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Food consumption and bioenergetics of the Lime Blue butterfly (*Chilades lajus* (stoll)) (Lepidoptera: Rhopalocera: Lycaenidae) in Sri Lankamalleswara Reserve forest, Southern Andhra Pradesh – India

PALEM HARINATH
KANIKE SURYANARAYANA
SRI PURUSOTHAMA VENKATA RAMANA[1]
Department of Zoology, School of Life Sciences
Yogi Vemana University Kadapa
Andhra Pradesh, India
M. VENKATA REDDY
Department of Zoology
Sri Krishnadevaraya University- Anantapur
Andhra Pradesh –India

Abstract:

The life history of the long-tailed blue butterfly also called Lime blue, Chilades lajus (Stoll) larval stages in relations to diet consumption, exploitation and the length of life phase on its host plant Citrus aurantifolia have been designated for the first time. The Lime blue, Chilades lajus was one of the species of Lycaenidae butterfly (classification) originated in the Palaeotropics and it was usually seen in exposed scrub and grassland environments. The study was conducted between January to December 2015 at Sri Lankamalleswara reserve forest, Kadapa, India. Chilades lajus completes its life cycle in 15-22 days (Eggs 2-3 days, Larvae: 8-12 days, Pre-Pupa:2-3 days, Pupa: 3-4 days). The standards of food indices

[1] Assistant Professor & Corresponding author Email: spvramana.butterfly@gmail.com

across the instars include approximate digestibility (AD): 66.64 – 96.01%; growth rate (GR): 0.29 - 0.99, consumption index (CI): 2.32 – 8.48, efficiency of conversion of digested food (ECD): 3.53 – 64.77%; efficiency of conversion of ingested food (ECI): 3.39 – 42.77% as measured in the research laboratory. The relatively high values of ECD and ECI partially explain the ecological success of this butterfly in the present study environment.

Key words: Life history, *Chilades lajus*, *Citrus aurantifolia*, food indices, Sri Lankamalleswara reserve forest, Andhra Pradesh.

INTRODUCTION:

Butterflies are known for the incontestable beauty of their wing colors, and contribute to the aesthetic quality of the environment. They constitute an important pollinator resource, and on this account, their conservation management has become a global risk [1; 2]. Little has been said by conservationists about the conservation of butterflies, which are worthy of protection [3]. To consider any conservation management programme accurate life history information of all butterflies in an area was required. Such knowledge in most cases of Indian butterflies was seriously inadequate [4; 5]. Sincere efforts in this direction have been in action for the butterflies of South India [6,7; 8] We describe here the details of immature stages, larval performance on the Lime blue, *Chilades lajus* was one of the species of Lycaenidae butterfly (classification) originated in the Palaeotropics (Fig: 2), and it was usually seen in exposed scrub and grassland environments. There are 23 species in the genus Chilades, distributed variously from Africa and India to China, and south through from Malaysia and the islands of south-east Asia to Australia.

The genus Chilades includes one of the smallest known butterfly species on Earth - the Madagascan Grass Blue

Chilades (Freyeria) *miniscula*, which has a wingspan of 9 mm. *Chilades lajus* was wide spreaded and common species, found from India and Sri Lanka to the Philippines.

Although stated to be generally common in India mostly in the deciduous forests, particularly forest clearings and open country [4; 9], the species was not that common in most places [10, 11, 12; 13] and also in the north coastal Andhra Pradesh (South India) representing the present study area. Perhaps its rarity in most areas has qualified this species to be listed as endangered in Schedule I of Indian Wildlife (Protection) Act, 1972. The species with declining populations may have reducing opportunities to find mates and reproduce, hence likely to disappear from the locality [14; 15]. Rearing such species and releasing the same into the wild will help restocking the depleting populations and also serve as a conservation measure of the species. This strategy requires complete knowledge of life history, phenology of both adults and early life stages, larval host plants, host plant selection and utilization, and also adult food resources for its successful implementation. Although some description of the early life stages was given in [9], data regarding duration of each life stages, life cycle duration, and voltinism was not studied. Here we describe complete knowledge on the stated parameters for its possible use in the conservation management of this the Lime Blue butterfly.

MATERIALS AND METHODS:

Study area:

The present study was carried out during January 2015 to December 2015, in the Sri Lankamalleswara Reserve forest (14°45' - 14°72' N & 79°07' - 78°80' E), Sothern Andhra Pradesh (Fig: 2). Both sites were regularly searched for the reproductive activity of the lime blue butterfly. Once located, detailed

observations were made in order to record the period of copulation and oviposition, followed by fresh egg collection without causing any damage to the larva. After oviposition, the leaf with eggs was collected into Petri dishes (15 cm × 2.5 cm depth) and transported to the research laboratory. The piece of the leaf with the eggs was then placed in a small Petri dish (10 cm × 1.5 cm depth), the inside of which was lined with moistened blotter to prevent the leaf from drying. Such Petri dishes were kept in hygienic, spacious cages fitted with wire gauge. Since ants were never detected, no special protection device was used to stop predation of eggs. They were inspected regularly at 6 hr intervals to record the time of hatching. Each of the freshly emerged larvae was transferred to a clean Petri dish the inside of which was lined with moistened blotter with the help of a camel hair brush.

The larvae were provided daily with a weighed amount of leaf pieces of the host plant. The faeces and the leftover of the foodstuff were collected and weighed each day (24 h). The growing larvae were observed regularly to note the instar change and features including length and weight measurements. As the larvae grew, they needed more space, increased space was provided by transferring the growing larvae to bigger Petri dishes (15 cm × 2.5 cm depth). Larval development in terms of food consumption indices was designed by Waldbauer (1968) as (Fig: 1) to estimate the growth and nutritional indices.

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 *Citrus aurantifolia* and also for adult frequency. Five replications were maintained for the study of all parameters. Fresh weight measurements were used for the purpose. The development of pupa from full grown larva and particulars of pupa including color, shape, size, weight and the time of adult eclosion were

also recorded. Millimetre graph paper was used for taking measurements. The laboratory temperature was 28 ± 20°C and relative humidity 80 ± 10% with normal indirect sunlight conditions that varied in duration between 12 hr during November/ January and 14 hr during June/July. In describing the details of adult characters, the butterflies that have emerged from the pupae in the laboratory, and those caught in the wild were used.

The prevailing weather conditions were borrowed from the ISRO Department of Physics on the Yogi Vemana University campus. Statistical analysis was done between counts of early life stages and the prevailing weather, using Origin Statistical Software (2010), and ANOVA was used to find out the variation in the effects of host plant species on larval performance.

CI (Consumption Index)	=	$\frac{\text{Weight of food consumed}}{\text{Weight of instar} \times \text{Number of feeding days}}$
GR (Growth rate)	=	$\frac{\text{Weight gain of instar}}{\text{Mean weight of instar} \times \text{Number of feeding days}}$
AD (Approximate digestibility)	=	$\frac{\text{Weight of food consumed} - \text{weight of faeces} \times 100}{\text{Weight of food consumed}}$
ECD (Efficiency of conversion of digested food)	=	$\frac{\text{Weight gain of instar} \times 100}{\text{Weight of food consumed} - \text{weight of faeces}}$
ECI (Efficiency of conversion of ingested food)	=	$\frac{\text{Weight gain of instar} \times 100}{\text{Weight of food consumed}}$

Fig (1): Waldbauer Formula.

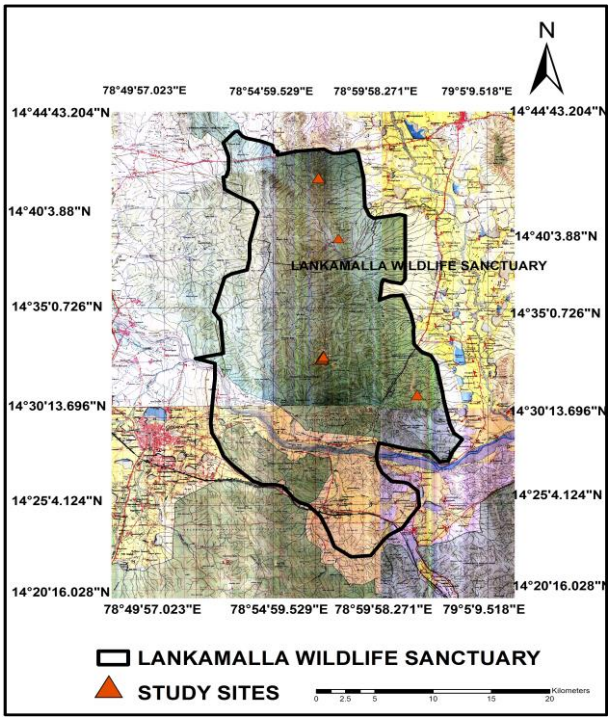

Figure (2). Study area Sri Lankamalleswara wildlife sanctuary

Distribution (Fig: 2):

This butterfly normally requires a tropical to the subtropical environment but sometimes ranges into sheltered, hot temperate areas. In Southern Andhra Pradesh, the butterfly was encountered at Sri Lankamalleswara Reserve forest, of the Eastern Ghats. The host plant, *Citrus aurantifolia* plants have been widely recorded in the Sri Lankamalleswara Reserve forest areas of Southern Andhra Pradesh and it occurs uncommonly in the tropical Southern Andhra Pradesh [17,18;19], and in the hot, humid, open grassland areas of Eastern Ghats of Southern Andhra Pradesh.

The early stages of the breeding populations of this butterfly in the reserve forest are heavily parasitized by flies

and wasps, and this would suggest that these populations are relatively sedentary [20; 21].

RESULTS AND DISCUSSION:

Description:

The female at the time of laying eggs was rather sluggish and simply walked after landing at the growing patches of the host plant *Citrus aurantifolia*. Oviposition usually occurred during 0900-1400 hr. The gravid female laid eggs singly on the underside of a leaf. About 6-12 eggs were laid, each on a different leaf, in a single egg-laying bout. As already stated, the eggs and larvae were reared on the actual host plant leaves and also on the leaves of two potential host plants in the laboratory and the early life stages were followed till the eclosion of the adult.

Adult stage (Figure: 3):

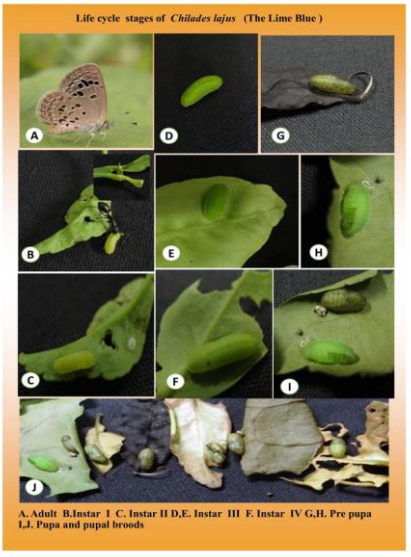

Figure: (3). Life cycle stages of *Chilades lajus*

Field characters:

The *Chilades lajus* (Stoll), Lime blue was univalent and seasonal. It was on wing almost throughout the year and breeds with high frequency during the periods of monsoon and post monsoon seasons. The lime blue butterfly (*Chilades lajus*) was a small butterfly found in India belonging to Lycaenidae or blue family. *Chilades lajus* was chiefly tropical and subtropical in distribution. The underside has numerous dark spots, but the most prominent of these are two and that are joined to each other at a right angle, forming an "L". In the males, the upper side of the wings was dull purplish blue and in the female it was brown with blue bases.

Habit:

These butterflies were distributed to the tropical and sub-tropical regions and also to some extent hot temperate regions. In Southern Andhra Pradesh, the butterfly was encountered at Sri Lankamalleswara Reserve forest, of the Eastern Ghats. The host plant, *Citrus aurantifolia plants* have been widely recorded in the Sri Lankamalleswara Reserve forest areas of Southern Andhra Pradesh and it occurs uncommonly in the tropical Southern Andhra Pradesh. It was found fighting mainly near to its larval host plant, *Citrus aurantifolia*. The behaviour of female can't be different at the time of egg laying. Intermittent foraging was observed while laying eggs.

Food resources:

Flies keep wings closer when they were hunting nectar. In the study area, the nectar host plants included Citrus species, and *Tribulus terrestris* L.*,* Less, *Tephrosia purpurea* (L.) *Boerhavia diffusa* L.*,* Pers.*, Vernonia cinerea* (L.), and *Rostellularia procumbens* (L.) Nees.

Oviposition host plants:

This butterfly was active in late monsoon and winter, but it occurs in probably throughout the year flying close to shrubs and small trees and settles often. They are univalent lays eggs singly on the underside of young *Citrus aurantifolia* leaves. They lay eggs during 11:00-16:00 hr.

LIFE HISTORY STAGES:

Adult:

It was a light green tail less blue. The underside has numerous dark spots, but the most prominent of these are two and that are joined to each other at a right angle, forming an "L". In the males, the upper side of the wings was dull purplish blue and in the female it was brown with blue bases. Found in the moist deciduous, semi-evergreen and evergreen forests, but only in open areas.

Egg stage:

The eggs are laid singly underside of the young Citrus leaves. The eggs are disc shaped and greenish with a blue tinge. The eggs are incubated at room temperature of about 29 ^{o}C hatched in about 3 days. The hatched larva feed on the egg shell.

Larval stage:

Instar I:

The caterpillar was pale green in the colour lies underside of the young leaves. It was semi-circle with a flat bottom and curved on the dorsal side. 1.9 – 2.3 mm (2.2 ± 0.02 mm). This stage lasts for 2 days. A unique aspect of this group was that caterpillars of several species share a very special relationship with ants in return for the protection that they receive from the ants

Instar II:
This stage lasted for two days; it measures around 2.9 – 3.3 mm (3.2 ± 0.02). It was light green with a mid-dorsal line. The head was black in colour.

Instar III:
This stage lasted for 2 to 3 days. It reached a length of 4 - 4.5 (4 ± 0.5 mm) and a width of 1.00 to 1.50 mm. The larva was green in colour and has greenish brown hair with the mid-dorsal line.

Instar IV:
This stage lasted for 2 to 3 days and grows to a length of 8 – 8.5 mm (8.3 ± 0.02 mm) in length and 1.8 – 2.00 mm in width. It was brown to green in colour with clear segments and reddish brown form on young leaves. A prominent dark spot was seen at the posterior end.

Pre-pupal stage:
This stage lasted for 2 days. At this stage, the larva stops feeding and get shortened in length and appears common hudge blue in colour.

Pupal stage:
This stage lasted for 6-7 days; the humped portion was seen in the middle region and the abdomen was broader than the anterior part. It was common hudge blue coloured with black spots.

Duration of life cycle:
The total development time from egg to adult eclosion ranged between 15-22 days (Eggs 2-3 days, Larvae: 8-12days, Pre-Pupa:2-3days, Pupa: 3-4 days)

Development success of eggs, larvae, and pupae:

The laboratory study results hatching success rate of 50 – 90% during January – November with higher rate recorded during July – November. The success rate of larval development varied between 50 - 89%, with higher rate occurring during July – November. Pupal development varied from 50 – 88%, with the higher rate being evident during July to October.

Table. 1. Development success of eggs, larvae, and pupae of *Chilades lajus on Citrus aurantifolia* leaves in the laboratory

Calendar month	Number of eggs incubated	Number of larvae hatched	Number of pupae formed	Number of adults emerged
Jan	8	6	4	2
Feb	10	8	6	4
Mar	13	10	6	6
Aprl	20	16	12	11
May	22	18	15	13
Jun	16	14	12	9
July	28	23	19	17
Aug	24	18	16	15
Sep	26	37	27	24
Oct	39	23	22	22
Nov	36	23	22	22
Dec	12	8	6	5

Population Index: (Fig: 4)

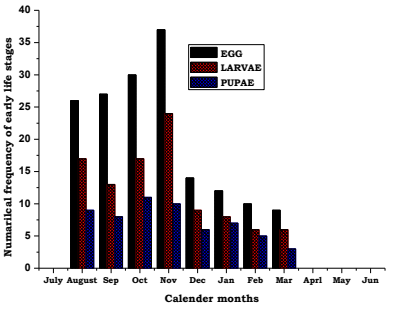

Figure: (4). Month wise distribution and numerical frequency of three different early stages of *Chilades lajus on Citrus aurantifolia* leaves in the field.

The numerical frequency of the natural occurrence of the life stages – eggs, larvae, pupae and adult on the host plant are given in Fig.4. All the stages were spotted out throughout the year in the study locality. However, there was a higher frequency of occurrence of the life stages from August to November and March to May which corresponds with the warmer temperature.

Table: 2. Population index of different life stages of *Chilades lajus on Citrus aurantifolia* leaves in the field.

Calendar month	Adults abundance	Number of Eggs	Number of Larvae	Number of Pupae
July	Rare	0	0	0
August	Very Common	26	17	9
Sep	Very Common	27	13	8
Oct	Very Common	30	17	11
Nov	Very Common	37	24	10
Dec	Very Common	14	9	6
Jan	Common	12	08	07
Feb	Common	10	6	5
Mar	Common	9	0	0
Aprl	Rare	0	0	0
May	Rare	0	0	0
Jun	Rare	0	0	0

FOOD CONSUMPTION AND GROWTH:

Table: 3. Food consumption and utilization, growth and food utilization efficiencies of *Chilades lajus* on *Citrus aurantifolia* leaves

Instar number	Wt.of food ingested (mg)	Wt.of faeces (mg)	Wt. gain by larvae (mg)	GR (mg/day)	CI (mg/day)	AD (%)	ECD (%)	ECI (%)
I	12.80 ± 01.24	0.48 ± 00.04	0.41 ± 00.05	0.29	8.48	96.1	3.53	3.39
II	19.94 ± 05.28	4.20 ± 01.33	2.08 ± 00.72	0.3	5.84	84.72	6.09	5.16
III	32.17 ± 05.07	6.44 ± 01.52	3.36 ± 03.16	0.32	3.81	78.23	12.94	10.12
IV	85.84 ± 07.21	29.17 ± 02.05	37.00 ± 02.35	0.99	2.32	66.64	64.77	42.77

Efficiency of conversion of digested food (ECD), Efficiency of conversion of ingested food (ECI), Growth rate (GR), Consumption index (CI), Approximate digestibility (AD)

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: 3. Of the total amount of food consumed, the percentage shares of the successive instars were 12.80, 19.94, 32.17, 85.84 % and the proportions of weight gained by the successive instars were 0.41, 2.08, 3.36, and 37.00. 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 from the food consumed (Figure: 5) resulted in 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 last instar. The values of growth rate (GR) increased from first to the last instar. Values of CI ranged between 2.32 – 8.48 mg/day/mg and those of GR ranged between 0.29 – 0.99 mg/day/mg.

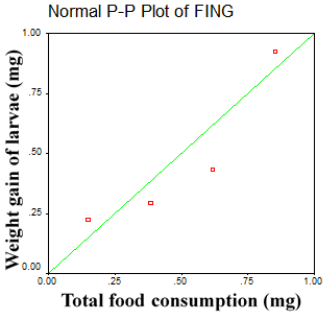

Fig: (5). Relationship between food consumption and growth in *Chilades lajus on Citrus aurantifolia*

Indices of food utilization:

The total development time from egg laying to adult eclosion was 21.30 ± 1.14 days at about 28 ± 2°C. This behavior is in line

with the expectations of short life cycles in tropical butterflies [3]. Since temperature influences instar duration and the overall development time [22, 23, 24; 25] the duration of the life cycle may vary from our records depending on the prevailing temperatures. As no temperature extremities occur at Kadapa, the duration of life cycle did not vary much over the overlapping seasons. Since temperature influences instar duration and the overall development time, the duration of the life cycle may vary from our records depending on the prevailing temperatures and relative humidity (Fig:6). As no temperature extremities occur at Kadapa, the duration of life cycle did not vary much over the overlapping seasons.

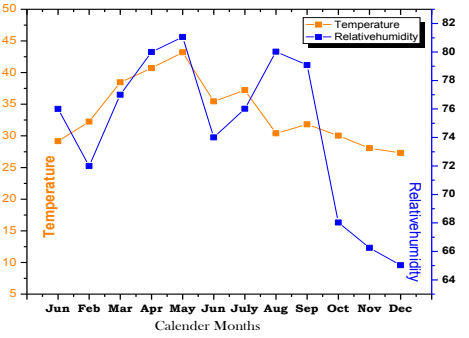

Fig: 6. Relative humidity and temperature month wise data at Kadapa

Over the entire period of its growth, a larva consumed on average over 3.23 grams of leaf material, with increased consumption in the last two instars. This tendency of greater consumption by the last two instars has been reported in lepidopterous larvae in general [16, 22, 26, 23, 27; 28], and it compensates the energy expenditure of non-feeding pupal stage [29]. The values of CI are near to the range (2.32 – 8.48) predicted for forb foliage chewers [26]. Food consumption rate depends on the conversion efficiency of ingested food to biomass (ECI), the rate increasing as the conversion efficiency decreases

or vice versa [30]. In this sense, the high CI value (8.48) of instar I am probably due to low conversion efficiency and this character was reflected in the low values of ECI for instar I compared to other successive instars. Higher growth rates occur with penultimate than with final instars [31]. The GRs of penultimate and final instars of *Chilades lajus* are in line with the above decreasing trend.

Table: 3 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 – 65.62% and those of ECI varies from 3.39 – 42.77%. Thus, there was an inverse relationship between the values of AD and those of ECD and ECI.

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 the life cycle from egg to adult eclosion of the lime blue butterfly *Chilades lajus*.

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 houses are popular exhibits in Zoos and have an immense educational and conservational potential. The present study also indicated that captive rearing the larvae at about 28 ± 20^{o}C permits enough stock of adults for restocking the areas poor in populations of the Lime Blue butterfly.

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