

**EFFECT OF SHADED COLORS CAGE ON MATING BEHAVIOR,  
EGGS WEIGHT, AND ADULT SURVIVAL OF  
BLACK SOLDIER FLY (*Hermetia illucens* L.)**

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**ABSTRACT**

The growing economic demand for Black Soldier Fly (BSF) or *Hermetia illucens* larvae in the animal feed industry surpasses the current low production of BSF eggs. The insufficient production and population of BSF pose a challenge, necessitating the development of technology and strategies for mass rearing of *H. illucens*. To address this, efforts were made to enhance egg productivity by manipulating mating behavior and egg weight through exposure to different light spectra based on *H. illucens* photoreceptors. Four plastic cage shaded colors (clear, yellow, red, and blue) to optimize egg production and observing mating behavior were conducted from November 29, 2022 to January 7, 2023, at the Animal Physiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Indralaya, Indonesia. The data collected were the number of mating pairs, copulation duration, average egg weight, and the adult lifespan of *H. illucens*. The shade color was found to significantly influenced mating behavior and egg weight, with the blue shade treatment yielding the best results. Specifically, the blue shade stimulated mating behavior, resulting in the highest number of mating pairs ( $7.71 \pm 1.38$ ) and an mean copulation duration of  $33.47 \pm 1.56$  min. While optimum egg weight increased by  $0.25 \pm 0.12$ , and it had no impact on the lifespan of *H. illucens* from eggs to death.

**Keywords:** Black soldier fly, *Hermetia illucens*, light spectrum, photoreceptors, ommatidia.

## ABSTRAK

Peningkatan permintaan ekonomi larva Lalat Askar Hitam (BSF) (*Hermetia illucens*) dari industri makanan haiwan ternakan tidak selari dengan kualiti dan kuantiti penghasilan telur BSF telur yang masih rendah. Kurangnya penghasilan dan populasi BSF menjadi permasalahan utama. Oleh itu, teknologi dan strategi pemeliharaan *H. illucens* perlu dijalankan secara massa. Usaha yang dijalankan adalah meningkatkan penghasilan telur dengan cara meningkatkan kelakuan mengawan dan berat telur melalui pendedahan ke atas spektrum cahaya yang berbeza berdasarkan fotoreseptor *H. illucens*. Empat warna sangkar plastik telah diuji iaitu jernih, kuning, merah dan biru dalam mendapatkan penghasilan telur yang maksimum. Kajian ini telah dijalankan bermula November 2022 hingga 7 Januari 2023 di Makmal Fisiologi Haiwan, Jabatan Biologi, Fakulti Matematik dan Semulajadi, Universiti Sriwijaya, Indralaya, Indonesia. Kajian dijalankan merangkumi penyediaan haiwan kajian, pemerhatian kelakuan mengawan, mengira pasangan megawan, tempoh kopulasi, purata berat telur dan lamanya *H. illucens* dewasa sampai mati. Warna perlindungan naungan didapati memberi kesan dalam mempengaruhi kelakuan mengawan dan dalam meningkatkan berat telur. Warna perlindungan biru telah memberikan hasil terbaik berbanding warna lain. Biru juga mempegaruhi kelakuan mengawan dengan pasangan mengawan  $7.71 \pm 1.38$  dengan purata tempoh kopulasi  $33.47 \pm 1.56$ . Berat telur optimum meningkat kepada  $0.25 \pm 0.12$  tetapi tidak memberikan kesan kepada rentang hidup dari telur sampai mati *H. illucens*.

**Kata Kunci:** Lalat Askar Hitam, *Hermetia illucens*, spektrum cahaya, fotoreseptor, omatidia.

## INTRODUCTION

The Black Soldier Fly (BSF) or *Hermetia illucens* Linneus is a tropical fly species with significant economic benefits for processing various by-products into insect-based products. *Hermetia illucens* larvae offer fatty acids for biofuels, minerals, chitin, and chitosan for biobased product production, and protein for animal feed (Julita et al. 2020). The rising economic demand for *H. illucens* larvae in the animal feed industry exceeds the current low egg production. Insufficient production and population pose challenges, addressed in a study by Mohamad Ibrahim & Hadura (2021) using animal manure and plant material for increased production in semi-captivity. Therefore, developing technology and strategies for mass rearing *H. illucens* is essential. Efforts to boost egg productivity include enhancing mating behavior and egg weight through exposure to different light spectra (Giunti et al. 2018; Hoffman et al. 2021).

One way to increase the population and mass production of *H. illucens* is to obtain optimal mating. *Hermetia illucens* is very sensitive to light and is attracted to direct sunlight for mating. Artificial light also has a significant effect on mating behavior. It is reported that sunlight, and temperature have a significant influence on *H. illucens* mating success and fertilized egg production. This is due to the relationship between temperature and color wavelength. Temperature influences the mating behavior, egg weight and life span of *H. illucens* (Sakai et al. 2002). Light has different energy and wavelengths based on its spectrum. The energy of light will provide different temperatures or heat based on the color of the light, while the wavelength of light has propagation power so that it can enter the *H. illucens* photoreceptor. Sufficient and appropriate light will also help maximize mating behavior and egg weight (Shumo et al. 2019). Therefore, technological strategies are needed for the rearing of *Hermetia illucens*. The objectives of this study were to (i) determine the effect of shade color

on mating behavior, egg weight and length of life of *H. illucens* imago, (ii) determine which cage shade is the most optimal for increasing mating success, egg weight and length of life of black soldier fly imago (*H. illucens*).

## MATERIALS AND METHODS

### Materials of Study

The tools used in this study are stationery, clear cups, cutters, scissors, cameras, fly frame cages in the form of four-level shelves with steel material measuring (190 cm high x 80 cm long x 40 cm wide), mesh cages measuring (30 cm long x 30 cm wide x 30 cm high), rubber bands, wire mesh, wooden blocks (ovitrap) measuring (length 18, width 3, height 0.5 cm), 1000 ml container, lux meter, tacks, clear plastic, red, blue, yellow, analytical scales, kg scales, tissue, HTC-2 ThermoHygrometer (VelVeeta) and 10x10 cm waring. While the materials used in this study were water, sugar, 200 g fish feed, and a sample of 2 grams of BSF eggs.

### Working Steps

#### *Provision of Test Animals*

The larvae are reared in a dark room using a tub until the pre-pupa stage. The prepupae are placed in the pupation medium in the form of palm shells. Pupae before becoming adult flies are moved into the cage for imago emergence. Newly emerged flies are separated by sex and pupal emergence. Flies were caught using clear cups manually and kept in love cages measuring 30 cm x 30 cm x 30 cm (30 cm x 30 cm x 30 cm) with a net (nylon size 200 × 300 mm, mesh size 2 × 2 mm). Ten pairs of black soldier flies were included with a male to female ratio of 1:1.

#### *Cage Planning*

A rack-shaped cage frame was made with four levels of steel measuring length (220 cm x 40 cm x 190 cm). For each type of artificial light, a cage measuring 210 cm x 30 cm x 30 cm is used and divided into 7 sub-enclosures (mating cages) measuring 30 cm x 30 cm x 30 cm which are separated by a cage divider. This is shown in Figure 5. The design of the shade of the cage is done by cutting the colored plastic customized with the size of the cage shade using clear solation on the roof, right, left, and back of the cage. The same treatment was carried out on the four cages with different plastic colors. The cage is placed under the roof and the walls are made using UV plastic and the sides of the cage are changed. This is so that all parts of the cage get full sunlight.

#### *Egg Production Site*

The place for laying BSF was made using thin strips of wood measuring length 18 cm x 3cm x 0.5 cm). After that, 3 pieces of thin wooden planks were arranged and given a gap with small tacks. Then the end of the wood is tied using a rubber band. The prepared wood is placed on top of the fish feed media has been provided. Then weighed to calculate the weight of BSF.

#### *Making Attractive Media*

This study uses fish feed media in the form of pellets (Beauty PP812 Premium Patee) because it is easy to obtain and commonly used. According to Anggia and Ramadhani (2021), fish feed media is in the form of pellets (Beauty PP812 Premium Patee), which is similar to commercial chicken feed given during the process of growth and development of the larvae before pupation is the most preferred substrate for BSF. Fish feed is dissolved in water in a 1:1 ratio. Then the media is put in each cage.

## **Observation**

### ***Number of Mating Pairs of *Hermetia illucens****

Observation of BSF was done by qualitatively describing mating behavior, counting the number of mating pairs and the duration of copulation (in minutes) per day. Entered as many as 10 pairs of males and females with a sex ratio of 1:1 flies into each cage with a different shading color. The treatment is placed in a room exposed to sunlight. Done measurement temperature using a digital thermometer HTC-1 and light intensity sun (Lux), use Lux meter every 09.00, 12.00, and 15.00 WIB in each test cage.

### ***Calculation of the Egg Weight of Black Soldier Fly Eggs (*Hermetia illucens*)***

In each cage provided a place placement of fly eggs (ovitrap) viz logs (trap) sized (18 cm x 3 cm x 0.5 cm). Each ovitrap consists of three pieces of wood arranged and given a gap with small tacks. Then on each wood tied using a rubber band. Media laying eggs using a substrate. The fish feed used is mixed with water.

### ***Survival Time Observations***

The long survival time observation of the BSF begins when pupa become imago. Then the long-lived observations of the BSF are done by counting and recording the number of flies that die per day until all the flies are present inside the dead cage.

## **Data Analysis**

Presentation of data on the number of mating pairs, duration of copulation, egg weight, and life span of the black soldier fly is presented in the form of tables, graphs, and documentation in the form of photos and videos. Analysis of BSF egg weight data statistical analysis was performed using one-way – analysis of variance (ANOVA) at the 95% confidence level ( $P < 0.05$ ) using SPSS 25.0 software. If each treatment shows a significant difference, it can be continued with a further test, namely the test Duncan Multiple Range Test (DMRT) to determine the effect of each treatment on the parameters observed. measurement data factor environmental parameters: daily average temperature (EC), using a digital humidity thermometer HTC-1, and sunlight intensity (Lux), using a lux meter LM-3000.

## **RESULTS AND DISCUSSION**

### ***Mating Behavior of Black Soldier Fly (*Hermetia illucens*)***

The results of these observations are described descriptively regarding the stages of *H. illucens* mating behavior that occurs in several cage colors (clear color, yellow color, red color, and blue color). Observations were made for 40 days in the presence of full sunlight from 08.00 to 15.00 WIB.



Figure 1. Mating behavior of black soldier flies. a. unresponsive female flies; b. males approaching flying females; c-e. Male-female copulation activity; f. during copulation the female flies remain motionless and the males randomly wave their antennae; g. copulation ends; h-i. Interrupted copulation activity with other males

Mating of *H. illucens* occurs after the reproductive maturation phase. Female flies started to perform flight activities, while other females showed no flight and were therefore unresponsive (Figure 4a). The flight activity of females stimulates the response of males to approach. Males approaching a flying female (Figure 4b), touch the female's chest with their feet and lead their mate downwards or towards the sidewall (Figure 4c). During the fall and landing, the female stops moving her wings, while the male continues to flap his wings and exposes his aedeagus. Upon landing, the male generally places himself on the female's back (Figure 4d).

After landing, the male begins to perform wing-fanning behavior while mounting the female. The duration of wing-fanning reflects mating success as shorter wing-fans are more successful than long ones. After wing-fanning, the male moves backwards onto the female's body in the opposite direction trying to make genital contact. During this process, the male touches the female's thorax with his front pair of legs, while he starts tapping the female's abdomen with the tarsi of his hind pair of legs. During the approach of genital insertion, the female may remain still, try to run away, fly away, or get off the male by flapping her wings up and down rapidly to avoid it.

The receptive female accepts the acceptance position of remaining stationary, with wings folded at the sides of the abdomen at a 60° angle, then the male moves backwards and arches his abdomen downwards to achieve genital contact and copulation. Once copulation has begun, the male rotates 180° in a reverse position with the head on the opposite side and makes genital contact (Figure 4e). During copulation, the female remains motionless and the male randomly performs antennal waving, moving the antennae alternately up and down, occurring synchronously for a few seconds or for a long time (Figure 4f). The minimum copulation duration in this study was 8 min and the maximum copulation duration reached 49 min.

Copulation ends when the male retracts the aedeagus and initiates grooming activity (Figure 4g) before flying away (Figure 4h). The mated female also showed grooming activity on the ovipositor before leaving the site. This behavior has been observed previously by Ucu et al. (2020) Copulation without fanning behavior has not previously been observed. During mating activity, intruder males may be attracted to the same female, seducing the approached female by placing themselves on her back. However, the intruder is always replaced by the first male and copulates anyway. This is documented in (Figure 4i). Non-receptive females are not receptive and constantly push the male away, trying to run or fly away from the approaching male. This mating behavior has been reported by Giunti et.al (2018).

### Number of Black Soldier Fly Mating Pairs

Table 1. Mean Number of Mating Pairs of *Hermetia illucens* (pairs)

Treatments	Mean Number of Mated Pairs
P1	6.14 <sup>b</sup> ±3.38
P2	3.57 <sup>a</sup> ±2.14
P3	3.85 <sup>a</sup> ±2.34
P4	7.71 <sup>c</sup> ±1.38

Note: P1 = natural cage shade color, P2 = yellow cage shade color, P3 = red cage shade color, P4 = blue cage shade. Different superscripts in the same column indicate significantly different at the 5% level, indicating the results of a significant effect on Duncan's further test with a 95% confidence level.

P4 (shading blue) has a more significant value, namely 7.71 pairs. This is caused by several factors, including temperature. The resulting temperatures from highest to lowest were P4 30°C, P1 34.35°C, P2 35°C and P3 36.40°C. The color difference in the shading of the cage will produce a different temperature. This is due to the temperature generated in each color of the shade of the cage which is absorbed by the BSF. The temperature that affects the number of mating pairs of BSF ranges from 30°C-36.40°C. According to Chia et al. (2018), approximately 99% of mating and oviposition occur in the temperature range of 27.5 to 37.5°C. Temperature and light intensity are also affected by the intensity of light and dark light on the daily rhythm. The highest temperature and light intensity were obtained at 12.00 followed by 15.00 and 09.00 as the lowest temperature and intensity. The average copulation behavior in the four treatments occurred at 10:00 - 11.00 WIB, this is in accordance with previous research in Wardhana (2016), which reported BSF mating activity generally occurred at 8.30 and reached its peak at 10.00.

In this case, hormones are an important factor. Hormones that function in the reproductive process are sex pheromones. According to Giunty et al. (2018), one of the factors

that influence marriage is the behavior of male lekking which is influenced by sex pheromones. Blue light also has a light intensity of 450 nm which has the same preference as the BSF photoreceptor organ and can increase the number of BSF mating pairs. Ommatidia are the structural units of insect compound eyes and have photoreceptor cells for light waves. A suitable light source that is between 450 nm - 700 nm wavelength is ideal for reproductive activity in BSF mature.

P1 (clear shade) is the second highest average, namely 6.14 (Table 1). This is because the clear color is natural light for BSF to carry out the marriage process. BSF under the clear plastic receives a mixture of different wavelengths of sunlight. According to Heussler, et al. (2018), BSF occurs naturally depending on space and sunlight in the temperate sub-tropics. The value of the number of mating pairs P2 (yellow shading) and P3 (red shading) showed the lowest average values of 3.57 and 3.85. This is because the extreme increase in temperature causes BSF could not regulate the thermoregulator in their body. So that the BSF run out of energy before mating.

The BSF is not sensitive to yellow and red light, this is in accordance with Kluber's previous research et al. (2020), based on the spectral sensitivity of the ommatidia photoreceptor compound eye of the BSF can sense blue, ultraviolet, and green light which affect mating behavior. The maturity of the reproductive organs is also an important factor affecting the number of mating pairs of BSF. According to Guitti et al. (2018); Zhongyi et al. (2020), females avoid interactions with males and are unresponsive due to their physiological status, i.e. immature reproductive organs.

### Duration Copulation Flies Black Army

Table 2. Mean Copulation time of *Hermetia illucens*

Treatments	Copulation Duration (minutes)
P1	26.27 <sup>b</sup> ±4.37
P2	19.17 <sup>a</sup> ±8.61
P3	18.37 <sup>a</sup> ±2.95
P4	33.47 <sup>c</sup> ±1.56

Note: P1 = natural cage shade color, P2 = yellow cage shade color, P3 = red cage shade color, P4 = blue cage shade. Different superscripts in the same column indicate significantly different at the 5% level, indicating the results of a significant effect on Duncan's further test with a 95% confidence level.

P4 (blue shading) is more significant than the other treatments, namely 33.47 minutes (Table 2). In the old copulation parameter, it is suspected that there is a lack of correlation between temperature and the duration of copulation. This is supported by the absence of reports on research previously. There is a relationship between temperature and duration of copulation. But correlated with the number of mating pairs. This is due to the increasing number of install marry, for the more time it adds up. In this study, the shortest duration of copulation was only 8 minutes and the longest duration was 49 minutes, exceeding previous studies. According to Guitti et al. (2018), states that the minimum duration of copulation is 20 minutes and the maximum duration of copulation is 42 minutes.

Body size *H. illucens* the male is thought to affect the duration of copulation. In this study, the size of the *H. illucens* of various males. The number of sperm produced is directly

proportional to the body size of *H. illucens* male. The more sperm the black soldier fly produces *H. illucens* male, the longer the duration of copulation. The duration of copulation will maximize the fertilization of eggs in females. Male flies that have large body sizes tend to be more fertile than those with small sizes. In the present study, it was found that no re-mating was recorded for any of the tested females during the observation period. *Hermetia illucens* males and females are monogamous. Males will die after mating and females will die after laying eggs (synovigenic). This was also reported in Salam's previous study et al. (2022), that the *H. illucens* females are reported to lay only one egg during their lifetime, after which they die.

### Black Soldier Fly Egg Weight

Table 3. *Hermetia illucens* Mean Egg Weight (gram)

Treatments	Egg Weight (gram)
P1	0.15 <sup>a</sup> ±0.10
P2	0.10 <sup>a</sup> ±0.07
P3	0.09 <sup>a</sup> ±0.05
P4	0.25± 0.12

Note: P1 = natural cage shade color, P2 = yellow cage shade color, P3 = red cage shade color, P4 = blue cage shade. Different superscripts in the same column indicate significantly different at the 5% level, indicating the results of a significant effect on Duncan's further test with a 95% confidence level.

P4 correlated with the duration of copulation seen from the most significant value of 0.25 g (Table 3). In this study, the results of the appropriate correlation between copulation duration data and egg weight data were obtained. This is due to long-time copulation affecting the number of sperm transferred by the BSF male to female. The more sperm that are transferred, the more optimally the female egg will be fertilized. BSF body size female affects egg weight. The number of eggs produced by flies with large bodies is more than flies with small bodies.

Another factor that affects egg weight is temperature. The lowest average egg weight was found in treatment P3 (red shading), this was due to the effect of temperature on each treatment. The temperature measured in the shade of the red cage exceeds the optimal temperature of 36.6°C. Temperature also plays a role in stimulating egg laying.

Treatment with blue shading resulted in the highest egg weight of 0.26 g. This is due to the large number of mating fly pairs and the longest copulation duration at P4 (shaded blue). This is in accordance with Schneider's previous research (2020), Blue light which has a wavelength of around 440 nm and green around 540 nm, and some of their ratios, has been shown to be very important for successful mating and egg production of BSF. Oonincx et al. (2016) also found that BSF ommatidia contains photoreceptor cells that are sensitive to blue, green, and UV light and can significantly increase egg weight.

It was found that female flies only mate and lay eggs during their lifetime and only one female fly could produce more than one group of eggs in one oviposition, indicating a difference in egg weight between the mating cages. In general, eggs began to be produced on the fourth or fifth data collection with the peak of egg production appearing on data collection on the 31st day reaching 0.56 g. According to Heussler et al. (2018), The peak of oviposition ranges from four to eight days.



**Survival time of *Hermetia illucens***Table 4. Mean of Survival time of *Hermetia illucens*

Treatments	Female Length of adults to death	Male Length of adults to death
P1	10.00 <sup>a</sup> ±2.23	9.28 <sup>a</sup> ±2.69
P2	10.14± 2.19	10.42 <sup>a</sup> ±1.61
P3	12.00 <sup>a</sup> ±1.95	12.28 <sup>b</sup> ±2.28
P4	12.87 <sup>a</sup> ±3.05	11.00 <sup>a</sup> ±2.30

Note: P1 = natural cage shade color, P2 = yellow cage shade color, P3 = red cage shade color, P4 = blue cage shade. Different superscripts in the same column indicate significantly different at the 5% level, indicating the results of a significant effect on Duncan's further test with a 95% confidence level.

The length of life of females in P4 (blue shade) has a more significant value than other treatments which is 12.87 days. While the highest adults to death of male *H. illucens* is found in P3 (red cage shade) which is 12.28 days followed by P4 (blue cage shade), P2 (yellow cage shade) then P1 (natural cage shade) as the lowest average value of 9.28 days (Table 4). The relationship between parameters in this study is that *H. illucens* is stimulated using several colors of light to accelerate the mating process. Each color of light will affect the temperature in the experimental cage. Optimal temperature accelerates mating. The faster the first mating occurs, the shorter the life span of *H. illucens*. Male flies will die after mating, while females will die after laying eggs. This is supported by Julita et al. (2019), stating that *H. illucens* is known as a monogamous species that mates only once during its lifetime.

P3 data (shading red) in males and females show no association with other parameters. This is due to the body temperature adaptation of the BSF to the ambient temperature. According to Oliveira et al. (2015), the ideal life cycle conditions for breeding are temperatures between 28-30°C, but this does not rule out the possibility of black army flies (*Hermetia illucens*) evolving and adapting to defend themselves.

One of the influencing factors is BSF nutrition at the time of larvae. Wardhana (2016) and Lamin et al (2022), reported that the quality of larval development media was positively correlated with larval length and the percentage of survival of adult flies. In this study, it was found that the BSF females have the longest lifetime. This is directly proportional to P4 data (blue shading) on the number of mating pairs, P4 data (blue shading) on copulation duration, and P4 data (blue shading) on egg weight. BSF male had the longest survival time at P3 (shading red). Apart from nutritional factors at the time of larvae, it can also be caused by BSF that do not copulate because extreme temperatures exceed optimal temperatures so that sperm release does not occur in males. This resulted in male flies having a long life span and many unresponsive individuals without mates marriage so that they do not marry until they die.

This is according to Macavei et al. (2020); Zhongyi et al. (2022), In the tropics, the BSF mate and lay eggs throughout the year, while in warm climates natural reproduction is limited to a few generations when above-average temperatures prevent flies from mating. Another factor that is thought to affect the longevity of males at P3 (red shade) is not meeting responsive females causing a phenomenon called single flies. This has been reported in a previous study by Giunti et al. (2018).

## **CONCLUSION**

Shading color affects stimulating mating behavior and increasing egg weight. The blue shading treatment gave the best results compared to other plastic shading colors. That is stimulating mating behavior, increasing egg weight, and prolonging the lifespan of BSF imago.

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### **Conflict of Interest**

The authors declare that they have no conflict of interest.

### **Ethics Declarations**

No ethical issue required for this research.

### **Data Availability Statement**

The author confirm that the data supporting the findings are available within the article. Raw data available from the corresponding author upon reasonable request.

### **Authors' Contributions**

SL and AA conceived this research and designed experiments; JI and NF participated in the design and interpretation of the data; SL, AA, and NF performed experiments and analysis; SL, AA, and RP wrote the paper and participated in the revisions of it. All authors read and approved the final manuscript. All authors read and approved the final manuscript.

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