 1865) (Lepidoptera: Lycaenidae) from Visakhapatnam, east coast of south India

Matala Bhupathi Rayalu

Lecturer, Department of Botany, Government Degree College, Kovvur, East Godavari District, Andhra Pradesh, India

### Abstract

This study presents a comprehensive analysis of the life history and larval performance of the Dark Grass Blue butterfly, *Zizeeria karsandra* (Moore 1865), focusing on food consumption, utilization, and life cycle duration on its host plant, *Tribulus terrestris* L. Conducted in 2023 in Visakhapatnam, South India (17°42'N, 82°18'E), this research details that the butterfly completes its life cycle in approximately 16 to 18 days ( $17.20 \pm 0.84$  days), with specific stages lasting as follows: egg stage - 3 days, larval stage - 8 to 10 days, and pupal stage - 5 days. The nutritional indices across the larval instars were measured, revealing the following results: Approximate Digestibility (AD) ranged from 66.64% to 96.10%, Efficiency of Conversion of Digested Food (ECD) ranged from 3.53% to 64.77%, and Efficiency of Conversion of Ingested Food (ECI) ranged from 3.39% to 42.77%. These measurements were taken at a controlled temperature of  $28 \pm 2^\circ\text{C}$  and a relative humidity of  $80 \pm 10\%$ . The high ECD and ECI values observed in this study may contribute to the ecological success of *Z. karsandra* in this environment.

**Keywords:** Life history, *zizeeria karsandra*, captive rearing, immature stages, food utilization indices

### Introduction

The genus *Zizeeria* Chapman 1910, belonging to the family Lycaenidae, is commonly known as the Blues family. This genus encompasses small butterflies renowned for their delicate appearance and striking blue or purple hues. Approximately 34 species are classified under this genus, with five species (*Zizeeria gaika* Trimen, *Zizeeria karsandra* Moore, *Zizeeria maha* Koll., *Zizeeria otis* Fabr., and *Zizeeria trochilus* Freyer) found in India. The Dark Grass Blue, *Zizeeria karsandra*, is widely distributed across various regions of the Old World, ranging from the Middle East through South Asia, Southeast Asia, and into Australia. In India, it is prevalent across diverse habitats, including grasslands, gardens, and urban areas [1, 2].

The larvae of *Z. karsandra* feed on plants from several families: Acanthaceae (*Hygrophila auriculata*, *Strobilanthes*), Amaranthaceae (*Amaranthus spinosus*, *A. viridis*), Chenopodiaceae (*Chenopodium album*), Oxalidaceae (*Oxalis*), Papilionaceae (*Medicago*, *Zornia diphylla*), Polygonaceae (*Polygonum plebeium*), Verbenaceae (*Lantana camara*), and Zygophyllaceae (*Tribulus terrestris*). This study aims to detail the adult nectar resources, the various immature stages, larval performance on its host plant *Tribulus terrestris*, and the entire life cycle from egg to adult emergence of the butterfly *Zizeeria karsandra* (Moore 1865).

### Materials and Methods

The study was conducted in Visakhapatnam, located on the east coast of South India (17° 42' N latitude and 82° 18' E longitude), during the year 2023. The methodology for captive rearing involved collecting eggs from wild-mated female butterflies, rearing the larvae to adulthood in captivity, and then releasing the adult butterflies or pupae back into the wild, as outlined by Crone *et al.* [3].

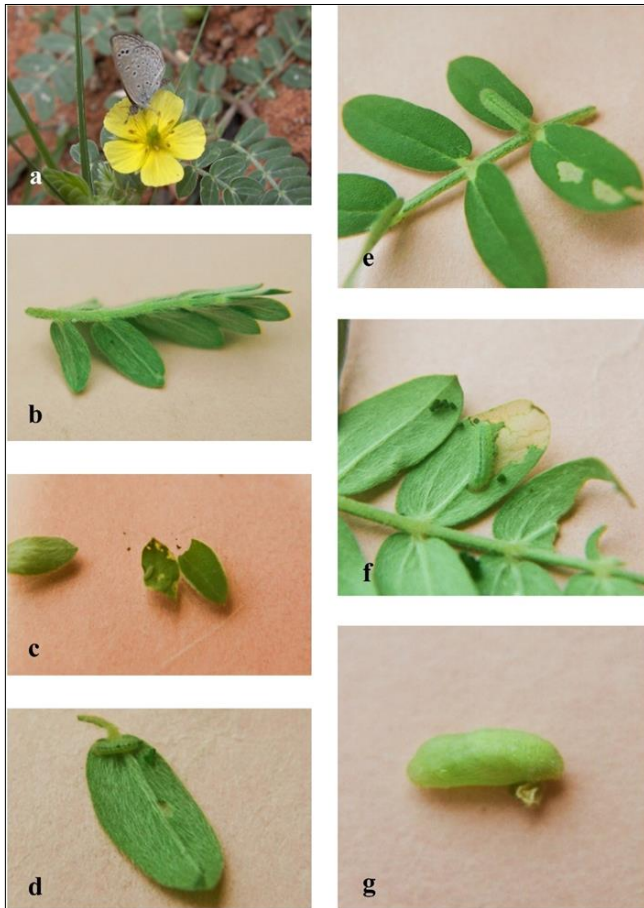
The reproductive activity of the Dark grass blue butterfly, *Zizeeria karsandra*, was monitored regularly between 0800 and 1500 hours at two locations: the Andhra University

campus and the Zoo Park area, situated 5 kilometers from the campus. Upon locating adult butterflies, detailed observations were conducted to record the periods of copulation and oviposition. Leaves containing eggs were collected in Petri dishes (15 cm × 2.5 cm depth) and transported to the laboratory. These leaves were then placed in smaller Petri dishes (10 cm × 1.5 cm depth) lined with moistened blotter paper to prevent desiccation. The Petri dishes were kept in a clean, spacious cage fitted with a wire gauge. As no ants were detected, no additional protective measures against egg predation were deemed necessary. The eggs were examined at 6-hour intervals to record hatching times.

Freshly emerged larvae were transferred to clean Petri dishes lined with moistened blotter paper using a camel hairbrush. They were fed daily with a weighed amount of tender leaf pieces from the host plant. Daily collections and weight measurements of faeces and leftover food were recorded. The growing larvae were observed regularly for changes in instar, and their length, breadth, and weight were measured. As the larvae grew, they were provided with larger Petri dishes (15 cm × 2.5 cm depth) to accommodate their increased space requirements. Larval performance, in terms of food utilization indices, was calculated as described by Waldbauer [4].

All parameters were studied in five replicates using fresh weight measurements. The development from larva to pupa, including characteristics such as color, shape, size, weight, and the timing of adult emergence, were documented. Measurements were taken using millimeter graph paper. The laboratory conditions were maintained at a temperature of  $28 \pm 2^\circ\text{C}$  and a relative humidity of  $80 \pm 10\%$ , with normal indirect sunlight varying between 12 hours during November to January and 14 hours during June to July.

To describe the adult butterfly characteristics, both laboratory-emerged specimens and those captured in the wild were utilized.



**Fig 1:** Life stages of *Zizeeria karsandra* (a) Adult (b) Egg (c) Instar I (d) Instar II (e) instar III (f) instar IV (g) Pupa

**Results**

**Adult Stage (Fig. 1a)**

**Field characters:** Upperside dark blue with brown margins. A series of black round spots is found on the hindwing. Underside grey. Forewing with a spot in the middle of the cell and discal series of eight black spots. Hindwing with four spots at sub-basal line. Head, thorax and abdomen are brownish, while thorax and abdomen are white on ventral side. Wing span 18-24 mm.

**Habit:** It was found in flight mainly near to its larval host plant, *Tribulus terrestris*. The behaviour of female can't be different at the time of egg laying. Intermittent foraging was observed while laying eggs.

**Food resources:** It kept its wings closed while foraging at flowers for nectar. In the study area, the nectar host plants included *Vernonia cinerea* (L.) Less., *Boerhavia diffusa* L., *Tephrosia purpurea* (L.) Pers., *Tribulus terrestris* L., and *Rostellularia procumbens* (L.) Nees.

**Oviposition host plants:** The plant used for ovipositing in the study area was *Tribulus terrestris* L. (Figure 2i).

**Egg Stage (Fig. 1b):** Oviposition takes place during 1000 – 1300 h. The gravid female laid eggs singly on the undersurface of the leaflets and occasionally on the midrib. There was no discrimination among young and mature leaves while laying eggs. About 6 – 8 eggs were laid at a time but on different leaflets or leaves. The eggs were sky blue in color, and flattened round disc like shape, and measured 0.3 mm in height. They hatched in three days of incubation. It passed through four distinct instars over a period of 8 – 10 ( $9.20 \pm 0.84$ ) days.

**Laval Stage (Fig. 1c-f)**

**Instar I:** This stage lasted 2 – 3 days. On the first day of hatching, the instar measured 1.10 – 1.70 ( $1.46 \pm 0.23$ ) mm in length. It grew to 1.60 – 1.90 ( $1.76 \pm 0.15$ ) mm in length and 0.70 – 0.90 ( $0.82 \pm 0.08$ ) mm in width. Head black in color and measured 0.40 – 0.50 ( $0.46 \pm 0.05$ ) mm in diameter. Body cream colored. Larva mainly chooses to reside on the underside of leaflet. It feeds mainly on the epidermal layer of the leaves. By the next day larval body turned into light green.

**Instar II:** This stage lasted for 3 – 4 days. The larva attained a length of 3.00 – 4.20 ( $3.44 \pm 0.45$ ) mm and a width of 1.20 – 1.60 ( $1.34 \pm 1.15$ ) mm. Head greenish and measured 1.00 ( $1.00 \pm 0.00$ ) mm in diameter. Body was greener than previous instar. There was a thick green colored streak along the mid-dorsal surface of the body. Body was fully covered with minute transparent hairs. Larval structure – flattened on ventral side and convex on dorsal side. Segmentation was clear.

**Instar III:** This stage lasted for 2 – 3 days. The larva attained a length of 6.80 – 7.30 ( $7.10 \pm 0.20$ ) mm and a width of 2.10 – 2.90 ( $2.48 \pm 0.32$ ) mm. Head measured 1.10 – 1.50 ( $1.36 \pm 0.15$ ) mm in diameter. Characters are same as previous instar.

**Instar IV:** This stage lasted for '1' day. The larva attained a length of 8.00 – 9.00 ( $8.30 \pm 0.41$ ) mm and a width of 2.80 – 3.00 ( $2.96 \pm 0.09$ ) mm. Head measured 1.90 – 2.20 ( $2.02 \pm 0.11$ ) mm in diameter. Characters are same as instar II. Body contracted before pupation.

**Pupal Stage (Fig. 1g):** This stage lasted '5' days. It was 7.00 – 8.00 ( $7.30 \pm 0.45$ ) mm in length and 2.30 – 2.80 ( $2.54 \pm 0.21$ ) mm in width at its broadest point. It was green in color without any ornamentation or markings. Its weight was 19.20 – 36.80 ( $26.46 \pm 6.57$ ) mg. The particulars of all these biological observations are given in the Table 1.

**Duration of Life Cycle:** It ranged between 16 – 18 ( $17.20 \pm 0.84$ ) days. (Egg: 3; Larva: 8-10; Pupa: 5 days).

**Table 1:** Biological observations of early life stages of *Zizeeria karsandra* on *Tribulus terrestris*.

Stage	Length(mm)			Width (mm)			Duration (days)	
	Min.	Max.	AV.±S.D.	Min.	Max	AV. ±S.D.	Range	AV.±S.D.
<b>Egg</b>	0.30	0.30	0.30 ± 0.00	0.50	0.50	0.50 ± 0.00	3 – 3	3.00 ± 0.00
<b>Larva</b>								
Instar I	1.60	1.90	1.76 ± 0.15	0.70	0.90	0.82 ± 0.08	2 – 3	2.40 ± 0.55
Instar II	3.00	4.20	3.44 ± 0.45	1.20	1.60	1.34 ± 1.15	3 – 4	3.20 ± 0.45

Instar III	6.80	7.30	7.10 ± 0.20	2.10	2.90	2.48 ± 0.32	2 – 3	2.60 ± 0.55
Instar IV	8.00	9.00	8.30 ± 0.41	2.80	3.00	2.96 ± 0.09	1 – 1	1.00 ± 0.00
<b>Total larval period days</b>								
Pupa	7.00	8.00	7.30 ± 0.45	2.30	2.80	2.54 ± 0.21	5 – 5	5.00 ± 0.00

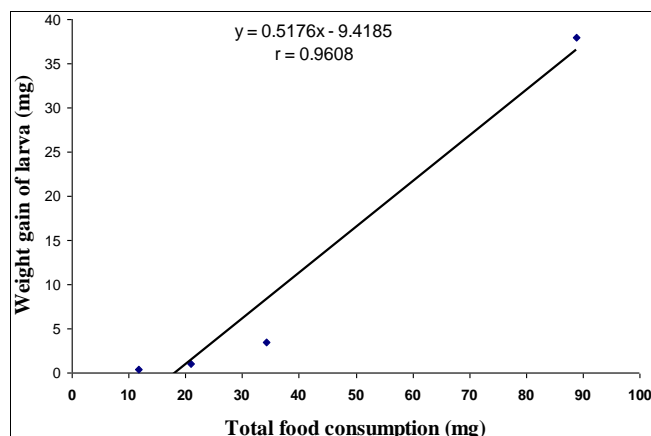
**Food Consumption, Growth and Utilization:** The data on the amount of food consumed by each of the four instars and the corresponding data on weight gained by different instars are given in Table 2. Of the total amount of food consumed, the percentage shares of the successive instars were 7.58, 13.44, 21.94, 57.04% and the proportions of weight gained by the successive instars were 0.93, 2.51, 8.06, 88.49%. Thus, there was over 78% of the total food consumption in the third and fourth instars together and 96% of total weight gained in the third and fourth instars together. Plotting the weight gained against the food consumed (Figure 2) a direct relationship between food consumption and growth across the four instars could be seen. The values of consumption

index (CI) decreased from first to final instar. The values of growth rate (GR) increased from first to final instar. Values of CI ranged between 2.32 – 8.48 mg/day/mg and those of GR between 0.29 – 0.99 mg/day/mg.

Table 2 also included the data on AD, ECD, and ECI. The estimated values of AD ranged between 66.64 – 96.10%. The values decreased as the instars progressed. The values of ECD and ECI increased progressively from the first instar to the last instar. The values of ECD varied from 3.53 – 64.77% and those of ECI from 3.39 – 42.77%. Thus, there was an inverse relationship between the values of AD and those of ECD and ECI.

**Table 2:** Food consumption, growth and food utilization efficiencies of *Zizeeria karsandra* on *Tribulus terrestris*.

Instar	Wt. of food ingested (mg)	Wt. of faeces (mg)	Wt. gained by larva (mg)	GR	CI	AD (%)	ECD (%)	ECI (%)
				(mg/day/mg)				
I	11.80 ± 01.24	0.46 ± 00.04	0.40 ± 00.05	0.29	8.48	96.10	03.53	03.39
II	20.94 ± 05.28	3.20 ± 01.33	1.08 ± 00.72	0.30	5.84	84.72	06.09	05.16
III	34.17 ± 05.07	7.44 ± 01.52	3.46 ± 03.16	0.32	3.81	78.23	12.94	10.12
IV	88.84 ± 07.21	30.17 ± 02.05	38.00 ± 02.35	0.99	2.32	66.64	64.77	42.77



**Fig 2:** Relationship between food consumption and growth in *Zizeeria karsandra* on *Tribulus terrestris*.

**Discussion**

The overall development time from egg laying to adult emergence for *Zizeeria karsandra* was 17.20 ± 0.84 days at approximately 28 ± 2°C. This observation aligns with the expected short life cycles characteristic of tropical butterflies [5]. Since temperature significantly affects both the duration of each instar and the total development period [6, 7, 8, 9], variations in life cycle duration could occur depending on the prevailing temperatures. However, due to the absence of extreme temperature variations in Visakhapatnam, the life cycle duration remained relatively consistent across different seasons.

Throughout their growth period, the larvae consumed an average of 155.75 mg of leaf material, with the highest consumption occurring during the last two instars. This increased consumption in the final instars is a common trend observed in lepidopterous larvae [4, 6, 7, 10, 11, 12] and compensates for the energy expenditure during the non-feeding pupal stage [13]. The Consumption Index (CI) values

were found to be within the expected range (2.32 – 8.43, average 5.11 mg/day/mg) for foliage chewers [14]. The food consumption rate is influenced by the efficiency of converting ingested food to biomass (ECI), with the rate increasing as conversion efficiency decreases and vice versa [14]. The high CI value (8.48 mg/day/mg) observed in the first instar likely results from its low conversion efficiency, which is reflected in the lower ECI values for this instar compared to subsequent ones.

The Approximate Digestibility (AD) values obtained in this study are consistent with the range of AD values (19 – 81%) reported for lepidopterous larvae [15]. The average AD percentage of over 81.42% supports Slansky and Scriber’s [14] assertion that foliage chewers often achieve high AD values. High AD values are also expected when the food source is rich in nitrogen and water [15]. Similar findings have been reported in studies on *Pieris brassicae* (L.) [16], *Ariadne merione merione* (Cramer) [17], *Byblia ilithyia* Drury [18], *Helicoverpa armigera* (Hübner) [19], *Phalanta phalantha* Drury [20], *Spodoptera frugiperda* J.E. Smith [21], and *Zizula hylax hylax* Fabricius [22].

The Efficiency of Conversion of Digested Food (ECD) values increased from early to later instars [14], with the lowest ECD observed in the first instar and the highest in the fourth instar. These ECD values, although lower compared to AD values, are not unusual [4] and indicate a lower efficiency in converting digested food to body tissues. This low utilization of food may be attributed to deficiencies in essential nutrients [23] or factors increasing metabolic energy expenditure [24]. The Efficiency of Conversion of Ingested Food (ECI) values closely followed the pattern of ECD. The ECI values (3.39% – 42.77%) fall within the expected range for foliage chewers (1% – 78%) [14, 19, 21]. The ECI values also mirrored the AD pattern as suggested by Waldbauer [4]. The relatively high ECD and ECI values, especially in the last two instars (12.94%, 64.77%; 10.12%, 42.77%), indicate efficient tissue and ecological growth,

which contribute to the successful thriving of *Zizeeria karsandra* in the studied environment.

### Conclusions

This study offers valuable insights into the oviposition larval host, *Tribulus terrestris* L., and evaluates the larval performance in terms of food consumption, growth, and utilization, as well as the complete life cycle duration from egg to adult emergence for the Dark grass blue butterfly, *Zizeeria karsandra*. The plants consumed by larvae significantly affect the performance of adult butterflies [25]. Consequently, the findings from this research can be effectively applied to the conservation management of this butterfly species in parks, zoos, butterfly houses, and natural fields. Butterfly houses, which are popular zoo exhibits, possess substantial educational and conservation value [26, 27]. Additionally, the study suggests that rearing larvae in captivity at approximately  $28 \pm 2^\circ\text{C}$  can maintain a sufficient stock of adults for repopulating areas with low butterfly populations [28] of the Dark grass blue butterfly.

### Acknowledgements

The authors are very thankful to the authorities of Andhra University, Visakhapatnam, A.P., India for providing infrastructure and laboratory facilities to conduct this research work.

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