

# EFFECTS OF POPULATION DENSITY ON SURVIVAL, DEVELOPMENT TIME, FECUNDITY AND HATCHABILITY OF SAMIA CYNTHIA RICINI BOISDUVAL

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### ABSTRACT

Samia cynthia ricini Boisduval Lepidoptera:Saturniidae is a lepidopteran insect that has a gregarious behaviour during their larval stage. This research aimed to determine the minimum population limit of larvae that can support the survival of *S. c. ricini* by observing the development time, fecundity, and hatchability of eggs produced by adult moths under different larval densities rearing conditions. The research was conducted by observing the lifecycle and development of *S. c. ricini* at different population densities. The results showed that density had effect on the development time of *S. c. ricini* egg only. Population density also affects the survival and adult fecundity of *S. c. ricini*. A density of 30 is the minimum population required to support the success of *S. c. ricini's* life. Samia cynthia ricini reared at this density had the highest survival rate, faster egg development time, heavier cocoon shell weight and highest fecundity.

Key words: Samia cynthia ricini, castor, crowded population, density, development time, eri silkworm, fecundity, gregarious, insect rearing, lifecycle

Population density is an essential factor in the life of organisms including insects. Population density provides an opportunity for intraspecies competition of space and resources, which directly affects survival rate, development time, dispersal, reproduction, behaviour, and even morphology. According to Riddick and Wu (2015), Coleomegilla maculate more survived when maintained at a density of 20 compared to other densities. Lazarević et al. (2004) reported that Lymantria dispar showed a longer development time at high density compared to low density. Yu et al. (2019) Diabrotica virgifera virgifera (LeConte reported that female adult moth Diabrotica virgifera virgifera derived from larvae reared at high density had a further dispersal ability than moths derived from larvae reared at low density. The effect of density on morphology was reported in the migratory locust Locusta migratoria (Nijhout and Wheeler, 1982), aphid Acyrthosiphan pisum (Tsumuki et al., 1990), and Anticarsia gemmatalis (Fescemyer and Hammond, 1986).

Samia cynthia ricini Boisduval (Lepidoptera: Saturniidae) is economically valuable insect for humans (Hani and Das, 2019). The fifth instar caterpillar larva can produce silk fibres that envelop the pupa, which can be utilized as the main material of the textile industry, surgical threads, parachutes, and others. Silkworm fibres have a special feature that has not been replaced by artificial one to date (Nuraeni and Putranto, 2008). Silkworms have long been cultivated by humans such as mulberry silkworm *Bombyx mori*. However, the *S. c. ricini* silkworm is a new type of silkworm cultivated in Indonesia (Trisnawati and Nurkomar, 2020). The main host plants are castor and cassava as their secondary host plant (Nangia et al., 2000). Although the resulting silk is very different from mulberry silk, the consumer interest in samia silk is quite high.

In our previous pilot study, high mortality was found in solitary-reared early instar larvae, but when S. c. ricini were reared at a density of 30, they could live successfully into adulthood (Nurkomar et al., 2022). We hypothesized that the larvae of S. c. ricini have a gregarious behaviour during their larval stage. However, there hasn't been information about the minimum larval population that can support the survival of S. c. ricini. Research on the effects of S. c. ricini population density has been carried out but is limited to the parameters of mortality rates, larval and the resulting cocoons weights. Meanwhile, how population density affects the development and reproductive ability of S. c. ricini has never been reported. These parameters are very important for the efficiency of mass rearing purposes. This study aimed to determine the minimum population limit of larvae that can support the survival of S. c. ricini by observing the development time, fecundity, and hatchability of eggs produced by adult moths

under various conditions of different larval population densities.

# MATERIALS AND METHODS

The most effective diet for supporting the development of S. c. ricini was castor bean (Ricinus communis) (Devaiah et al., 1985). Leaves were collected from the neighbourhood around campus of Universitas Muhammadiyah Yogyakarta, Bantul, Yogyakarta, Indonesia. The leaves were then dried with sterile tissue paper before usage after being cleaned with tap water. Because each instar has a unique physical trait and feeding behaviour, the food was supplied in varying amounts, depending on the larvae's development stage. For the first and second instar larvae, the diet was administered once daily in the morning; for the third and fourth instar larvae, it was administered twice daily in the morning and afternoon; and for the fifth instar larvae, it was administered three times daily in the morning, afternoon, and evening.

The research was started by preparing eggs according to the population density treatments tested i.e., 1, 5, 10, 20, 30, 40 and 50 eggs. The density used was determined based on the preliminary studies that found that S. c. ricini could not be reared solitary. Thus, rearing experiments were carried out under various conditions of different larval population densities. This number was also determined based on the availability of larvae and materials used in rearing. Each treatment was replicated five times. Eggs were kept on a petri dish (86 x 13 mm) until hatching. When hatching, the larvae were transferred to the larval rearing container. To reduce the impact on the environment, identicalsized containers were used. The rearing container used for the first to fourth instar larvae was a tray with a transparent lid (length: 30 cm, width: 11.5 cm, height: 3.5 cm). The larvae were moved into an adult rearing cage when they reached the fifth instar (length: 37 cm, width: 30, height: 33 cm) to facilitate the pupation process. For the moth to emerge with perfect wings, the constructed cocoon was then strung up using thread in a sagittal plane position. Observations were made on the survival rate, the development time of each phase (egg, larva, pupa, and adult), larval body colour, cocoon shell weight, adult fecundity, and hatchability of the egg produced by the adult. The development time was observed daily from the egg until adult died. Observations of the larval body colour were made at each instar change. Cocoon shell was measured after the moth emerged. Adult fecundity was calculated out

by calculating the number of eggs laid by the adult daily until death. Hatchability of eggs was observed from 30 eggs taken randomly from each treatment.

Survival data were analysed with Kaplan Meier's Survival Analysis (May, 2009). The data of the development time, cocoon weight, fecundity, and hatchability of eggs were analysed using the Generalized Linear Model (GLM). The appropriate model used for the related data was determined by using Akaike Information Criteria (AIC) value. The difference in the average value of the data was further tested using Tukey HSD at the level of  $\alpha = 5\%$  with Holm>s adjustment (Hothorn et al., 2008). Statistical analysis was performed using R Statistics and data visualized using ggplot package (Wickham, 2009). Because no larvae grew in the treatment at densities 1, 5, and 10, the observational data were excluded from the analysis. Data analysis was performed using R Statistics version 4.0.2 (R Core Team, 2015).

# **RESULTS AND DISCUSSION**

The results showed that in completing its life cycle, S. c. ricini had a gregarious behaviour during the larval stage. It is crucial to know the lowest population density while propagating S. c. ricini since it can be employed for mass propagation activities, especially for small-scale farmers and lab-scale research. The results showed that population density affected the survival rate of S. c. ricini (P<0.0001, Fig. 1). The highest survival rate was observed in larvae reared at a density 30, with 92% survival rate. The survival rate decreased to 87.5% and 83.2% at a density of 40 and 50. respectively. Meanwhile, the lowest survival rate was observed at a density of 20, which only reached 78%. When the larvae reached the fifth instar, the survival rate generally started to decline. Larvae reared at densities of 1, 5, and 10 died at the beginning of the first instar. The minimum population density for S. c. ricini was 20 larvae. However, to obtain optimum survival rate, larval propagation should be carried out at a population density of at least 30 larvae. The amount of density needs to be adjusted to the size of the rearing container used so that the population is not less or excessive because population density can affect the survival rate of larvae. Diamantidis et al. (2020) reported that high larval density decreased the survival rate of larvae and pupae of the fruit fly Tephritidae short-lived biotype. Hooper et al. (2003) also stated that an increase in larval density decreased the survival rate of Chironomus riparius larvae. Larval stage is particularly susceptible to population density. This stage is the main stage in obtaining nutrients from feed. High density increases intraspecific competition that limits resources for each individual so that it can affect the development time as exemplified in the larvae of Drosophilae fly (Klepsatel et al., 2018).

Population density affected development time of *S. c. ricini* eggs (glm  $F_{6.28}$ =255.39; P=2.2e-16). The development time of *S. c. ricini* eggs reared at a density of 30 was a day faster (6.49 days) than the egg development time in other treatments (Fig. 2). Meanwhile, larvae (Im F<sub>3.16</sub>=2.774, P=0.075) developed in relatively the same time. The larva developed in 17-30 days (an average of 19-20 days). It was reported that population density can also affect insect morphology. For example, Plusia gamma larvae have little colour variation, which tend to be brightly coloured in solitary conditions and tend to be more diverse with dark colours in gregarious conditions (Long, 1953). In this study, there was no effect of population density on the difference in body size of each larval instar or body colour. However, the larvae of S. c. ricini have various body colour. The larvae of the fifth instar of S. c. ricini have six different shades of colours, such as plain,



Fig. 2. Developmental time of *Samia cynthia ricini* at different population densities. Mean followed by the same letter were not significantly different based on Tukey's test ( $\alpha$ =5). S.E. Standard Error

small and large speckled with a basic body colour of white and green tosca, respectively. Natch et al. (2017) mentioned that despite the phenotype variations in the body of *S. c. ricini* larvae, the genetic diversity was quite low. Appearance of colour natural populations may have polymorphisms because of biased mutation, pleiotropy and trade-offs, gene flow, variable selection through time and/or space, and negative frequencydependent selection that can fend off genetic drift's loss of diversity (Gray and McKinnon, 2007; Majerus, 1998; McKinnon and Pierotti, 2010; Punzalan et al., 2005) where individuals of a rare type (i.e. a rare morph or a rare species).

Population density had no effect on the formation and development time of pupae (glm  $F_{316}=0.4289$ ; P=0.7351). Pupae developed for  $\pm 16$  days at each treatment. The formation and development of pupae in each density treatment tended to be the same since all density treatments examined in this study employed a room with the same temperature and humidity. Temperature and humidity are factors affecting the formation of the pupa of S. c. ricini (Vaidya et al., 2014). Hussain et al. (2011) also reported that temperature and humidity affected the formation of Bombyx mori pupae and other insects, such as Aedes aegypti (Yahya et al., 2019) and Stomoxys spp. (Issimov et al., 2020). Nevertheless, all the observed larvae developed into pupae, resulting in cocoons with considerably different weights for each population density examined (Im  $F_{3,16}$ =28.48; P=1.187 e-06). The pupa from the larvae reared at densities of 20 and 30 had a cocoon shell that weighed 0.3 g, while the pupa from the larvae reared at densities of 40 and 50 had a cocoon shell that weighed 0.25 g. (Fig. 3). Cocoon shell weight is a crucial factor in the effective production of silkworms, as it will have an impact on yields and selling prices (Winardi, 2021). Cocoon shell weight is influenced by the ability of the larvae to produce silk fibre-forming proteins during the fourth and fifth instar (Cholifah et al., 2012). The results showed that the heaviest cocoon weights resulted from larvae reared at densities of 20 and 30, with an average cocoon weight of 0.30 g and 0.32 g, respectively. Meanwhile, the density of 40 and 50 resulted in the same average cocoon weight, which was 0.25 g. Cocoon fibres are produced from protein molecules. As was the case with *B. mori*, high density may prevent protein function and cause its weight to decrease. (Putro et al., 2016). In addition, Vaidya et al. (2014) reported that the weight of the S. c. ricini cocoon was affected by temperature and humidity in the rearing container.



Fig. 3. Cocoon shell weight of *Samia cynthia ricini* at different population densities. Mean followed by the same letter were not significantly different based on Tukey's test ( $\alpha$ =5). S.E. Standard Error

The population density also had no significant effect on adult longevity (lm  $F_{316}$ =3.153; P=0.0538). The entire pupa developed into an adult moth that lived between two and six days. Some adults produced by a density of 50 had damaged wings. This might occur because of the adult not having enough room as it emerges from the cocoon, which affects the wing's irregular shape. However, population density significantly affected the fecundity of adult female moths ( $\lim F_{3.16}$ =3.931; P=0.028). The fecundity of adult females increased as the population density increased. Adult females derived from larvae reared at densities of 30 and 40 could produce more eggs than those reared at a density of 20 or 50 (Fig. 4). Park et al. (2016) also reported similar result in which the fecundity of adult females of Hermetia illucens increased as density increased. In contrast, Xing et al. (2021) reported that the fecundity of Sitobion avenae decreased along with the decrease in the population density.

The oviposition by adult females was also influenced by the interaction of the population density and females age (lm F<sub>23.96</sub>=4.625; P=4.976 e-08). Based on the pattern of daily oviposition, females often exhibit low reproductive rates, with a tiny initial egg production. However, it gradually increases to the maximum (more eggs are laid) and then decreases again (Fig. 5) as a result of physiological degradation at the end of life, or what is known as reproductive senescence (Bradshaw and McMahon, 2018). Adult females descended from larvae reared at a density of 50 did not exhibit this pattern in their daily oviposition, even though fewer eggs were deposited on the final day. Finally, there was no difference in the hatchability of eggs produced by female at all density treatments tested ( $\lim F_{3.16}$ =1.277; P=0.3159). A total of 75-90% of eggs could hatch (Fig. 6).

In conclusion, population density affects the survival of *S. c. ricini*. A density of 30 is the minimum population density to support the success of *S. c. ricini*'s life. *S. c. ricini* reared at a density of 30 had the highest survival rate, faster egg development time, heavier cocoon shell weight, and highest fecundity.







Fig. 5. Adult daily fecundity of *Samia cynthia ricini* at different population densities. A. 20 density, B. 30 density, C. 40 density, and D. 50 density



Fig. 6. Hatchability of eggs produced by adult Samia cynthia ricini at different population densities. Mean followed by the same letter were not significantly different based on Tukey's test ( $\alpha$ =5). S.E. Standard Error

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# AUTHOR CONTRIBUTION STATEMENT

IN and DWT contribute to conceptualization, methodology, data analysis and research supervision. FKW performed the experiments. FKW and IN write the original manuscript, IN and DWT reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

#### **CONFLICT OF INTEREST**

No conflict of interest.

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