Multidisciplinary

Comparative study on the life cycle of lime butterfly *Papilio demoleus* (*Linnaeus, 1758*) in tummalapalle mining region and palakondalu forest region of Andhra Pradesh – India

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Abstract

Butterfly assemblages and life history characteristics are impacted by habitat quality changes brought about by mining operations. This study assessed the effects of mining disturbance on the life cycle of *Papilio demoleus* and butterfly diversity in Kadapa District, Andhra Pradesh, India. In the Palakondalu forest (control) and Tummalapalle (mining zone), field surveys were carried out. The morphometric and developmental parameters of *P. demoleus* were measured from the egg to the pupal stages, and the richness, abundance, and variety of the assemblies were documented. With restoration of butterfly communities improved, although they were less varied in mining areas. Forest area eggs were larger (1.02 ± 0.05 mm) and incubated for 4.2 ± 0.7 days, whereas mining area eggs (0.95 ± 0.06 mm, 4.8 ± 0.8 days) demonstrated habitat-specific variations in *P. demoleus***.** Fifth instars in the mining zone (38.6 ± 2.4 mm, 6.1 ± 0.6 days) were smaller and slower than those in the forest (45.0 ± 2.6 mm, 5.2 ± 0.4 days). Pupae exhibited comparable trends. Overall, the forest took 29–30 days to develop, whereas the mining regions took 34–36 days. Heavy metals, microclimatic changes, and potential radiation are examples of mining-related stressors that delay development and decrease morphometric features, whereas habitat restoration increases butterfly performance and variety.

Keywords: *Papilio demoleus,* Tummalapalle, Palakondalu forest, Life cycle, Mining disturbance

Introduction

Butterflies are sometimes called "dancing flowers" because of their eye-catching coloring, varied wing patterns, and elegant flying. Beyond their aesthetic value, they are widely acknowledged as sensitive bioindicators of ecosystem health and serve an important ecological role (Rosenberg, 1986; Beccaloni & Gaston, 1995; New *et al*., 1995; Oostermeijier & Van Swaay, 1998) [30, 1, 20, 21]. Their life cycle includes four distinct stages, just like that of all Lepidoptera: egg, larva, pupa, and adult (Shekahda *et al*., 2021) [32]. Urbanization, industrialization, and agricultural intensification were examples of contemporary anthropogenic pressures that have accelerated habitat degradation, driven species declines, altered water and soil quality, and reduced floral resources (McKinney, 2002; Garg *et al*., 2009; Singh *et al*., 2009; Lintott *et al*., 2014) [16, 5, 34, 15]. Such disruptions have the potential to upset ecological networks and pose a direct threat to biodiversity.

The citrus butterfly, *Papilio demoleus* L. (Papilionidae), sometimes referred to as the lemon or checkered swallowtail, is one of the butterflies of agricultural importance and a significant pest of citrus crops in Asia (Ebeling, 1959) [4]. Citrus, a member of the Rutaceae family, is indigenous to tropical and subtropical regions of Asia, including China and India, with northeast India serving as a major hub for diversification. After China and Nigeria, India is the world's third-largest producer of citrus, growing grapefruit, sweet orange, lime, and mandarins (Saljoqi *et al*., 2006) [31]. States like Maharashtra, Andhra Pradesh, Punjab, and Haryana are important production hubs in India. There were two subspecies of *P. demoleus, and P. malayanus,* which are found throughout the Asia-Pacific region (Guerrero *et al*., 2004) [6].

Although there is ample documentation of its morphology and development, little is known about its ecological tolerance and adaptability to damaged ecosystems, especially mining landscapes.

Mining operations are one of the most serious causes of habitat deterioration in the world. Thus, conservation biology has made the restoration of damaged ecosystems—including mine sites—a top concern [Rodrigues *et al*., 2006, Pressey *et al*., 2007] [29, 27]. As the second-largest order in the Insecta, Lepidoptera play a major role in terrestrial biodiversity [New & Collins, 1991] [19]. Due to their heavy reliance on microhabitat conditions and host plants, they are extremely vulnerable to changes in the environment, such as pollution, vegetation loss, and climate variability [Blair, 1999; Kremen C, 1992; Hellman JJ. 2002.] [2, 14, 7]. As model organisms, butterflies have also been employed extensively to investigate the ecological responses of other insect species, including crustaceans, ants, beetles, and spiders [Pierce *et al*., 2002; Thomas JA, 2005; Syaripuddin K, 2015] [25, 26, 35].

There were over 1,439 butterfly species known to exist on the Indian subcontinent, 100 of which are endemic, and at least 26 taxa are listed as globally threatened (Red List of Threatened Animals and Insects, 2020) [IUCN, 1990] [10]. This emphasizes their significance as conservation priority and ecological indicators [Komac *et al*., 2013] [13]. Despite the fact that many studies have looked at how disturbance regimes affect insect communities, little is known about how mining operations especially affect butterfly assemblages [Hogsden KL & Hutchinson TC 2004; Čermáková Z *et al*., 2011; Tropek R, *et al*., 2012] [9, 3, 36]. It is essential to comprehend these relationships since butterflies are used as tools to assess the effectiveness of restoration initiatives as well as indicators of ecological health. In order to determine how butterfly assemblages, in particular *P. demoleus,* react to habitat changes caused by mining in Kadapa District, Andhra Pradesh, the current study was conducted. It specifically seeks to examine the distribution, make-up, and life cycle characteristics of butterfly communities at locations with different degrees of restoration and mining activity.

Methodology

The study was carried out in two different habitats in the Andhra Pradesh, India, Kadapa District. The first location was the Tummalapalle mining zone) (14º28'24.573"N 78º42'45.447"E), which is located in Pulivendula, Kadapa District, and is distinguished by extensive uranium mining and related disturbances. The second location was the Palakondalu forest land region (14°25'56.967''N and 78°52'49.159"E), which is a naturally occurring ecosystem with a variety of vegetation that has not been much altered. These locations were chosen to assess the impact of stress from mining on *Papilio demoleus* biology and butterfly assemblages.

Observations and laboratory rearing

The Department of Zoology's Entomology Laboratory and Net House conducted biological research on the citrus butterfly (*P. demoleus*). The temperature and relative humidity in the lab were kept between 29 and 35 °C and 60 and 75%, respectively. Less than a year-old *P. demoleus* larvae were taken from *Limonia acidissima* and Citrus limon plants and brought to the lab. To prevent cross-contamination and cannibalism, each larvae were raised independently on sterile petri dishes with a diameter of 2.5 cm. To facilitate aeration, muslin cloth was placed over each petri dish.

Every day, larvae were fed fresh leaves of *Limonia acidissima*, with replacements made to keep the leaves from drying out. To ensure hygienic conditions, petri dishes were cleaned with soap solution and changed every two to three days. Larvae were permitted to pupate inside the plates, typically adhering to the dish borders or muslin lining. In order to preserve humidity and promote adult emergence, freshly produced pupae were carefully placed into separate glass jars, each of which had a moist filter paper at the base. Morphometric measurements (width, length) and developmental durations (egg, larval instars, pre-pupal, pupal stages) were recorded by daily observations. For the purpose of comparing mining and forest populations, data were tabulated and mean values with standard deviations were computed.

Local caterpillar host plants

Ruta graveolens (Rutaceae, common name: Herb-of-grace), *Citrus microcarpa* (Rutaceae), *Citrus maxima* (Rutaceae, common name: Pomelo), *Citrus aurantifolia* (Rutaceae, common name: Lime), *Limonia acidissim*, *Citrus limon*, *Maxima Citrus* (Pomelo), Mandarin, or *Citrus reticulata*, sweet orange, or *Citrus sinensis* and other Citrus species.

Adult butterfly physical description

Both sexes possess yellow markings and spots on top, some of which create an irregular, sporadic macular band that continues from the mid-dorsum of the hind wing across the forewing. Each wings possess a sub marginal cluster of tiny yellow dots. Both sexes have a red mark on the hind wing area of the butterfly. The male has a very thin black gap between a narrow blue lunule that caps this area. The female, on the other hand, has a relatively large black spot between the blue lunule and the red spot. Both sexes are primarily yellow underneath, with irregularly shaped dots and black streaks. There was blue striate lining a number of black dots on the hind wing. On the forewings and Hindwings, there are a number of orange post-discal bars.

Field notes on butterfly behaviour

The lime butterfly is a rather widespread species in Singapore, occurring in both urban and woodland settings. Often observed flying throughout and near residential areas, the swift adults typically visit potted plants in common spaces outside of homes and apartments and flowers growing in gardens. There are numerous citrus plants in the residential neighbourhood, both planted and natural, where the females can be observed making ovipositing visits. During the cooler morning and late afternoon hours, when they retreat to snooze amid the leaves, it is easier to approach the hyperactive adults for photographs.

Results and discussion

Initial Phases (Egg stage): According to Phartiyal *et al*. (2012) [24], Rao (2015) [28], and Patel *et al*. (2017) [23], the eggs of Papilio demoleus were laid singly, primarily on the underside of delicate citrus leaves and branches, and had a pale creamy-golden appearance with a coarsely roughened surface. The average egg diameter in the forest area was 1.02 ± 0.05 mm, while in the mining zone it was slightly smaller at 0.95 ± 0.06 mm. The incubation period was 4.2 ± 0.7 days in the forest and 4.8 ± 0.8 days in the mining habitat. After hatching, the neonate larva (~2.75 mm long) ate the eggshell before feeding on the host plant.

Larval instars

First instar: In the first instar, the caterpillars reached about 5 mm in length before molting. The forest population measured 5.3 ± 0.4 mm in length, 1.6 ± 0.05 mm in width, and lasted 2.7 ± 0.5 days, while the mining population was smaller 4.7 ± 0.5 mm in length, 1.4 ± 0.06 mm in width, duration 3.2 ± 0.6 days.

Second instar: The second instar was characterized by a more noticeable saddle patch and pale thoracic spots. Larvae in the forest averaged 9.2 ± 0.1 mm in length, 2.9 ± 0.1 mm in width, duration 2.6 ± 0.5 days, while in mining areas they were smaller 8.4 ± 0.2 mm, 2.5 ± 0.1 mm, duration 3.1 ± 0.6 days.

Third instar: The white saddle patch grew larger during the third instar, and light lateral patches showed through. However, mining larvae were shorter 12.6 ± 0.6 mm, 3.2 ± 0.2 mm, 3.5 ± 0.5 days, while forest larvae reached 14.0 ± 0.5 mm length, 3.8 ± 0.2 mm breadth, and 2.9 ± 0.3 days duration.

Fourth instar: In the fourth instar, the larvae grew to a length of 26.2 ± 2.0 mm, a width of 5.8 ± 0.2 mm, and duration of 3.2 ± 0.4 days in forest populations. They also had a slimmer body and a darker ground color. By comparison, mining larvae had an average length of 22.8 ± 1.8 mm, breadth of 5.1 ± 0.2 mm, and duration of 3.9 ± 0.5 days.

Fifth instar: Transverse bands and recognizable eye-spot markings appeared on caterpillars in their fifth instar. The body color eventually changed to a consistent shade of green. In comparison to mining larvae, which were significantly smaller 38.6 ± 2.4 mm, 6.1 ± 0.3 mm, lasting 6.1 ± 0.6 days, forest larvae reached 45.0 ± 2.6 mm length, 6.8 ± 0.2 mm width, and lasted 5.2 ± 0.4 days.

Pre-pupal stage: Before pupa A silk pad and girdle were used to secure the larvae as they shrunk and became immobile at the end of the fifth instar. Larvae from the mining zone were smaller (24.0 ± 1.5 mm, 6.9 ± 0.3 mm, 1.2 ± 0.1 days), whereas forest larvae in pre-pupa were 27.2 ± 1.7 mm in length and 7.7 ± 0.4 mm in breadth, lasting~1.0 day.

Pupal stage: Pupae were either brown with heavy mottling or green with a diamond-shaped patch. In comparison to mining zone pupae, which were smaller and developed more slowly (27.1 ± 2.1 mm, 8.3 ± 0.3 mm, 9.6 ± 0.7 days), forest pupae had an average length of 30.6 ± 2.4 mm, breadth of 9.1 ± 0.3 mm, and duration of 8.3 ± 0.5 days. After nine to ten days, the pupae darkened considerably and the adult emerged.

Comparative summary

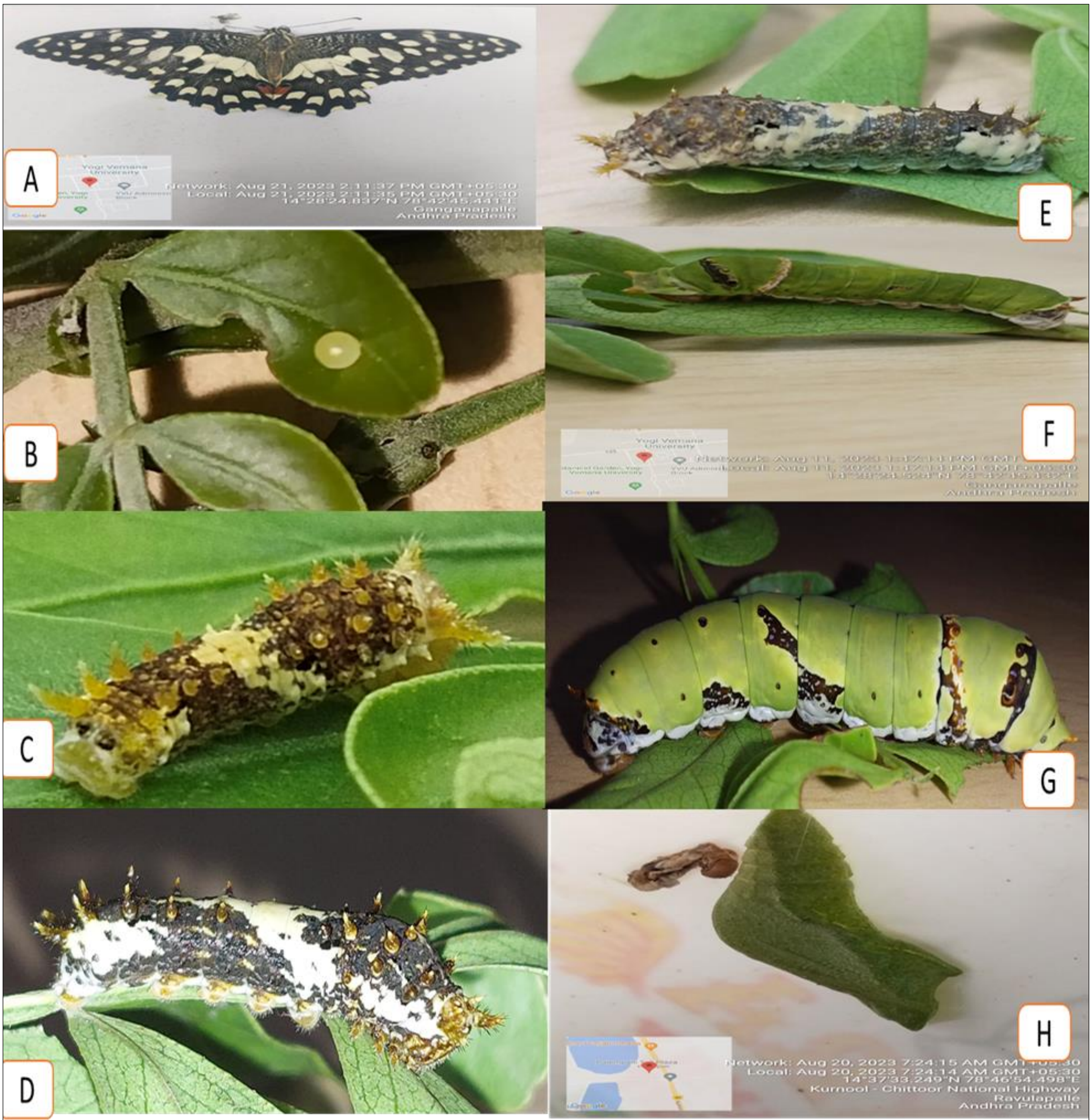
In general, larvae and pupae from forest areas were bigger and matured more quickly, finishing the immature stages in roughly 29 to 30 days. On the other hand, populations in mining zones showed least morphometrics and took longer to develop, taking 34–36 days to reach the pre-adult stage. These variations imply that stressors associated with mining (such as changed microclimate, decreased host plant quality, or trace metal pollution) have an adverse effect on *P. demoleus* larval and pupal development.

Table 1: Lifecycle measurements of *Papilio demoleus*

Stage	Metric	Units	Forest area (Mean ± SD)	Mining zone (Mean ± SD)
Egg	Diameter	mm	1.02 ± 0.05	0.95 ± 0.06
	Duration	days	4.2 ± 0.7	4.8 ± 0.8
1st instar	Length	mm	5.3 ± 0.4	4.7 ± 0.5
	Width	mm	1.6 ± 0.05	1.4 ± 0.06
	Duration	days	2.7 ± 0.5	3.2 ± 0.6
2nd instar	Length	mm	9.2 ± 0.1	8.4 ± 0.2
	Width	mm	2.9 ± 0.1	2.5 ± 0.1
	Duration	days	2.6 ± 0.5	3.1 ± 0.6
3rd instar	Length	mm	14.0 ± 0.5	12.6 ± 0.6
	Width	mm	3.8 ± 0.2	3.2 ± 0.2
	Duration	days	2.9 ± 0.3	3.5 ± 0.5
4th instar	Length	mm	26.2 ± 2.0	22.8 ± 1.8
	Width	mm	5.8 ± 0.2	5.1 ± 0.2
	Duration	days	3.2 ± 0.4	3.9 ± 0.5
5th instar	Length	mm	45.0 ± 2.6	38.6 ± 2.4
	Width	mm	6.8 ± 0.2	6.1 ± 0.3
	Duration	days	5.2 ± 0.4	6.1 ± 0.6
Pre-pupa	Length	mm	27.2 ± 1.7	24.0 ± 1.5
	Width	mm	7.7 ± 0.4	6.9 ± 0.3
	Duration	days	1.0 ± 0.0	1.2 ± 0.1
Pupa	Length	mm	30.6 ± 2.4	27.1 ± 2.1
	Width	mm	9.1 ± 0.3	8.3 ± 0.3
	Duration	days	8.3 ± 0.5	9.6 ± 0.7

Changes when compared -

- The forest area is larger and develops more slowly (normal growth).
- Mining zone: Extended duration of larvae and pupae (stress effect), reduced size in all stages.
- The entire immature phase:
  - Forest = about 29 to 30 days
  - Mining zone: around 34–36 days



PAPILIO DEMOLEUS

Life cycle of ***Papilio demoleus*** conducted in the region i.e., **Palakondalu forest area** (Figure 1) (14°25′56.967′′N and 78°52′49.159"E) Kadapa region.

A. Adult; B. Egg; C. Instar I; D. Instar II, E. Instar III, F. Instar IV; G. Instar V; H. Pupa.

Fig 1

PAPILIO DEMOLEUS

Life cycle of ***Papilio demoleus*** conducted in the region i.e., **Tumalapalle mining area surroundings** (Figure 2)(14°28'24.573"N 78°42'45.447"E) Pulivendula region.

A. Adult; B. Egg; C. Instar I; D. Instar II, E. Instar III, F. Instar IV; G. Instar V; H. Pupa.

Fig 2

Discussion

There were notable morphometric and developmental differences between the Tummalapalle mining zone and the Palakondalu forest when *Papilio demoleus* populations are compared. Eggs, larvae, and pupae all reached bigger sizes and shorter developmental times in the forest habitat, finishing the immature stages in 29–30 days. On the other hand, populations found near mining zones had smaller larval and pupal sizes, smaller eggs, and longer development times (34–36 days). These results imply that environmental stresses brought on by mining, such as changed microclimate, heavy metal deposition, and low-level radiation, have quantifiable effects on the growth and performance of butterflies. Similar trends have been observed in Fukushima-derived *Zizeeria maha*, where long-term radiation exposure led to transgenerational impacts, decreased fitness, and developmental abnormalities (Hiyama *et al*., 2012; Otaki, 2022) [8, 22].

Similarly, it has been demonstrated that heavy metals including Cd, Pb, and Zn absorbed by host plants affect herbivore development, decrease survival, and change the distribution of energy in Lepidoptera (Kafel *et al*., 2013; Morkunas *et al*., 2018) [11, 18]. For instance, monarch larvae showed higher mortality rates when exposed to elevated zinc levels (Shephard *et al*., 2022) [33], whereas moths exposed to Cd/Zn across several generations showed decreased growth and survival. These results are consistent with decreased body size and slower growth seen in *P. demoleus* mining populations. As also noted near Chernobyl and other disturbed landscapes, reduced pupal size can result in smaller adult butterflies with lower fecundity, ultimately reducing local abundance. Prolonged development also increases the risk of predation (Møller & Mousseau, 2009) [17].

An integrated approach is necessary for mitigation. Our findings support the notion that habitat restoration and revegetation enhance butterfly abundance and richness at the landscape level. Remedial methods like lime application, biochar, and organic amendments can immobilize heavy metals and enhance the quality of host plants at the soil–plant interface (Khan *et al*., 2017) [12]. Monitoring butterfly bioindicators in conjunction with soil and leaf assays can provide early warning of ecological stress, and Phytoremediation belts employing hyper accumulator plants may further reduce bio available metals. Chronic effects can be reduced in radiation-affected areas by mapping dose rates and giving restoration of low-dose zones priority. Overall, our findings highlight that mining operations affect butterflies at the species and community levels, yet restoration techniques provide a practical means of regaining assemblage diversity and life history performance.

Conclusion

The current study shows that the life cycle of *Papilio demoleus* and butterfly assemblages were significantly impacted negatively by mining operations in Tummalapalle. In comparison to mining-zone populations, which were smaller, took longer to mature (34–36 days), and grew more slowly overall, forest populations had larger egg, larval, and pupal morphometrics and shorter developmental periods, finishing their pre-adult life cycle in 29–30 days. These variations can be linked to stressors associated with mining, which cumulatively hinder growth and survival. These stressors include changes in the microclimate, heavy metal contamination, and prolonged radiation exposure. The adaptability of butterfly populations when ecological conditions are restored is demonstrated by the fact that, at the community level, butterfly richness, abundance, and diversity were decreased in disturbed mining environments but improved with revegetation and restoration. Therefore, reducing ecological stress brought on by mining and guaranteeing the preservation of butterfly diversity and ecosystem health need efficient habitat rehabilitation, soil and plant remediation, and ongoing bioindicator monitoring.

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