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Eco-biology of the dark grass blue butterfly, *Zizeeria karsandra* (Moore) (Lepidoptera: Rhopalocera: Lycaenidae) from the Eastern Ghats of Southern Andhra Pradesh

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Abstract
The life history of the dark grass blue butterfly, *Zizeeria karsandra* (Moore) and its larval performance in terms of food consumption and utilization, and the length of life cycle on its host plant *Amaranthus spinosus* (Amaranthaceae) are described for the first time in Southern Andhra Pradesh. The dark grass blue, *Zizeeria karsandra* was a species of Lycaenidae butterfly usually seen in open scrub and grassland habitats. The study was conducted during January to December 2014 at Sri Lankamalleshwara wild life sanctuary (Kadapa) (79º 07' – 78º 80' E) of Southern Andhra Pradesh. *Zizeeria karsandra* completes its life cycle in 17 – 23 (19.20 ± 0.84) days. (Egg: 3-4; Larva: 9-13; Pupa: 5-6 days). The values of nutritional indices across the instars were AD (approximate digestibility) 98.00 - 63.43%; ECD (efficiency of conversion of digested food) 3.52 – 59.45%; ECI (efficiency of conversion of ingested food) 05.00 – 40.80%, measured at the temperature of 29 ± 2 ºC and RH of 80 ± 10% in the laboratory. These relatively high values of ECD and ECI explain at least partially the ecological success of *Zizeeria karsandra* in the present study environment.

Keywords: Life history, *Zizeeria karsandra*, Sri Lankamalleshwara wild life sanctuary, captive rearing, immature stages, food utilization indices.

Introduction
Butterflies offer inexhaustible stimulating material for those who are curious in carrying out applied and pure research [1]. They are valuable pollinators in the local environment and help to pollinate more than 50 economically important crops [2] and are one of the important food chain components of birds, reptiles, spiders and predatory insects. They are also good indicators of a healthy environment and healthy ecosystems because they are sensitive to changes in microclimate, temperature, solar radiation and the availability of host plants for oviposition and larval development. They serve as an excellent model to study evolutionary processes, insect migration, ecological genetics, insect reproductive behaviour, herbivory and insect/plant co-evolution, and as teaching tools in nature study. Accordingly, a large number of scientists from temperate and tropical regions of the world have carried out research on butterflies - their systematics and faunistics, autecology, community ecology (including niche dimensions), thermoregulation, pollination potentials, life cycles, voltinism, nutrition ecology, migration, conservation, etc. The Royal Entomological Society's (London) publication - The Biology of Butterflies [3] referred 2356 articles related to the researches in various fields of butterfly biology, ecology and conservation management. Other reviews on oviposition behaviour [4], butterfly conservation [5-8], nutritional ecology and bioenergetics [9-11] covered exhaustive literature. Jiggins (2003) *et al.* noted that the life histories of nearly 70% of the Indian species require description. [12] Studies on life history undertaken with greater vigour, originality and care are among the immediate research needs of any effective butterfly conservation program [5]. As such, reproductive effort involves a combination of larvae-derived and adult-derived nutrients or energy. The proportion of larvae-derived nutrients allocated to adults reproductive resources are determined during the metamorphosis. While discussing the basic relationships between resource allocation at metamorphosis and mean adult nutrient intake and reproductive output in holometabolus insects, [13, 14] underlined the importance of life history studies on such insects. It was well known that butterflies feed as adults and as larvae. However, as many experts observed, little was known of early stages of Indian butterflies [15-18]. Today the life history of 70% Indian butterflies was still unknown.

In recent years such studies are being promoted in India [19-25]. The mean temperature begins to rise from latter part of February and it ranged between (38 – 45 ºC) during May/June. Monsoon rains cool the tropical heat from June/July onwards, with the mean temperature remaining relatively high through October and thereafter decreasing to a minimum (26–30 ºC) in January/February. As it was expected of a semi environment, the maximum-minimum range in temperature is narrow, and the difference rarely exceeds 22 ºC. Mean monthly temperature, relative humidity, rainfall (Table. 4; Fig. 6) and sunlight data for the study years borrowed from the ISRO (Super computer facility) at the Yogi Vemana University campus are given in Table 4.

Materials and Methods

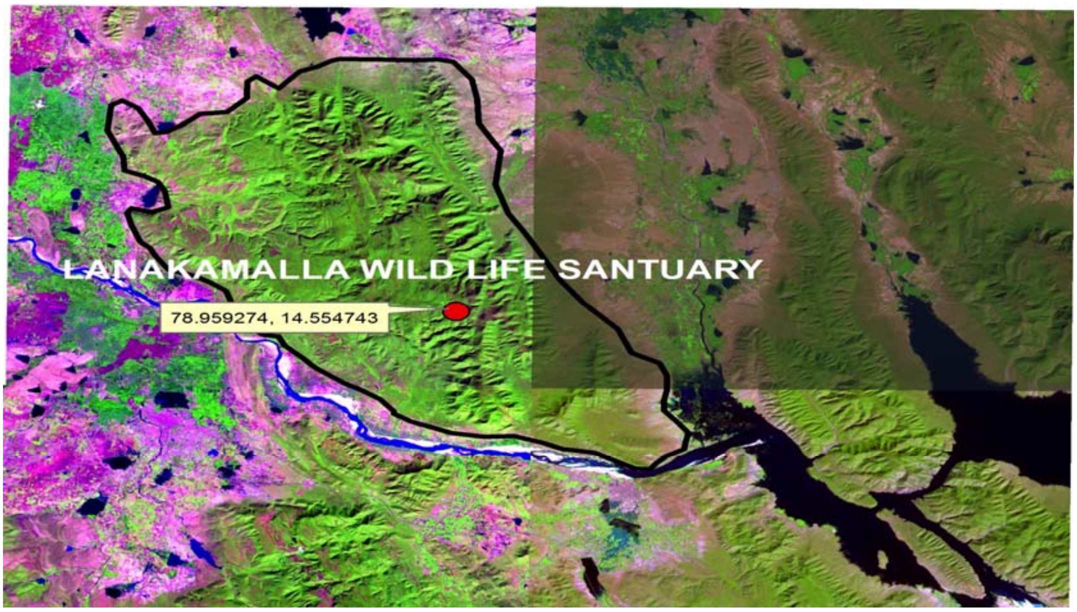


Fig 1: The study area

The Sri Lankamalleshwara wild life sanctuary with first rich vegetation at Kadapa (79º 07’-78º 80’ E) (Fig.1) was taken as the study site. The host plant used by the gravid females for oviposition and as subsequent larval food was *Amaranthus spinosus* (Amaranthaceae). The females were followed from 0800 h to 1700 h on several occasions for their oviposition, the eggs laid were collected in Petri dishes along with the leaf and brought to the laboratory for the study of life cycle and the life stages. Eggs with the leaf were placed in a Petri dish (10 cm diameter and 1.5 cm depth) inside lined with filter paper. The temperature was 29 ± 2 ºC, relative humidity 80 ± 10%, and the natural daylight of 12-13 hr duration. The eggs thus kept were examined daily at 5 hr intervals to record the hatching time. The larva was reared on fresh host leaves daily. The number and duration of different instars, their weight, length and morphological features and particulars of food consumption were recorded. The fresh weight measurements thus obtained were used to estimate the growth and nutritional indices according to Waldbauer (1968). To find out the phenology of early life stages, intensive searches were made thrice every month for the occurrence of eggs, larvae and pupae on the oviposited host *Amaranthus spinosus* and also for adult frequency. Statistical analysis was done between counts of early life stages and the prevailing weather, using Origin Software (2015).

$$\text{CI (Consumption Index)} = \frac{\text{Weight of food consumed}}{\text{Weight of instar} \times \text{Number of feeding days}}$$

$$\text{GR (Growth rate)} = \frac{\text{Weight gain of instar}}{\text{Mean weight of instar} \times \text{Number of feeding days}}$$

$$\text{AD (Approximate digestibility)} = \frac{\text{Weight of food consumed} - \text{weight of faeces} \times 100}{\text{Weight of food consumed}}$$

$$\text{ECD (Efficiency of conversion of digested food)} = \frac{\text{Weight gain of instar} \times 100}{\text{Weight of food consumed} - \text{weight of faeces}}$$

$$\text{ECI (Efficiency of conversion of ingested food)} = \frac{\text{Weight gain of instar} \times 100}{\text{Weight of food consumed}}$$

Flight period in Southern Andhra Pradesh (Fig. 2):
In the Eastern Ghats of Southern Andhra Pradesh flight period of *Zizeeria karasandra* was recorded as late September-early October in the Far North, early March in the Penna river land, and late April-early May in the Far Northeast. In Tirumala hill region and Thalakona evergreen forest it has been seen flying from January to May. There was also a record from the spring's area to the north of Seshachalam Bio-reserve forest at the end of December. In Southern Andhra Pradesh it was likely the butterfly would fly throughout the warmer months wherever its host plants remain in a green, growing condition and this situation would be dependent on seasonal rains or the presence of water. The butterfly was seen all year round in the tropical north of Sri Lankamalleshwara wild life sanctuary except from May- July. A brood can be completed in less than three weeks during early spring in the hot far south area of Southern Andhra Pradesh.

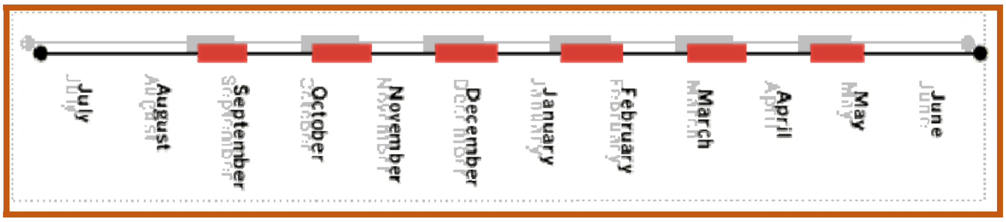


Fig 2: Flight period of *Zizeeria karsandra* recorded in study areas

Results and Discussion

Field characters

Adult

Upper side dark blue with brown margins, a series of black round spots were found on the hind wing. Under side with grey colour, forewing with a spot in the middle of the cell and discal series of eight black spots. Hind wing with four spots at sub-basal line. Head, thorax and abdomen are brownish, while thorax and abdomen are white on ventral side. Wing spans 18-24 mm.

Habit

It was found flying mainly near to its larval host plant, *Amaranthus spinosus* (Amaranthaceae). 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

Amaranthus spinosus Linn., (Amaranthaceae), commonly known as Spiny amaranth or Pig weed, was an annual or perennial herb, native to tropical America and found throughout India. The plant used for ovipositing in the study area was *Amaranthus spinosus* L., (Amaranthaceae).

Egg Stage

Oviposition takes place during 1000 – 1300 h. The gravid female laid eggs singly on the under surface 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 colour, and flattened round disc like shape, and measured 0.3 mm in height. They hatched in 3-4 days of incubation. It passed through four distinct instars over a period of 9 – 12 (9.75 ± 0.64) days.

Larval Stage: (Table. 2)

Instar I: This stage lasted for 2 – 3 days. On the first day of hatching, the instar measured 1.70 -1.90 (1.86 ± 0.15) mm in length. It grow to 1.60 – 1.90 (1.76 ± 0.15) mm in length and 0.70 – 0.90 (0.82 ± 0.08) mm in width. Head black in colour and measured 0.40 – 0.50 (0.46 ± 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 2 – 3 days. The larva attained a length of 3.10 - 4.20 (3.44 ± 0.45) mm and a width of 1.20 – 1.60 (1.24 ± 1.15) mm. Head greenish and measured 1.00 (1.00 ± 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 7.30 - 7.80 (larval length) (7.10 ± 0.20 (Mean values)) mm and a width of 2.10 – 2.90 (2.48 ± 0.32) mm. Head measured 1.10 – 1.50 (1.36 ± 0.15) mm in diameter. Characters were same as previous instar.

Instar IV: This stage lasted for 3-4 days. The larva attained a length of 8.00 – 9.00 (8.20 ± 0.41) mm and a width of 2.95 – 3.20 (2.96 ± 0.09) mm. Head measured 1.90 – 2.20 (2.02 ± 0.11) mm in diameter. Characters were same as of instar III. Body contracted before pupation.

Pupal Stage (Fig. 3**)**

This stage lasted for 5-6 days. It was 7.00 – 8.00 (7.30 ± 0.45) mm in length and 2.30 – 2.80 (2.54 ± 0.21) mm in width at its broadest point. It was green in colour without any ornamentation or markings. Its weight was 19.20 – 36.80 (26.46 ± 6.57) mg. The particulars of all these biological observations are given in the Table 2.

Duration Of Life Cycle: The life cycle completes in 17-23 (19.20 ± 0.84) days. (Egg: 3-4; Larva: 9-13; Pupa: 5-6 days).

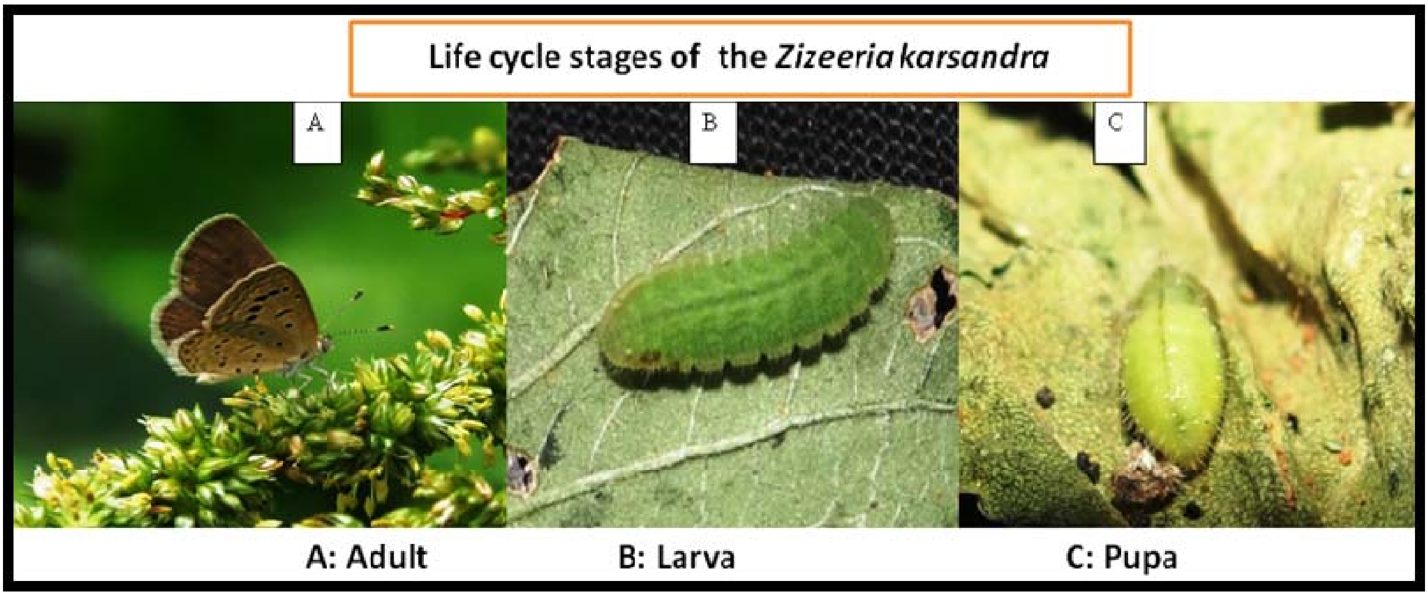
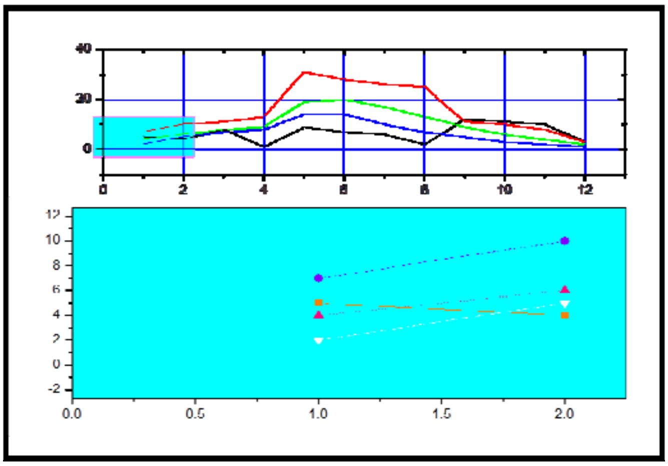


Fig 3: Photographs of the sequential stages in the life history stages of *Zizeeria karasandra*
a) Adult **b)** Instar IV **c)** Pupa

Population Index: (Fig. 4)
The numerical frequency of the natural occurrence of the life stages – eggs, larvae and pupae, on host plant *Amaranthus spinosus* (Amaranthaceae) was given in Fig. 4, all the stages could be spotted out during July–March, which correspond to post monsoon in study locality.



X axis- total months, **Y axis** – Numerical frequency of life stages
Fig 4: Population index of different life stages of *Zizeeria karsandra* on *Amaranthus spinosus*

Food Consumption and Growth

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 1. Of the total amount of food consumed, the percentage shares of the successive instars were 12.0, 19.14, 33.12 and 82.05% and the proportions of weight gained by the successive instars were 0.51, 1.07, 3.52 and 34.00%. Thus, there was over 74% 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 (Fig. 5) 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 8.16-2.16 mg and those of GR between 0.30 – 0.95 mg.

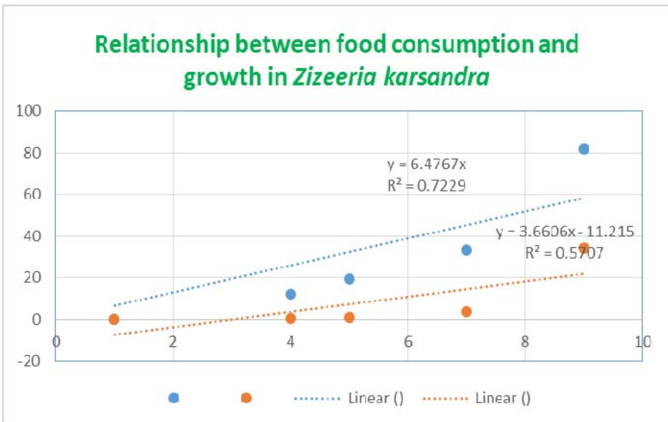


Fig 5: Food consumption, growth and food utilization efficiencies on *Zizeeeria karsandra* larva on *Amaranthus spinosus* (Amaranthaceae) leaves

Table 1: Growth and food utilization efficiencies of *Zizeeria karsandra* larva on *Amaranthus spinosus* (Amaranthaceae)

Instar Number	Wt. of food ingested (mg)	Wt. of faeces (mg)	Wt. gain by larva (mg)	Performance indices				
				GR mg/day	CI mg/day	AD %	ECD %	ECI %
I	12.0 ± 0.22	0.56 ± 0.04	0.51 ± 0.03	0.30	8.16	98.00	03.52	05.00
II	19.14 ± 1.21	3.10 ± 01.17	1.07 ± 2.02	0.31	4.50	85.20	06.20	06.40
III	33.12 ± 3.40	6.84 ± 01.72	3.52 ± 0.17	0.34	3.10	74.50	11.60	12.30
IV	82.05 ± 5.90	29.05 ±0 2.91	34.00 ± 2.40	0.95	2.16	63.43	59.45	40.80

GR = Growth rate, CI = consumption index, AD = Approximate digestibility, ECD = Efficiency of conversion of digested food, ECI = Efficiency of conversion of ingested food

Table 2: Instar wise growth and duration of *Zizeera karsandra* larva on *Amaranthus spinosus*.

Stage	Length (mm)		Width (mm)		Duration (Days)	
	Max. Min.	Mean SD.	Min. Max.	AV. ± S.D.	Range.	AV. ± S.D.
Egg Larva	0.32 0.32	0.32 ± 0.00	0.51 0.51	0.51 ± 0.00	3	3.00 ± 0.00
Instar I	1.70 1.90	1.86 ± 0.15	0.70 0.90	0.82 ± 0.08	2 – 3	2.30 ± 0.55
Instar II	3.10 4.20	3.44 ± 0.45	1.20 1.60	1.24 ± 1.15	3 – 4	3.10 ± 0.45
Instar III	7.30 7.80	7.10 ± 0.20	2.10 2.90	2.48 ± 0.32	2 – 3	2.60 ± 0.55
Instar IV	8.00 9.00	8.20 ± 0.41	2.95 3.20	2.96 ± 0.09	1 – 1	1.00 ± 0.00
Total larval					8 – 10	9.10 ± 0.84
Period (Days) Pupa	7.00 8.00	7.30 ± 0.45	2.30 2.80	2.54 ± 0.21	5	5.00 ± 0.0

Table 3: Larval development, growth and food energetics of *Zizeeria karsandra* on *Amaranthus spinosus* in the laboratory

Butterfly species	Host plant	Larval survival to adult stage (%)	Development time of larva (days)	Pupal wt. (mg)	*Growth rate (%)	Nutritional indices (mean of all instars)		
						AD (%)	ECD (%)	ECI (%)
Zizeeria karsandra	*Amaranthus spinosus*	43.67 (n=12)	08.89	258.60	89.45	82.4 220.83 16.36		

* Growth rate (%) = [(mp/mh)1/t ± 1] x 100. Samata 2006[23].
Where mp = Pupal weight; mh = hatchling weight; t = larval development time

Indices of Food Utilization

Table. 1 also included the data on approximate digestibility (AD), efficiency of conversion of digested food (ECD), and efficiency of conversion of ingested food (ECI). The estimated values of AD ranged between 98.00-63.43%. The values decreased as the instar stages progressed. The values of ECD and ECI increased progressively from the first instar to the last instar. The values of ECD varied from 3.52-59.45%and those of ECI from 05.00- 40.80%. Thus there was an inverse relationship between the values of AD and those of ECD and ECI

Table: 4 Mean Temperature, Relative humidity, Rainfall and the length of photoperiod during study period at Kadapa**.**

Life cycle stage ↓	Calendar month											
	J	F	M	A	M	J	J	A	S	O	N	D
Early life stages	6	6	8	7	22	41	115	60	32	20	13	15
Adults	*	*	*	*	**	***	***	***	***	**	**	*
Temperature	29.15	32.24	38.45	40.7	43.21	35.45	37.23	30.41	31.81	30.03	28.05	27.31
Relative humidity	76	72	77	80	81.05	74	76.02	80.02	79.08	68.03	66.25	65.03

* Rare, ** Common,*** Very common

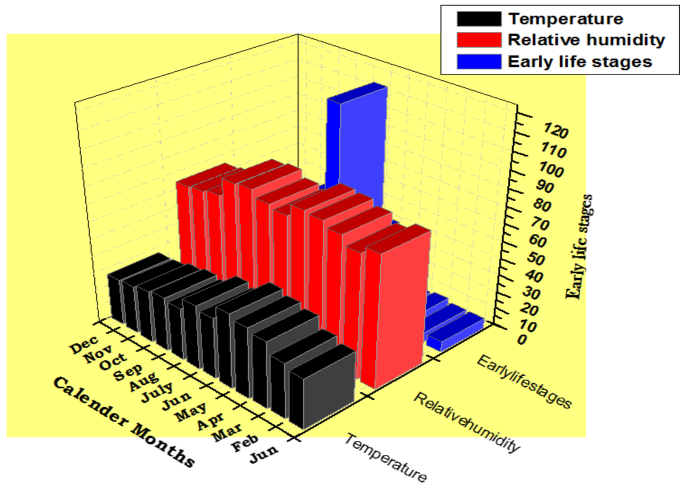


Fig 6: Mean Temperature, relative humidity, rainfall and the length of photoperiod during study period at Kadapa.

Discussion

The life cycle passed through four instars over a period of days after which the final instar undergoes pupation. The increasing food consumption at successive instars was in inverse relationship with consumption index & growth rate (Table. 3). CI decreased with the age of larvae, the former from a high of 8.16 to 2.16 the values of CI, the range 0.31-6.60 predicated for tree foliage chewers [26, 27]. Food consumption rate depends on the conversion efficiency of ingested food to biomass (ECI), the rate increasing as the conversion efficiency decrease or vice versa [28, 29] and high CI value of instar I is due to conversion efficiency and this character was reflected in the low value of ECI of instar I compared to other successive instars.

The AD values decreased from instar I to IV. The highest being instar I (98%). The average AD% is 90 and these high AD substantiate the statement of [28-30] those foliage chewers often attain high AD values. Such high AD values also are expected when food item was rich in water tree foliage [31, 32]. The values of food ECD increase from early to late instars [28]. Such trend was broadly apparent with the ECD. This was indicative of low efficiency of conversion of digested food to body tissues. There was no trend of increase or decrease in ECI values suggested by [28, 32]. The values of ECI may increase, decrease or show little changes depending on the extent to which the changes in AD and ECD compensate each other. The ECI values in the present study varied from 5.0 to 40.80. They showed a continuous increase from first instar to fifth instar. These values are within the range of 9-34% reported for foliage and grass chewing Lepidoptera respectively [28, 33] the total period of development from egg to emergence of adult was estimated to be 17 - 23 days. This was a relatively short period and may enable the butterfly to have 11 -12 broods yearly [34-36]. During this period laboratory study of hatching success rate ranged between 50-90% larval development success rate between 50-80%, pupal development success between 50-80%. With the three life cycle stages there was a higher success rate between July to March.

Thus, the present study provides information on the oviposition larval host and larval performance in terms of food consumption, growth and utilization, and the length of life cycle from egg to adult emergence of the dark grass Blue butterfly, *Zizeeria karsandra* (Moore) butterfly. The present data may be profitably utilized in the successful conservation management of this butterfly species either in parks, Zoos and butterfly houses or in the field. Butterfly parks are popular exhibits in Zoos and have an immense educational [37, 38] and conservational potential [37, 39, 40].

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