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Effect of Temperature under Continuous Darkness Photoperiod on Egg Hatching and Embryonic Development of Eri Silkworm (*Samia cynthia ricini*)

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Abstract
Background. The eri silkworm (*Samia cynthia ricini*) is a non-mulberry silkworm with considerable potential for natural silk production. Unlike some silkworm species, *S. c. ricini* eggs do not undergo obligatory diapause and generally hatch within 8–10 days after oviposition, creating challenges in synchronizing egg hatching with distribution and rearing schedules.
Methods. This study evaluated the effects of temperature under a continuous-darkness photoperiod (24D:0L) on hatching time, hatching delay, hatchability, and embryonic development of *S. c. ricini*. A total of 1,163 fertile eggs were assigned to four temperature treatments: control, 15 °C, 25 °C, and 30 °C, with three replicates per treatment. Temperature and relative humidity were monitored throughout the experimental period, while hatching performance and embryonic development were observed periodically.
Result. The results showed that temperature significantly affected hatching delay and hatchability ($P<0.05$). Eggs maintained at 15 °C exhibited the longest hatching period (23.96±0.07 days) and a hatching delay of 15.96±0.07 days, although hatchability was relatively low (9.68±9.13%). At 25 °C, eggs hatched within 10.21±0.76 days with a hatchability of 22.55±19.13%, while embryonic development proceeded normally. In contrast, exposure to 30 °C resulted in extremely low hatchability (0.91%) and considerable embryonic damage. Embryological observations further demonstrated that low temperature temporarily arrested development, whereas high temperature adversely affected embryo survival.
Conclusion. These findings indicate that temperature manipulation under continuous darkness can regulate the timing of eri silkworm egg hatching. A temperature of 15 °C effectively delays hatching, whereas approximately 25 °C provides more favorable conditions for subsequent embryonic development and larval productivity. This approach may improve synchronization of egg distribution, incubation, and eri silkworm production management.

Keywords: embryonic development; eri silkworm; hatchability; hatching delay; photoperiod; temperature

INTRODUCTION

Indonesia is known as a country rich in natural resources. Biodiverse and non-biodiverse resources drive the country's economy; one sector is the textile industry. Silk textile products are an example of natural textile products derived from silkworm cocoon

fibers. Economic development through silkworm cultivation in Indonesia has clear prospects. Therefore, communities in Indonesia can develop silkworm cultivation to produce quality silk products. Silk is a natural material with various advantages, especially in strength, durability, and high aesthetic value. Keten *et al.* (2010) stated that silk yarn has high mechanical strength and stretch, making it one of the strongest natural fibers. These advantages make silk not only commercially valuable but also have great potential to support the economy through its extensive processing and distribution.

Silk natural fiber textile products are divided into two types based on the silkworm feed: mulberry fiber and non-mulberry fiber (Endrawati, 2017). Mulberry fiber comes from the mulberry leaf-eating silkworm, namely Bombyx mori, while non-mulberry fiber comes from the non-mulberry caterpillar *Samia cynthia ricini*. Non-mulberry caterpillar types such as *S. c. ricini* are fed on castor leaves (*Ricinus communis* L.) and cassava leaves (*Manihot* sp.). However, the feed that is generally used by *S. c. ricini* silkworm farmers in Indonesia is cassava leaves (*Manihot* sp.) (Arinda, 2022). S. *c. ricini* silkworms can eat various types of plants or are *polyphagous*, which have primary feed and secondary feed (Lisa, 2019).

Silkworm S. c. ricini is an insect from the order Lepidoptera, the family Saturniidae, which undergoes a complete metamorphosis. Silkworm *S. c. ricini* metamorphoses from eggs, larvae, pupa, and then becomes moths (butterflies). Silkworm cultivation aims to produce silk fibers from the cocoon stage, which are used as yarn and converted into textile products in the form of silk fabric. The need for silk cloth continues to increase year to year, but production continues to decline. Indonesia's natural silk production is still very low; dry cocoon production only reaches 250 tons year-1, while the current national cocoon demand is 2400 tons year-1, so that the fulfillment of cocoon needs in Indonesia still requires imports from abroad (Elpisah, 2019). Low silkworm productivity is influenced by several factors, including genetic, environmental, and technical factors in the maintenance process (Nursita, 2011).

Silkworm S. c. ricini, also known as silkworm eri, is one of the most exploited, domesticated, and commercialized non-mulberry silkworms (Shifa *et al.*, 2014). Silkworm eri is one of the silkworm types widely cultivated in Indonesia. Therefore, silkworm eri cultivation has spread to several regions, including Kulon Progo, Wonogiri, Malang, and Bali (Lisa, 2019).

The S. c. ricini *moth* is able to produce ±300 eggs in one life cycle, which is about 46 days. In one life cycle, the silkworm eri consists of several phases, including 10 days of the egg phase, 5 days of the instar larval phase I, 3 days of instar II larvae, 2 days of instar III larvae, 3 days of instar IV larvae, 6 days of instar V larvae, 17 days in the pupa phase, then laying eggs for 2-3 days in the moth phase (Das and Das 2018). S. *c. ricini* silkworm eggs do not have a diapause phase, so eggs begin to hatch on the 8th day after oviposition. Based on this, controlling the hatching of thorny silkworm eggs is needed to help distribute and control hatchability.

One way to control silkworm egg hatching is the photoperiod method. The photoperiod method is light control by regulating the length of dark and light time on an object in one day. Controlling the hatching of silkworm eggs is carried out by providing dark and light periods of a predetermined length. Light intensity (photoperiod) can control the length of hatching time by affecting the diapause phase in insects (Narasimhulu *et al.,* 2020). Diapause is an adaptation that insects use to survive unfavorable environmental conditions, characterized by decreased metabolic activity. This process can occur at the embryonic, larval, or pupal stages (Russpita, 2023). Therefore, a significant method is needed to control the hatching of thorny silkworms; the photoperiod method can control the length of hatching time.

Photoperiod is carried out with a variety of irradiation periods, including a 24D (dark):0L (light) cycle, which uses 24 hours of light with a specific light intensity (*lux*). In this case, temperature in the photoperiod method is determined to find the optimal temperature for best hatching. Temperature greatly affects egg-hatching success. The ideal temperature for the hatching of *S. c. ricini* silkworm eggs ranges from 24-28 °C with a humidity of 70%-85% (Joan *et al.,* 2022; Russpita, 2023). Temperature and humidity that do not match silkworms' ideal needs will decrease appetite, weaken larvae and make them susceptible to disease, and stunt growth (Purnamasari, 2021). Therefore, a study is needed to examine the optimal temperature for hatching S. c. ricini silkworm eggs, including by providing treatment at temperatures of 15 °C, 25 °C, and 30 °C, and comparing with controls.

METHODS

Time and Place

This research was conducted from August to September 2024 at the Natural Silkworm Laboratory, Department of Animal Production Science and Technology, Faculty of Animal Husbandry, IPB University, and the Laboratory of Biosystematics and Animal Ecology, Faculty of Mathematics and Natural Sciences, IPB University.

Tools and Materials

The tools used in this study are maintenance boxes/trays, petri dishes, pipettes, preparation glass, paranet nets, rubber ropes, cotton, salt, *seriframes*, maintenance racks, *thermohygrometers*, 30 °C ovens, *Olympus CX33* microscopes, small bottles, lamps, batteries, 0.00 mm digital calipers, 0.00 g digital scales, labels, *counters*, stationery, cleaning tools, and Minitab version 22. The ingredients used are *Samia cyinthia ricini* silkworm eggs (1163 eggs, cassava leaves (Manihot utilissima), 70% alcohol solution, and NaOH 5M solution.

Working Procedure

Preparation of the Egg Maintenance and Selection Room

The room and all equipment used are cleaned and disinfected to avoid contamination by feces or disease-causing bacteria that can reduce silkworm mortality. Each leg of the maintenance rack is given a container filled with oil to prevent predators or other insects from climbing onto the rack and interfering with the maintenance process. Then each box is placed in 3 Petri dishes as a hatching medium with 4 treatments and 3 repeats. In addition, each maintenance box is given saline solution to neutralize moisture, and the box is closed to protect the silkworm eggs from external threats.

A total of 1163 fertile eggs were selected for use in 4 photoperiod treatments of silkworms in the study. After the moth lays eggs, the eggs are left to rest for 1-2 days or about 36-40 hours. This period is necessary to ensure early embryo development, and treatment of the new eggs can be given after this period. Thus, at the start of treatment, the eggs are 3 days post-oviposition. Silkworm eggs are selected to ensure the egg samples used are fertile. The physical characteristics of fertile silkworm eggs are perfectly oval, not wrinkled, cream-colored, and attached to each other; brown or black egg characteristics indicate that the eggs are dead, for eggs that do not stick to each other indicate problems in the hatching process, eggs with these characteristics cannot be used in the study (Das and Das 2018).

Photoperiod Treatment

The photoperiod treatment is light (*light* / L) and dark (*dark* / D). The treatment (24D:0L) does not require light intensity (*lux*) because in this treatment each test box is not given light and is conditioned to be completely dark. This observation was made based on Narasimhulu *et al.* (2020), which found that photoperiod treatment with 0 light hours and 24 dark hours (24D:0L) can cause a delay in hatching in *S. c. ricini silkworm eggs* as well as in light treatment (24D:0L). Observations at light intensity (24D:0L) were carried out at 4 temperatures: control, 15 °C, 25 °C, and 30 °C. During the observation, the temperature and humidity of each maintenance box were observed. The air temperature and humidity were recorded every morning and evening using a *thermohygrometer*. Controlling the temperature to keep it stable is by putting cotton that has been soaked in water on the edge of the maintenance box.

Observations were carried out for one month, and egg development was observed every day. Measurements were taken for temperature and humidity control twice a day, namely in the morning (07.00-09.00 WIB) and in the afternoon (15.00-17.00 WIB), using a thermometer and hygrometer.

Observed Variables

Hatchability

Hatchability is calculated as a percentage: the number of eggs hatched during hatching divided by the number of eggs treated. According to Russpita (2023), the hatching percentage can be measured by:

$$\text{Hatchability (\%)} = \frac{\text{Number of eggs hatched}}{\text{Number of fertile eggs}} \times 100\%$$

Average Hatching Time and Hatching Delay

The average hatching delay time is the time from egg treatment to hatching, generally 8 days. According to Russpita (2023), the average hatching time and delay time can be calculated by:

$$\text{Average hatching time} = \frac{\text{Time x Number of hatching eggs day} - \text{n}}{\text{Number of fertile eggs}}$$

$$\text{Delay hatching time} = \text{Average hatching time} - \text{Average time Hatching Controle}$$

Larval Mortality

One of the indicators of success in silkworm maintenance is the mortality rate or mortality of silkworms. Good silkworm productivity is indicated by low mortality. Mortality is calculated from the number of caterpillar deaths since hatching at each different temperature. The formula for calculating silkworm mortality is as follows.

$$Bi = \frac{xi - zj}{xi} \times 100\%$$

Description:
Bi = mortality at the i instar (%)
xi = number of caterpillars living on the first instar (tail)
zj = the number of caterpillars living on the jth instar (tail)

RESULTS AND DISCUSSION

Temperature and Humidity

Temperature and humidity are highly determinant of success in egg hatching. The temperature and humidity control treatments of 15°C, 25°C, 30°C, and ambient temperature are listed in Table 1.

Table 1 Average temperature and humidity of hatching eggs of silkworm *Samia cynthia ricini* in the control treatment, temperatures of 15°C, 25°C, and 30°C

Treatment	Variables	Time		Average±SB
		Morning	Afternoon	
Controls	Temperature (°C)	27.00±1.72	27.71±1.02	27.36±1.37
	Humidity (%)	58.43±2.94	54.57±5.22	56.5±4.08
15 °C	Temperature (°C)	17.06±2.80	15.16±1.04	16.11±1.92
	Humidity (%)	59.94±5.51	52.50±6.43	56.22±5.97
25 °C	Temperature (°C)	23.86±0.52	24.09±1.07	23.98±0.79
	Humidity (%)	57.63±2.77	58.88±5.19	58.25±3.98
30 °C	Temperature (°C)	29.55±0.44	-	29.55±0.44
	Humidity (%)	64.85±2.19	-	64.85±2.19
Environment	Temperature (°C)	26.82±1.46	27.07±0.93	26.95±1.19
	Humidity (%)	57.15±4.74	54.75±3.94	55.95±4.34

SB = standard deviation, Control = no photoperiod treatment

The average temperature and humidity during the egg hatching process in this study were control (27.36±1.37 °C and 56.5±4.08%), 15 °C treatment (16.11±1.92 °C and 56.22±5.97%), 25 °C treatment (23.98±0.79 °C and 58.25±3.98%), 30 °C treatment (29.55±0.44 °C and 64.85±2.19%), and environmental (26.95±1.19 °C and 55.95±4.34%) (Table 1). The ideal temperature for hatching *S. c. ricini* silkworm eggs ranges from 24-28 °C with a humidity of 70%-85% (Joan *et al*., 2022; Russpita, 2023). Punyavathi *et al*. (2022) reported that egg storage at too high or too low temperatures, as well as low humidity, can affect the embryo development of *S. c. ricini silkworm embryos* and affect the diapause phase.

The control temperature includes the ideal temperature needed for *S. c. ricini silkworm eggs* (27.36±1.37 °C), so the results of the control treatment can be compared with the hatching results of each treatment at different temperatures. The control temperature is different from the ambient temperature. Ambient temperature is the temperature around the research medium or larval rearing, while control temperature is the treatment temperature derived from ambient temperature. The control treatment did not receive a photoperiod treatment; therefore, the control temperature was not much different from the ambient temperature around the rearing, namely (26.95±1.19 °C). Temperature and humidity control is carried out because *S. c. ricini* silkworms are non-mulberry silkworms that are *poikilothermal*, so environmental conditions such as temperature, humidity, and feed will greatly affect the productivity of silkworms (Ramadani 2021). Temperatures that are not in accordance with the ideal temperature for hatching *S. c. ricini* silkworm eggs can make the embryo development metabolism stop, and this can delay egg hatching (diapause). Temperature treatments of 15 °C, 25 °C, and 30 °C are carried out to determine the temperature that can extend the hatching time of *S. c. ricini silkworm eggs.*

Average Hatching Time, Hatching Delay, and Hatchability

The average hatching time, hatching delay time, and hatchability of the photoperiod 24D:0L at the controls, temperatures of 15 °C, 25 °C, and 30 °C are shown in Table 2.

Table 2 Average hatching time, hatching delay time, and caterpillar hatchability silk *Samia cynthia ricini* on control treatment, temperature 15°C, 25°C, and 30 °C

Treatment	n	Hatching Time (day)	Time Procrastination (day)	Hatchability (%)
		Average±SB	Average±SB	Average±SB
Controls	323	7.72±0.22	0.28±0.22c	32.99±5.88a
15 °C	234	23.96±0.07	15.96±0.07[A]	9.68±9.13b
25 °C	320	10.21±0.76	2.21±0.76b	22.55±19.13b
30 °C	286	8.00±0.00	0.00±0.00c	0.91±0.00b

The number followed by different letters in the same column differs significantly (P<0.05); n = the number whole fertile eggs all repeated, SB = standard deviation, Control = no photoperiod treatment

The normality and Kruskal-Wallis tests on average egg hatching time showed no significant differences between treatments (P>0.05). The longest average egg hatching time was in the 15 °C treatment (23.96±0.07 days; Table 3). The average hatching time at the controls, temperature 25 °C, and temperature 30 °C were included in the effective hatching time of *S. c. ricini silkworm eggs*, which was the 8th to 10th day after oviposition (Arinda, 2022). Meanwhile, the one-way ANOVA and Tukey's follow-up test at the time of delay showed differences between treatments (P<0.05). In this study, a temperature lower (15 °C) than the optimal hatching temperature of silkworm eggs can extend the hatching time or can delay hatching by up to 15.96±0.07 days. Meanwhile, at the control and temperature of 30 °C, there was no additional day, and it can be interpreted that the temperature in these two treatments did not affect the diapause phase in *S. c. ricini* silkworm eggs. The results of this study indicated a significant difference (P<0.05) in the hatchability *of S. c. ricini eggs* at different temperatures. The control treatment resulted in a higher hatchability percentage than the other treatments, at 32.99±5.88% (Table 2). The low hatchability percentage was likely due to temperature and humidity. As with the 15 °C treatment, the lowest temperature among the treatments, it had a low hatching percentage (9.68±9.13%). The 30 °C treatment had the lowest hatchability percentage (0.91±0.00%). This temperature exceeded the ideal hatching temperature of silkworm eggs.

Murali and Singh (2022) state that maximum egg hatching generally occurs on the 10th or 11th day after oviposition. According to Russpita (2023), the time required for egg hatching is calculated starting from the time when the eggs of *S. c. ricini* are not treated. Observations are made for 25 days; if no eggs hatch during that period, observations will be stopped. Hatching time is related to the diapause phase that can occur in silkworms, where unfavorable environmental factors, such as inappropriate

temperatures, can affect the diapause process and, in turn, hatching time and egg hatching success. S. *c. ricini eggs* in the tropics generally hatch between the 8th and 10th day after oviposition (Arinda, 2022). The hatching time is much different from the effective time the eggs hatch due to the temperature and humidity of *the S. c. ricini eggs.*

Applying a temperature of 15 °C to the 24D:0L photoperiod showed significant results in affecting the diapause phase in the hatching of *S. c. ricini* silkworms. This is supported by Arinda (2022), who states that egg storage at low temperatures causes egg hatching time to be longer. Egg storage at low temperatures can slow down the egg hatching process. Rahmathulla's (2012) research shows that continuous dark conditions in the 24D:0L photoperiod can affect egg development and delay hatching (diapause).

The 24D:0L photoperiod control in this study had the highest hatchability, at 32.99±5.88% (Table 2). The 24D:0L treatment in the previous study showed significant hatchability results: in the Russpita (2023) study, hatchability was 78.66±17.47%, and in the Maretha (2024) study, hatchability in the 24D:0L treatment was 95.33±1.15%. Rahmathulla (2012) revealed that silkworm eggs are photosensitive and tend to move towards dimmer light. Narasimhulu (2022) revealed that the hatching of silkworm eggs at a light intensity of 24D:0L results in the hatching of silkworm eggs during the transition from dark to light, so that light is one of the factors that trigger hatching.

Exposure to 24D:0L light in the study can increase the humidity in the hatching medium. This is because longer bright periods keep the eggs constantly exposed to the lamp's heat and can reduce humidity in the surrounding environment (Russpita, 2023). Humidity in all treatments is not ideal for hatching thorny silkworms. This condition is caused by osmotic stress due to overly low or high temperatures. As a result, the egg's internal liquid binding stops, leading to dehydration, cell damage, and an inability to metabolize (Sari, 2014). Too high humidity can hinder egg hatching, while too low humidity can lead to embryo death due to dehydration or lack of water (Subrata *et al.*, 2013).

Embryo Storage

The duration of hatching of *S. c. ricini* eggs under photoperiod 24D:0L in the control treatment occurred for 7.72±0.22 days. Egg development from day 3 to day 4 (Figure 1, a1): the egg entered the germband phase I, where germinal bands began to separate from the surface of the egg; on day 5 (Figure 1, d), the egg entered the *germband*

III phase. The *germband* III phase is called *the spoon-shaped* stage or *spoon-like germband*. From the 6th to the 8th day (Fig. 1, j and h), the embryo formed setae, indicating that trichogenic cells had formed. On the 9th day (Fig. 1, k and l), the embryo has developed a spiracle, head pigmentation, and body pigmentation.

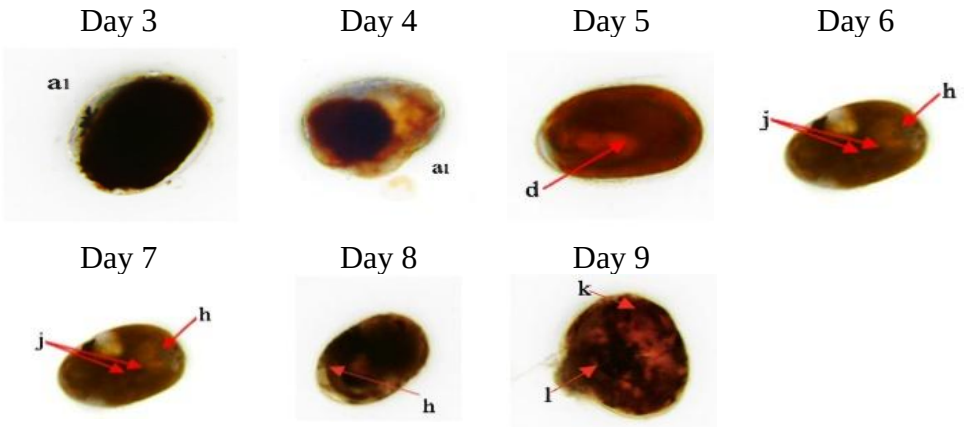


Figure 1 Egg development *Samia cynthia ricini* in photoperiod phase 24D:0L With control temperature: (a1) 3- to 4-day-old eggs, (d) 5-day-old eggs, (j h) 6- to 7-day-old eggs, (h) 8-day-old eggs, (k l) 9-day-old eggs. The image description is as follows: (a1) the germenlage separates from the surface of the egg, (d) the *protocephalon* and *protocorm* begin to separate, which is *the spoon-shaped* stage or *spoon-like germband*, (j) the embryo has formed a spiracle, (h) satae, (k) head pigmentation, (l) body pigmentation.

The hatching duration of *S. c. ricini* photoperiod 24D:0L eggs at 15 ℃ treatment occurred for 23.96±0.07 days. Egg development at 15 ℃ treatment on day 3 to day 14 (Figure 2, a1) the egg enters *the germband* phase I where germanlage begins to separate from the surface of the egg and in this phase the egg undergoes a diapause phase which is characterized by a cessation of metabolism in the egg, on day 15 (Figure 2, b) the separation of *the protocorm* and *protocephalon* occurs. From the 16th to the 17th day (Figure 2, e), the embryo forms an "S-shaped" stage; on the 18th day (Figure 2, d), the *germband* III phase is called *the spoon-shaped stage*. On the 19th day, the embryo resembles a circular shape; then, from the 20th to the 21st day, the limbs on the egg begin to appear, and from the 22nd to the 23rd day, the pigmentation of the head begins to appear. On the 24th day, the egg has perfect pigmentation.

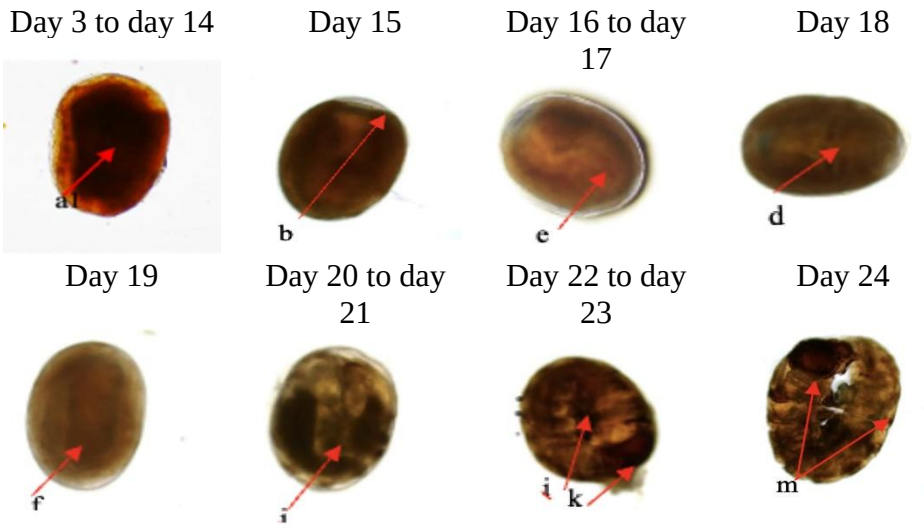
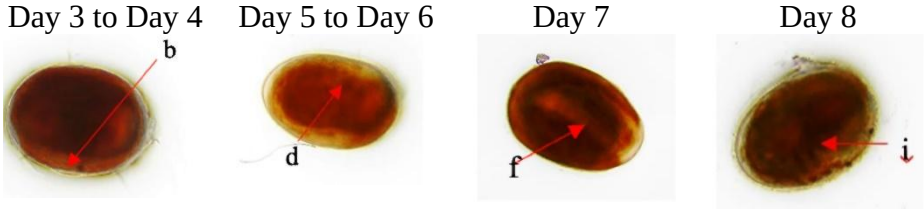


Image 2 Egg development *Samia cynthia ricini* in photoperiod phase 24D:0L with a temperature of 15 °C: (a1) eggs are 3 to 14 days old, (b) there is separation of *the protocorm* and *protocephalon* on the 15th day, (e) eggs are 16 to 17 days old (d) eggs are 18 days old, (f) eggs are 19 days old, (i) eggs are 20 to 21 days old, (i k) eggs are 22 to 23 days old, and (m) eggs are 24 days old. The description of the image is as follows: (a1) the germenlage separates from the surface of the egg, (b) the separation of *the protocorm* and *protocephalon*, (e) the embryo is s-shaped, (d) the *protocephalon* and *protocorm* begin to separate, i.e. *the spoon-shaped* stage or *germband* resembles a spoon, (f) the embryo resembles a circle shape, (i) the limb, (k) the pigmentation of the head, (m) the pigmentation of the perfect.

The hatching duration of *S. c. ricini* eggs under photoperiod 24D:0L at 25 °C treatment occurred for 10.21±0.76 days. Under 25 °C treatment, egg development from day 3 to day 4 (Figure 3, b) involves separation of the protocorm and protocephalon; from day 5 to day 6 (Figure 3, d), *germband* phase III is called *the spoon-shaped* stage or *spoon-like germband*. On days 7 and 9 (Figure 3, f), blastokinesis occurs, characterized by the embryo forming a circle; on day 8 (Figure 3, i), limbs begin to appear on the egg. On the 10th day, the embryo forms an "S-shaped". At 25°C, embryonic development proceeds normally, with hatching time ranging from 8 to 10 days.



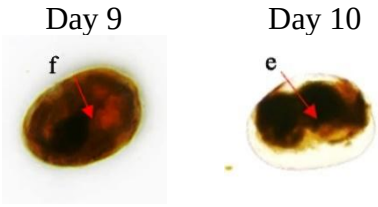


Image 3 Egg development *Samia cynthia ricini* in photoperiod phase 24D:0L with a temperature of 25 °C: (b) 3 to 4 day old eggs, (d) 5 to 6 day old eggs, (f) 7 day and 9 day old eggs, (i) 8 day old eggs, (e) 10 day old eggs. The description of the image is as follows: (b) the separation of *the protocorm* and *protocephalon*, (d) the separation of *the protocephalon* and *protocorm* begins to occur, namely *the spoon-shaped* stage or *germband* resembles a spoon, (f) the embryo resembles a circle shape, (i) the limb, (e) the embryo is s-shaped.

The duration of hatching *of S. c. ricini* eggs under photoperiod 24D:0L at 30 °C treatment occurred for 8.00±0.00 days, but in the embryo development study in this treatment, it was carried out until the 17th day. This is because the 3rd, 7th to 9th day, and 12th to 13th day of the embryo could not be observed, or there was damage to the embryo. Egg development at 30 °C treatment on day 4 (Figure 4, a1): the egg enters the germband phase I, where germanlage begins to separate from the surface of the egg; on day 5 (Figure 4, b), the protocorm and protocephalon separate. On day 6 (Figure 4, k, l, j), the embryo has formed a spiracle, and pigmentation of the head and body is visible; on the 10th and 1st days, pigmentation of the head and body of the larvae is seen. Day 12 is a damaged embryo that cannot be observed, as well as the observation of embryos on day 3, days 7 to 9, and days 12 to 13. On days 14 to 17, the embryo is already in a state of perfect pigmentation.

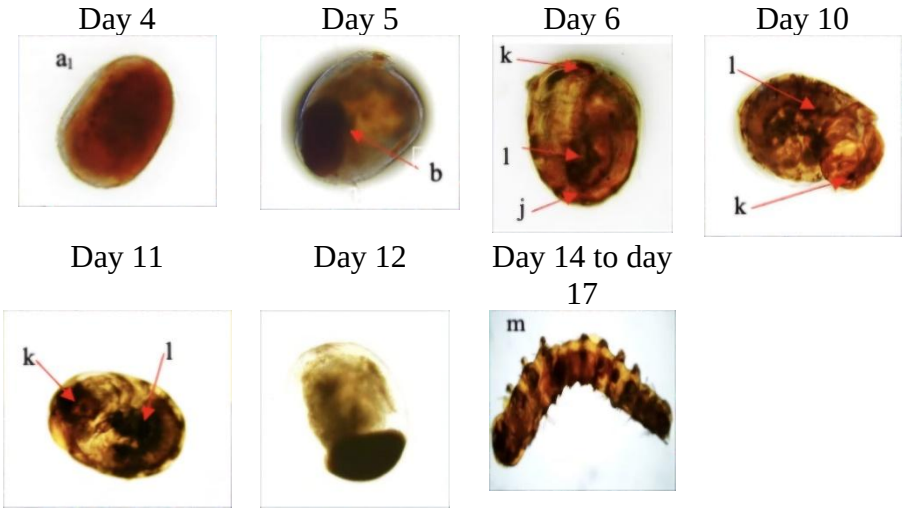


Image 4 Egg development *Samia cynthia ricini* on a photoperiod of 24D:0L with Control temperature: (a1) 4-day-old eggs, (b) 5-day-old eggs, (k l j) 6-day-old eggs, (l k) 10-day-old eggs, (k l) 11-day-old eggs, (m) 14 to 17-day-old eggs. The image description is as follows: (a1) germenlage separates from the

surface of the egg, (b) separation of *protocorm* and *protocephalon*, (j) embryo has formed a spiracle, (k) head pigmentation, (l) body pigmentation, (m) perfect pigmentation.

The embryo of *the silkworm Samia cynthia ricini* develops for 9-10 days (Elumalai *et al.,* 2021). Observation of the embryo is carried out after soaking the egg in a solution of 5 M NaOH; this is done so that the chorion, or the outermost membrane surrounding the embryo, becomes transparent and the nucleus of the egg can be seen. During observation, some embryos were found to be broken or damaged.

Embryonic development in silkworms takes place through a number of complex and coordinated stages. The process begins with fertilization, the union of the sperm and ovum cells that produces the zygote, which occurs about two hours after oviposition (Saheb *et al.,* 1990). About 10 hours after oviposition, the embryo undergoes an initial division that includes division of the nucleus as well as the cell membrane. Subsequently, the embryo enters *the germband* I phase, characterized by the separation of the germband from the surface of the egg, followed by germband II, when the protocephalon and protocorm begin to separate, and germband III, when the two structures become increasingly apparent. In the *germband* IV stage, the digestive tract begins to form (Miya, 2003). Development continues with the enlargement of the embryo, the separation of the mesoderm into the right and left parts, and the differentiation of the nervous system and organs of the body. The next stage includes the formation of the abdomen, labrum, and spiracle; segmentation of the cephalothorax; and the development of tricogenic cells that produce structures such as *setae* and *taenidium* (Nagaraju & Goldsmith, 2002). At the end of development, pigmentation occurs on the head and body, before the embryo hatches into larvae.

Embryonic development is most observed at 15 °C and 30 °C, but for different reasons. At 15 °C, developmental delays are caused by diapause, a phase of embryonic dormancy that temporarily halts development even though the egg is still alive. Meanwhile, at 30 °C, the length of observation time is limited due to the high embryo mortality rate, making it difficult to accurately identify developmental stages. Meanwhile, at 30 °C, the observation period is shortened because of the high embryo mortality rate, making it difficult to accurately identify developmental stages. This suggests that extreme temperatures, either too low or too high, can hinder the successful development of Eri's silkworm embryos.

CONCLUSION

Temperature manipulation under a continuous-darkness photoperiod (24D:0L) effectively influenced the hatching performance and embryonic development of eri silkworm (*Samia cynthia ricini*) eggs. The 15 °C treatment produced the longest hatching period (23.96±0.07 days) and delayed hatching by 15.96±0.07 days, demonstrating its potential to extend the egg incubation period, although hatchability remained low at 9.68±9.13%. In contrast, the 25 °C treatment supported more normal embryonic development, with a hatching period of 10.21±0.76 days and a hatchability of 22.55±19.13%. Exposure to 30 °C resulted in very low hatchability and considerable embryonic damage, indicating that excessively high temperatures are unsuitable for egg incubation. Overall, low-temperature exposure under continuous darkness can be used to regulate and delay eri silkworm egg hatching, whereas moderate temperatures are more favorable for maintaining subsequent embryonic development. These findings provide a basis for developing controlled incubation strategies to improve the synchronization of egg hatching, distribution, and eri silkworm production management.

Suggestions

It is necessary to conduct a follow-up evaluation regarding the selection of the space and tools used to carry out photoperiod treatment at the appropriate temperature and humidity so as not to affect the observed parameters. The tools and work procedures during the study have a major impact on the success of the research. Therefore, adjustments are necessary for further research.

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