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Optimization of the production of black soldier flies *Hermetia Illucens* by controlling biological parameters in Côte d'Ivoire

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Abstract

This study was conducted at the ENS vivarium located at the UFHB of Cocody in the city of Abidjan in the south of Ivory Coast, with a temperature of about 28° C and a humidity of about 70%. It aimed to study some biological parameters of the black soldier fly *Hermetia illucens*. For achieve this goal, it was a question of studying the different stages of development of the black soldier fly and also studying the evolution of its growth parameters. The flies were captured, kept in an aviary and observed from the fly stage to the end of the pupal stage (emergence of the first flies). Data on the duration of the different stages of development and the evolution of larval growth parameters (average weight, length, width, Fulton index, length/width ratio, survival and mortality rate of larvae) have been taken. This study revealed that the life cycle of *Hermetia illucens* is between 31 and 43 days, with an incubation time of eggs that lasts 2 days, a larval phase of 14 days, a pupation of 10 days and a duration of adult life of 8 days on average. In addition, this study reports a low mortality rate of larvae (3.45%), larvae much longer (19.46 mm), wider (6 mm) and plumper.

Keywords: Black soldier fly larvae, black soldier fly life cycle, larvae growth parameters.

Introduction

Access to food resources is a basic right for all people (Fung *et al.*, 2018). Food security improves economic growth in the region where it is practiced and enhanced (Savari & Zhoolideh, 2021). Food supply security depends on both an equitable application of the law and the availability of resources. Climate change effects such as changes in temperature and precipitation, increased frequency and intensity of extreme weather events, and ocean warming and acidification are impacting primary or traditional production of fisheries resources (Tirado *et al.*, 2010; Tomlinson, 2013). Current global population growth is likely to exacerbate the food security problem in developing countries with increasing demand for animal products such as milk, meat, eggs (Jones *et al.*, 2013; King *et al.*, 2017). Both aquatic and terrestrial animal husbandry is currently considered a high growth food segment (Silva & Barros, 2020). In industrialized countries, the concerns associated with this activity are health safety. To address these constraints, many orders of insects can be used as livestock feeds due to their high protein content (Ehounou *et al.*, 2019). Thus, insects are considered promising feed resources to meet the protein needs of animals (Gold *et al.*, 2018). Therefore, they are currently the focus of attention due to the numerous exploitation opportunities they offer. For these reasons, new production methods or new feeds must be implemented while preserving food quality, natural habitat, and biodiversity (FAO, 2005; Van Huis, 2013). Insect species that were evaluated as food resources included locust, grasshopper, locust, silkworm (Makkar *et al.*, 2014), and black soldier fly larva (Nyakeri *et al.*, 2017). Thus, the objective of this study is to investigate some biological parameters of the black soldier fly *Hermetia Illucens* in order to optimize its production for processing and use of the larvae for fish feed in Côte d'Ivoire. The realization of this study required first the determination of the different stages of development of the black soldier fly and then the monitoring of the evolution of some parameters of growth of the larvae.

Materials and Methods

Study site

This study was carried out in Cocody in the city of Abidjan in the south of Côte d'Ivoire. The corresponding climate is of the subequatorial type (Kouassi *et al.*, 2020) characterized by high rainfall and a high degree of humidity. Daytime temperatures vary on average from 27,7 °C to 29,8 °C annually (Ahoussi *et al.*, 2012) and abundant rainfall reaches 525 mm (Kouassi *et al.*, 2020). The temperature and humidity during this study were 28°C and 70%, respectively (Fatchurochim *et al.*, 1989; Nguyen *et al.*, 2013).

Experimental apparatus

Fly rearing device

The biological material consisted mainly of the black soldier fly from the Akouedo landfill (Abidjan) at different stages of development. One hundred black soldier flies were captured, released and maintained in a 3 x 3 x 6 m surface aviary and placed in sunlight for mating and oviposition according to the methods described by (Tomberlin *et al.*, 2005) (Fig. 1). The temperature of the fly rearing site was between 20 and 35°C. They received tap water twice a day using a device that keeps the relative humidity, measured with a hygrometer, above 30%.

Figure 1: Fly-raising device

Egg processing device

The oviposition sites were made from honeycomb cardboard with a surface area of 8 x 4 x 0.5 cm, held together by paper clips, all inside a food container (i.e. 17% crude protein, 2.5 % crude fat and 4.5% crude fibre) placed inside the aviary (Fig. 2). Every other day, cardboard flutes containing eggs were placed in food trays containing 20% corn flour, 30% alfalfa flour, 50% wheat bran. The larvae were then covered with coconut fiber at 70% humidity and a temperature of 27°C, four days later, the young larvae were sieved and distributed in 3 food trays of 300 larvae each (Tschirner & Simon, 2015)

Figure 2: Egg-laying device

Device for the progress of the pupation

The wandering stage larvae moved out of the food trays to a container of dry substrate (coconut fiber) where they completed their pupal development according to the method described by (Tschirner & Simon, 2015) (Fig. 3). Seven days after the onset of pupation, the tanks containing the pupae were transferred to an aviary.

Figure 3: Pupation device

Preparation of larvae feed

Thirty grams (30 g) of food scraps from the two restaurants at University of Felix Houphouët Boigny were ground using a Moulinex brand electric blender and then sieved using a 1mm manual sieve of mesh. This food is then allocated, to each plastic tank containing the larvae, on a daily and random basis until the larvae reach the wandering stage. The temperature and relative humidity inside the food tanks was 28°C and 70%, with a larval density of 4 larvae/cm2 (Diener *et al.*, 2009; Fatchurochim *et al.*, 1989; Nguyen *et al.*, 2013).

Parameters evaluated

The parameters evaluated were the duration of development (time required to reach the wandering stage), the length (L) and width (l) of the larvae (mm), the K factor and the mortality rate. Ten larvae on average were used for measuring the length and width of the larvae on a daily basis. Data collection on weight and length ceases when 40% of the larvae have reached the wandering stage, which is indicated by the completely black color of the larvae and lack of feeding (Tomberlin *et al.*, 2005).

Report L/W

Using a transparent graduated ruler, the length (L) and the width (W) of the fly larvae were measured. Then the ratio (L/W) was calculated.

Average weight of a larva (g)

To measure the average weight of a larva (g), the contents of each tank were weighed daily using a SARTORIUS-type electronic scale. The mass obtained was divided by the number of larvae provided:

$$Average\ weight\ (g) = \frac{Total\ weight\ of\ larvae}{Number\ of\ individuals\ collected}$$

Factor K

The K factor or Fulton condition coefficient or Fulton index is a coefficient revealing the physiological state (fusiform or plump) of fish of the same species, which indicates, among other things, their ability to reproduce and grow) (Mozsár *et al.*, 2015). It is measured according to the following formula:

$$K = \frac{Length^3}{100000 * average\ weight\ of\ larvae}$$

Mortality rate (%)

The number of surviving larvae was counted daily to calculate the daily mortality rate. The mortality rate is measured using the following formula:

$$Mortality\ rate\ (\%) = \frac{Initial\ number\ of\ individuals - Number\ of\ individuals\ harvested}{Initial\ number\ of\ individuals} X\ 100$$

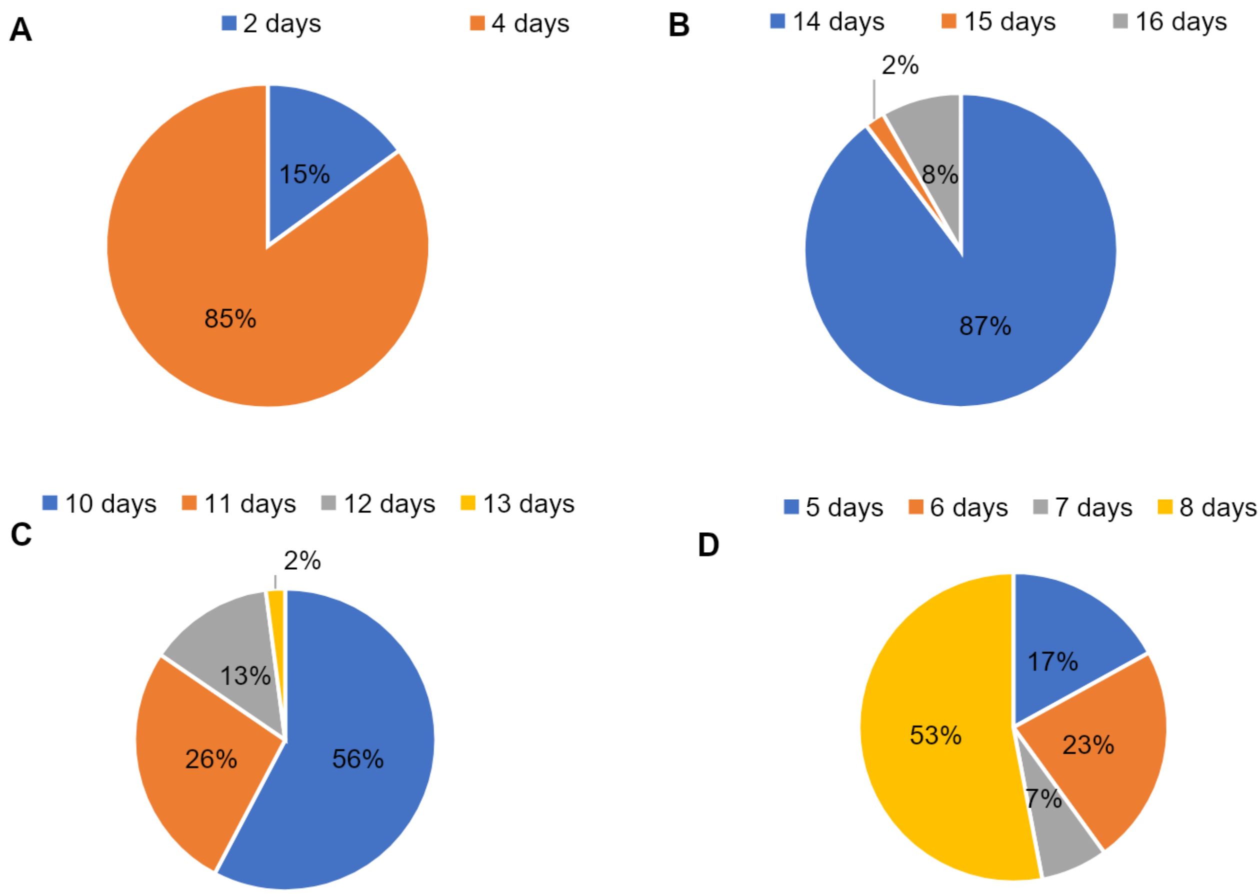
Statistical analysis

A one-way analysis of variance (ANOVA) was used to assess over time the time to reach errant developmental stages. The increase in larval length per day was analyzed using ANOVA while the rate of larval weight gain per day was analyzed using non-parametric ANOVA (independent samples Kruskal-Wallis). The weight, length and final width of the larvae were determined using an ANOVA (Tschirner & Simon, 2015). Also, the survival rate (%) was calculated by dividing the number of larvae after the test by the initial number of larvae.

Results

Different stages of development of *Hermetia illucens*

The study of the different developmental stages of the black soldier fly showed that the life cycle of *Hermetia illucens* is between 31 ± 0.11 and 43 ± 0.5 days with four different developmental stages: egg stage (2 to 4 days), larval stage (14 to 16 days), pupal stage (10 to 15 days) and adult stage (5 to 8 days). 85% of the eggs hatched in 2 days, 87% of the larvae completed their development in 14 days, 56% of the larvae completed their pupation in 10 days and 53% of the flies had a life span of 8 days (Fig. 4). In addition, 6 intermediate larval stages were observed according to the color of the individuals. L1 stage with white larvae measuring about 1 mm in length on average. L2 stage with pale yellow larvae measuring about 3 mm in length on average. L3 stage with the appearance of segmentation on the exoskeleton, larvae of light brown color and measuring between 6 and 8 mm in length on average. L4 stage with larvae darker than the L3 stage and measuring between 11 and 17 mm in length on average. L5 stage with larvae measuring between 17 and 18 mm in length which migrate to a drier place and L6 stage marked by totally dark larvae and measuring between 18 and 19 mm in length.



A: Egg ; **B**: Larva ; **C**: Pupa ; **D**: Adult

Figure 4: Spectra of distribution of individuals according to duration of the different life cycle stages of *Hermetia illucens*.

Evolution of some larval growth parameters

Length, width and Fulton index

Concerning the evolution of the few growth parameters of the larvae studied, the maximum average length of the larvae obtained on day 14 is 19.46±15 mm. For the maximum average width, it has a value of 6mm which does not vary until day 14. As for the K factor or Fulton index, we were able to observe a decrease in its values at the start of larval development (20.76± 1.47) until the end of the larval stage (2.3±0.21) (Table 1).

Table 1: Evolution of mean morphological parameters during the larval stage of *Herrmetia illucens*

Day	Length (L) (mm)	width (W) (mm)	L/W (mm)	Fulton Index
4	3,30 ± 0,10	1	3,30 ± 0,10	20,76 ± 1,47
5	6 ± 0,43	2	3,30 ± 0,11	10,88 ± 11,46
6	8,73 ± 0,11	3	2,91 ± 0,03	3,69 ± 1,96
7	11,70 ± 0,55	4,06 ± 0,11	2,80 ± 0,05	3,68 ± 2,09
8	13,96 ± 0,57	5	2,79 ± 0,11	3,11 ± 2,25
9	15,40 ± 0,26	5,73 ± 0,46	2,70 ± 0,26	2,31 ± 0,34
10	17,06 ± 0,15	6	2,84 ± 0,02	2,18 ± 0,35
11	18,10 ± 0,36	6	3,01 ± 0,06	2,94 ± 0,33
12	18,60 ± 0,30	6	3,10 ± 0,05	2,72 ± 0,19
13	19,16 ± 0,49	6	3,19 ± 0,08	2,55 ± 0,18
14	19,46 ± 0,15	6	3,24 ± 0,2	2,3 ± 0,21

Average larval weight, mortality rate, number of final individuals

The study of the evolution of the average larval weight of *Hermetia illucens* allowed us to distinguish 3 phases. A first phase from day 4 to day 6 characterized by a non-significant change in weight (0.007-0.01g). Then comes a phase of rapid growth from day 6 to day 11 (0.01-0.16g), and finally, a last phase during which the weight changes slightly (0.16-0.17g) until day 14 (Fig. 5). Regarding the number of remaining larvae (collected at the L6 stage of larval development), out of 300 larvae, 289 survived at the end of the work. As for the mortality rate, it evolves from day 4 to day 9 (0-3.33%) from which it remains constant (3.33%) until the end of larval development (Table 2)

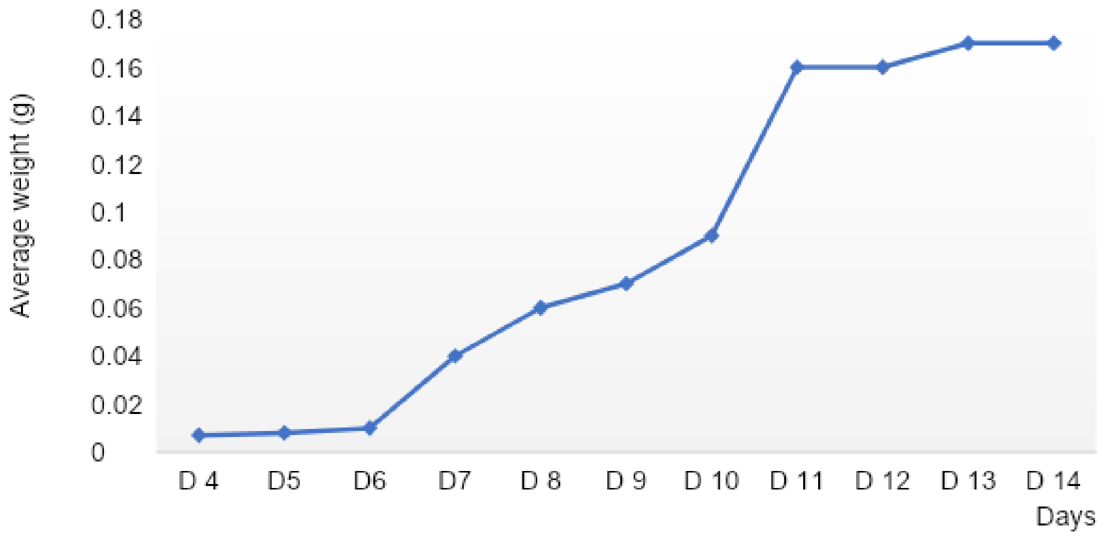


Figure 5: Evolution of the average weight of larvae

Table 2: Evolution of survival rate and average mortality rate during the larval stage of *Herrmetia illucens*

Days	Individuals	Mortality Rate (%)
4	300 ± 0,00	0
5	297 ± 0,57	1
6	295 ± 1,15	1.33
7	191 ± 1,15	3
8	290 ± 0,00	3
9	290 ± 0,00	3,33
10	290 ± 0,00	3,33
11	290 ± 0,00	3,33
12	289 ± 1,15	3,33
13	289 ± 1,15	3,33
14	289 ± 1,15	3,33

Discussion

The results obtained concerning the different stages of development of the black soldier fly indicate four different phases with different durations as in most Diptera (egg stage, larval stage, pupal stage and adult stage). The egg stage was relatively short and lasted on average 2 to 4 days. The eggs observed were oval in shape with a length of about 1mm. These results are consistent with those of Diclaro & Kaufman (2009). As for the duration of larval development, it is relatively short and lasts 14 days on average. This value is lower than that reported by Tomberlin *et al.* (2009), in which it took 22 days to complete the larval stage. This difference between these two results would be due to the fact that the humidity of the food substrate of the larvae in this study was 70%. Fatchurochim *et al.* (1989) in their work showed that a relative humidity of 70% favors the good development of the larvae.

Also our results indicate that the duration of pupation is relatively short and lasts 10 days on average. This value is much lower than that of Nguyen *et al.* (2013) who obtained an average of 16 days. This difference could be due to a moisture content of 60% and a humidity of 70%. (Yu *et al.*, 2009) showed in their study that the duration of pupation is directly influenced by the moisture content of the substrate in which the pupae are kept. As for the lifespan of adults, it is also short and varies from 5 to 8 days. This value is similar to that of Macavei *et al.* (2020) who reports that the adult life span of *Hermetia* under these same conditions is between 7 and 8 days.

The evolution of the growth parameters of the larvae of the soldier fly *Hermetia illucens* occurs at several levels. Regarding the length and width of the larvae, the results obtained at the end of the larval stage are relatively high (19.4 mm in length and 6 mm in width) compared to those of Koné (2020) (16.9 mm in length and 4.7 mm in width). These differences observed in the size of the larvae with the studies of Koné (2020) would be due to the use of kitchen residue for the food of the larvae which contains 10% lipids, 70% water and around 20% of proteins. This type of diet results in longer and wider larvae. It is also recommended for the good development of the larvae.

The Fulton's condition factor or Fulton's index (K factor) indicates that the larvae are increasingly filiform during larval development. We were able to observe that the values ranged from 20.76±1.47 (day 4) to 2.3±0.21 (day 14). These values reflect the growth status of the larvae (Myers *et al.*, 2014).

As for the evolution of the average larval weight, it is done in 3 stages. Nevertheless, the results obtained at the end of the larval stage show a high weight (0.17g) and slightly higher than those obtained by Tomberlin *et al.* (2009) (0.16±0.01g) and Koné (2020) (0.14±0.01g). The differences between its results may be due to various factors such as different daily food rations (50 g/day in the context of our study; 24 g/day). Indeed a daily food ration of 10 to 500 mg/larvae/day contributes to a rapid increase in the volume of the larvae.

The rapid larval growth observed at average weight between days 6 and 11 could result from a better balance between energy from protein and carbohydrate. Indeed, the work of Jucker *et al.* (2017) explain that the quantity and quality of protein and carbohydrates in the diet are the main factors influencing the balance of energy from these. Similarly, the average larval weight experienced a slight increase (day 11 to day 14) compared to the previous days (0.16 to 0.17g). This work shows that as the larvae move into the wandering stage, there is a decrease in feeding. Therefore, for better optimization of production, the feed intake should be reduced from day 11 onwards.

The results also show that the mortality rate at the end of the larval stage is (3.33%) and is lower than those of (Nguyen *et al.*, 2013) (53.33%) and Koné (2020) (4.1%). This low mortality rate would be linked to good ventilation conditions and relative humidity (70%). Indeed, Fatchurochim *et al.* (1989) reported that the mortality rate varied with the moisture content of the diet. According to these authors, a moisture content of 70% favors a low rate of larval mortality.

The number of live larvae is high and equal to 289. This value is higher than the value observed in Koné (2020) which is 254 larvae. This could be due to the influence of temperature (28°C) and humidity (70%) on the survival of the larvae. Sheppard *et al.* (2002), in their work showed that a relative humidity of 70% favors the good survival of the larvae.

Conclusions

The study on the biology of the black soldier fly *Hermetia illucens* in Ivory Coast, allowed us to note that the duration of its biological cycle is between 31 and 43 days. The duration of the egg stage is 2-4 days, 14-16 days for larval development, 10-15 days for pupation and 5-8 days for the adult stage. Also, work on the evolution of larval growth parameters revealed that at the end of larval development, the larvae were much longer (19.46 mm), wider (6 mm) and plumper. The average larval weight evolves in 3 phases with a maximum value of 0.17g obtained at the end of larval development (day 14). As for the mortality rate, it is low with a value of 3.33%. In addition, under the conditions of our study, the ideal for harvesting larvae would be the 13th day of age. Beyond this study, research perspectives are to be considered.

Conflicts of Interest

None.

Funding Statement

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