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STUDY OF SOME ASPECTS OF THE BIOLOGY OF *CHRYSOMYA ALBICEPS* WIEDEMANN, 1819 (DIPTERA: CALLIPHORIDAE), A NECROPHAGOUS INSECT BREEDED ON PIG (*SUS SCROFA DOMESTICUS* L., 1758) LIVER, IN SUBEQUATORIAL HOT AND HUMID, NATURAL ENVIRONMENT OF COTE D'IVOIRE

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ABSTRACT

The use of necrophagous insects for forensic expertise, requires knowledge of their life cycle. In this perspective, some aspects of the biology of Chrysomya albiceps have been studied in the subequatorial natural environment of Côte d'Ivoire. It was essentially a question of determining the development cycle time of Chrysomya albiceps as a function of the quantity of the nourishing substrate of its larvae. The work took place on the site of the National Center of Floristics (Félix Houphouët-Boigny University, Abidjan-Côte d'Ivoire). During this study which took place from March 26 to June 29, 2017, the average ambient temperature was 28.24°C and the average relative humidity was 84.28%. Ten pairs of C. albiceps were deposited on a 250 g portion of pig liver (Sus scrofa domesticus L.). The first female clutches, the eggs were counted. For the determination periods of each development phase, 200 stage 1 larvae were deposited on 5 different portions of fresh pig liver (50, 100, 150, 200 and 250 g). Observations were made up to the pupal phase. Then the pupae were placed in emergence cages. Calculated parameters were: larval phase, prepupal, pupal duration, pupation rate, and total development time. The emergence rate and the sex ratio were determined afterwards. The development of C. albiceps larvae showed three larval stages and one pupal stage. When the larvae ran out of food, they developed intra-specific predation behavior. The high rate of nutrient substrate mass loss was in favor of the substrate with the highest larval density. The total development times were 9.30 ± 0.11 and 12.17 ± 0.14 days, respectively on the 50 g and 250 g substrates. Adult emergence rates were 28.43 ± 0.82% and 96.73 ± 1.21%, respectively, on the 50g and 250g substrates. Adult lifetimes were 15.98 ± 0.30 and 24.05 ± 0.45 days, respectively for the 50 g and 250 g substrates. Large substrates favored good larval development and adult longevity of C. albiceps.

KEYWORDS: *Calliphoridae, Chrysomya Albiceps, Developmental Biological Cycle, Forensic Entomology, Post Mortem Interval, Subequatorial Climate of Côte d'Ivoire*

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INTRODUCTION

The post-mortem interval (PMI) is the time elapsed between the date of death and the date of discovery of the

corpse (Benecke, 2005; Wyss etChérix, 2006;Gaudry *et al.*, 2007). The entomological expertise, using the necrophagous insects present on a corpse, to date the deaths, is one of the most relevant disciplines of the forensic sciences because it aims at elucidating the murders and the scenes of crimes (Mougeat, 2012). Under these conditions, knowing the biology and ecology of these necrophagous insects, proves essential for the establishment, with precision, of a post-mortem interval.

Some biological parameters of some necrophagous insects, inventoried in the Guinean zone of Côte d'Ivoire, by Koffi *et al.* (2018a), have been the subject of some works, in particular, *Sarcophaga carnaria* (Diptera: Sarcophagidae) and *Lucilia sericata* (Diptera: Calliphoridae), respectively, by Dao *et al.* (2017) and Yapo *et al.* (2017). In addition, recent studies on necrophagous insects in Côte d'Ivoire, published by Koffi *et al.* (2017a, 2017b, 2018a, 2018b), have highlighted the large abundance of *Chrysomya albiceps*, a necrophagous Diptera commonly used in entomological expertise to date deaths. In Côte d'Ivoire, this Diptera Calliphoridae belongs to the very first group of insects that colonizes a corpse exposed in the open air, a few hours after death (Koffi *et al.*, 2017a). In addition, it is likely to colonize a corpse exposed outdoors, whatever the time of year (2018b). But, so far, no studies, relating to the biology, in the subequatorial natural environment of Côte d'Ivoire, of *Chrysomya albiceps*, have been realized.

This is the reason why, the main objective of this work was to study, in the subequatorial natural environment of Côte d'Ivoire, some parameters of the biology of *Chrysomya albiceps*. More specifically, it was a question of evaluating the duration of its development cycle, of determining the influence of the quantity of nutrient substrate, on the duration of its development cycle and of evaluating the effect of larval density on the loss of mass of this nutrient substrate.

MATERIAL AND METHODS

Study Site

The work of this study is carried out on the site of the National Center of Floristic (NCF) of the Félix Houphouet-Boigny University, in the autonomous district of Abidjan. The National Center of Floristic,a property of the Félix Houphouet-Boigny University of Cocody, was created on July 12, 1973, by the decree number 73-347. It is a space of research on the floristic diversities of Côte d'Ivoire and West Africa. Built on an area of 10 hectares, it is bordered on the north by the old road Bingerville, south by the ravine separating the Riviera Golf and campus. On the west side, are the campus, the FRU of economics and management, administrative and politicallegal sciences. It is limited to the east by the highway leading to the Henri Konan Bédié bridge. It has geographic coordinates, 05°20'North latitude and 03°65' West longitude (Figure 1).

Thanks to its geographical situation, the city of Abidjan is rocked by a hot and humid subequatorial climate. The main climatic parameters of temperature (°C), relative humidity (%) and rainfall (mm) recorded during the three years 2014, 2015 and 2016, are shown in Figure 2.

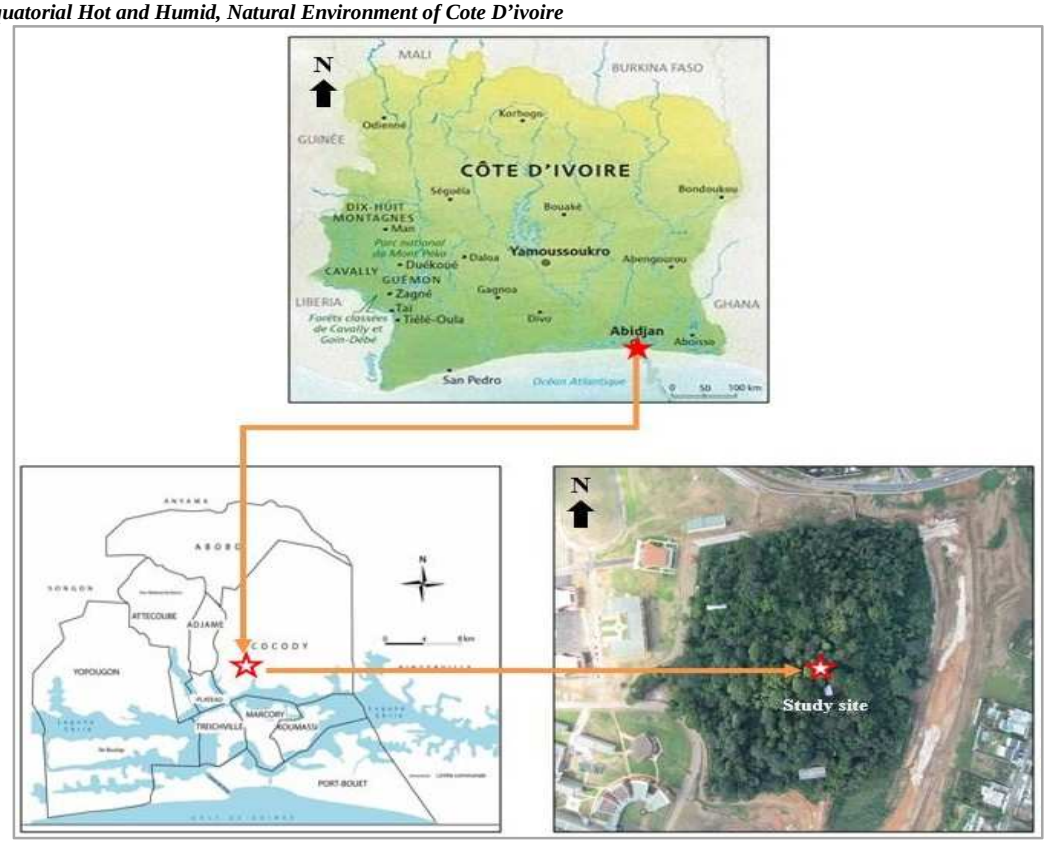


Figure 1: Location of the Study Site within the National Floristic Center (CNF) of the Félix Houphouët-Boigny University (http://www.Google-Maps.com/) (Accessed on 24/12/2016)

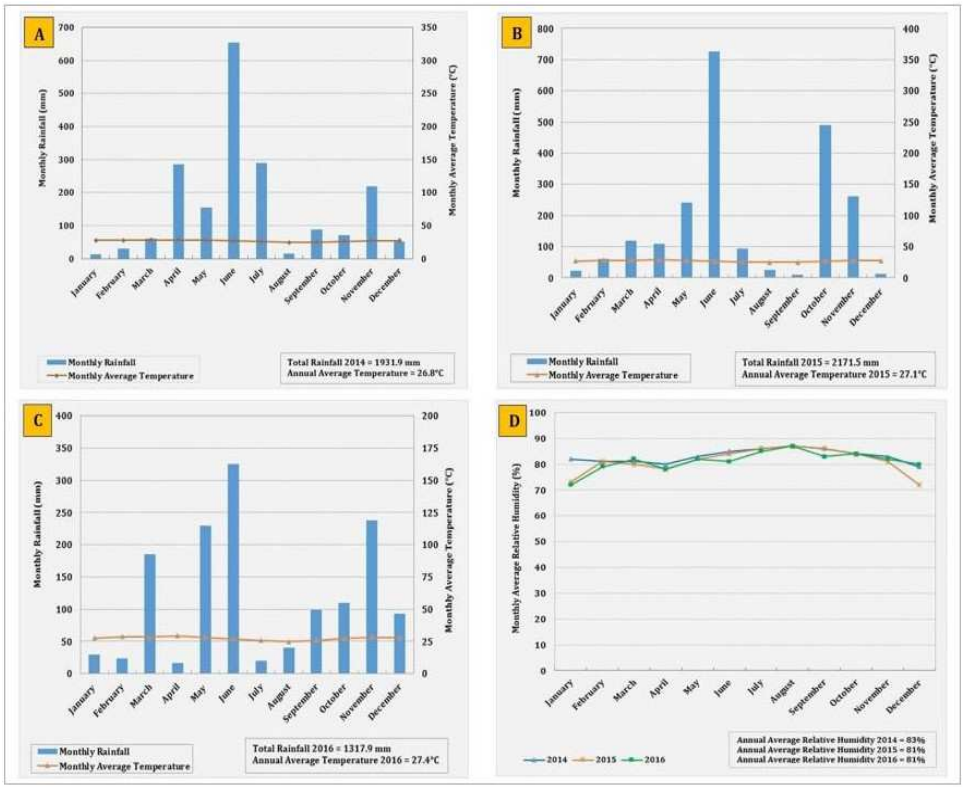


Figure 2: Monthly Average Temperatures and Monthly Rainfall Recorded in the City of Abidjan. A - 2014, B - 2015, C - 2016. D - Monthly Average Relative Humidity 2014, 2015 and 2016. (Data Provided by SODEXAM - Abidjan, Côte D'ivoire)

METHODS

Mass Breeding of Necrophagous Diptera

Adult individuals of Chrysomya albiceps were obtained through mass rearing. To do this, a piece of fresh pork liver, weighing 500 g and placed on a tray, was exposed for 4 hours in the open air (Dao *et al*., 2017). After the spawning of the various necrophagous Diptera, the substrate was introduced into a cylindrical breeding box, made of transparent plastic (height = 14 cm, diameter = 12 cm). The substrate was placed on a layer of about 3 cm of sterilized wet sand. The breeding box was then placed inside a large mosquito net cage (2 m high, 2 m long and 1.5 m wide). Throughout the experimental period, from March 26 to April 29, 2017, the thermo-hygrometer placed inside the experimental cage, recorded an average ambient temperature of 28.24 ± 0.13°C and an average relative humidity 84.28 ± 0.39%. The follow-up of the development cycle was done every day from 6 am to 6 pm...Throughout the larval development phase, a 30 -gram piece of liver was added each day to provide the larvae with sufficient food and to avoid competition for food. At the time of the prepupal phase, the prepupae were transferred to a transparent plastic emergence box and ventilated with a mosquito net (length = 32 cm, width = 22 cm and height = 20 cm). After the emergence of adults, a first sorting was performed on the basis of morphological characters using a vacuum bag. Then, some of the individuals were stung and identified using a binocular loupe and identification keys of the necrophagous Diptera (Szpila, 2014; Irish *et al.*, 2014).

Specific Breeding of *Chrysomya Albiceps*

The breeding of *C. albiceps* was carried out according to the method of Madeira (2001), in a mosquito net enclosure, under ambient conditions, with an average temperature equal to 28.24 ± 0.13°C and average relative humidity equal to 84.28 ± 0.39%. To reach sexual maturity, adults of *C. albiceps* were fed honeydew (50: 50) for five days. For stimulation of ovarian activity, 4 Petri dishes containing cotton soaked in fresh pig blood were placed in the enclosure for 24 hours. Then, a piece of pig liver weighing 250 g was used as a spawning substrate, to replace the blood.

Number of Eggs Laid Per Female

Ten adult pairs of *Chrysomya albiceps*, seven days old, were released into the rearing enclosure containing a 250 g portion of pork liver. After each egg-laying, the clusters of eggs were recovered and then counted under a binocular loupe. The tracking of the laying was done until the death of the last female. During this follow-up, the average number (F) of eggs laid (Oi) per female, was determined by the following relation:

$$\mathbf{F} = \frac{\sum \boldsymbol{Oiri}}{\sum \boldsymbol{ri}}$$

Oi = total number of eggs laid, ***ri*** = female population

Egg Incubation Duration

The eggs being very fragile for handling, the laying of ten new seven-day-old adult *Chrysomya albiceps* pairs was used to determine the incubation time. The substrate carrying the eggs was placed in a breeding cage containing sterilized sand. The eggs were regularly observed by hand magnifying glass until hatching. The mean incubation period (Pi), which is the time between egg-laying (P) and hatching (E), was obtained using the following formula:

$$\mathbf{Pi} = \frac{\sum tivi}{\sum vi}$$

ti = E – P, ***vi*** =number of hatched eggs

Larval and Pupal Development Duration

After hatching of eggs laid by adult females of *Chrysomya albiceps*, 200 stage 1 larvae were monitored daily, at regular intervals of two hours, until the prepupal phase. The prepupal phase is the time that separates the moment of obtaining the larvae of stage 3 (J_{L3}), that of the pupa (J_P); it was determined by the following relation:

$$\mathbf{DPr} = \frac{\sum fisi}{\sum si}$$

fi= J_P-J_{L3},***si*** =number of larvaeL3

After the prepupal phase, the pupae were removed from the breeding cage for another called emergence cage. The duration of the pupil phase (Dp) is the time that separates the prepupal phase (P) from the emergence of the adult (Ea). It was determined by the following formula:

$$\mathbf{Dp} = \frac{\sum gizi}{\sum zi}$$

gi =Ea-P,***zi*** = number of pupae

Pupation Rate, Emergence Rate and Sex Ratio

Obtained from eggs laid by adult females of *Chrysomya albiceps*, 200 stage 1 larvae were monitored daily from 6:00 am to 6:00 pm until adults emerged. For the determination of pupation rate Tn, the pupae were counted. This rate corresponds to the ratio of the total number of nymphs (Nn) obtained to the total number of stage 3 larvae (NL3), expressed as a percentage:

$$\boldsymbol{Tn}\ (\%) = \frac{\boldsymbol{Nn}}{\boldsymbol{NL3}} \times \mathbf{100}$$

Concerning the determination of emergence rate Tem, empty pupae were counted after the emergence of adult flies. It corresponds to the ratio of the number of images (Nim) to the total number of nymphs (Nn), expressed as a percentage:

$$\boldsymbol{Tem}\ (\%) = \frac{\boldsymbol{Nim}}{\boldsymbol{Nn}} \times \mathbf{100}$$

As for the sex ratio, it corresponds to the ratio of the number of males to the number of females.

$$\boldsymbol{Sex\text{-}ratio} = \frac{\boldsymbol{Nombre\ de\ mâles}}{\boldsymbol{Nombre\ de\ femelles}}$$

Imaginal Life of *Chrysomya Albiceps*

The imaginal life of *C. albiceps* was obtained from the 200 stage 1 larvae mentioned above. After following these larvae to imaginal emergence, the adults were fed honeyed water and fresh pig liver until the last individual died. During the follow-up, the nutrient substrate was renewed each morning, then the number of dead individuals and the date were recorded. The life is the time between the date of the emergence of the image and that of his death. Average life - times (days) for females and males were calculated from the following formula:

$$Dv = \frac{\sum xini}{\sum ni}$$

xi =individual lifetime, ***ni*** =insects population

Effect of the Quantity of Substrate on the *Chrysomya Albiceps* Development Cycle

Five portions of 50, 100, 150, 200 and 250 g of pork liver were placed in 5 different breeding boxes, made of clear plastic. In each box, 200 stage 1 larvae were introduced. Their evolution was observed until the emergence of adult flies. For this study, 3 repetitions were made. The calculated parameters were larval development time, prepupal duration, pupal development time, pupation rate, emergence rate from stage 1 larvae, and adult life duration.

Effect of larval density on the rate of mass loss of the substrate

Six 200 g portions of pork liver, including 1 control portion, were placed in six different clear plastic breeding boxes. Five different densities of stage 1 larvae, namely, 100, 150, 200, 250 and 300 larvae, were introduced into the five breeding boxes. In the 6th control box, no larvae were introduced. After 72 hours, the larvae were removed and the substrate of each of the boxes was weighed (P1) as well as that of the control boxes (P0). Three repetitions were made for each larval density as well as the control. The rate of mass loss was calculated and corrected by Abbott's formula:

$$Tc1\ (\%) = \frac{T1 - T0}{100 - T0} \times 100$$

T_{c1} = Corrected rate of loss of mass of a given portion of the liver after 72 hours

T_1 = Rate of loss of mass of a given portion of the liver after 72 hours

T_0 = Rate of mass loss of the control the liver portion after 72 hours

Data Processing

All data were processed using the Statistica 7.1 software. The different homogeneous groups were separated using the Newman-Keuls test at the 5% probability level. The curves and histograms were made with the Microsoft Excel 2007 spreadsheet.

RESULTS

Biological Parameters of Chrysomya Albiceps, Studied from Larvae Reared on a Potion of 250 g of Pork Liver

Number of Eggs Laid Per Female

The total number of eggs laid by the 10 females was 2580 eggs. The number of eggs laid per female of *Chrysomya albiceps*, was therefore 258.

Egg Incubation Time

The incubation time of the eggs of *Chrysomya albiceps*, on the 250 g portion of pork liver, ranged from 7.92 to 16.8 hours, for an average duration of 11.04 ± 0.02 hours.

Duration of Larval Development and Pupal

In this species, the average incubation time of eggs was 0.46 ± 0.02 days. The larval development of *Chrysomya albiceps* consisted of 3 stages. The passage from stage 1 larvae to stage 3 larvae averaged 3.93 ± 0.11 days. The prepupal phase lasted on average, 1.60 ± 0.09 days. As for the duration of the pupal phase, it was on average 6.17 ± 0.14 days. Therefore, the average development cycle time of *Chrysomya albiceps*, reared on the 250 g substrate of the porcine liver (reference substrate), was 12.17 ± 0.14 days. This average duration of the complete development cycle of *C. albiceps* was performed at an average temperature of 28.24 ± 0.13°C and a mean relative humidity of 84.28 ± 0.39% (Figure 2). After the emergence of adults, the average time taken by the image to achieve the first egg-laying was 7 ± 0.5 days.

Pupation Rate, Emergence rate, and Sex Ratio

The mean pupation rate of *Chrysomya albiceps* was 84.15 ± 0.71%. The average emergence rate of adults was 96.73 ± 1.21%. The mean sex ratio of adults emerged from *C. albiceps* was 1.02 ± 0.07.

Imaginal Lifetime of *Chrysomya Albiceps*

The mean adult longevity of *C. albiceps*, after emergence, was 24.05 ± 0.45 days.

Effect of the Nutrient Substrate Quantity on Some Biological Parameters of *Chrysomya Albiceps*

Larval Phase

The development cycle of *Chrysomya albiceps* consisted of 3 larval stages and one pupal stage. On the substrate of 50 g of pig liver, the mean larval development time was 3.43 ± 0.09 days. Regarding the average larval development times on the 100, 150, 200 and 250 g portions of pork liver, they were respectively 3.67 ± 0.13, 3.83 ± 0.09, 3.87 ± 0.09 and 3.93 ± 0.11 days. Analysis of variance and the Newman Keuls test, at the 5% threshold ($F = 3.685$, $df = 4$, P-value < 0.05), showed a significant difference between the average development times of the larvae grown on the different substrates quantities (Figure 3E). The amount of substrate therefore, seemed to influence the larval development time.

Prepupal Phase

After stopping feeding, stage 3 larvae left the feeder substrate to bury themselves in the sterilized sand. They have become motionless. Their integument became sclerified and took a color that gradually changed from light brown to dark brown. The larvae grown on substrates of 50, 100, 150, 200 and 250 g had a prepupal phase which respectively lasted 1.2 ± 0.07, 1.2 ± 0.08, 1.4 ± 0.09, 1.5 ± 0.09 and 1.6 ± 0.09 days. The analysis of variance and the Newman Keuls test at the 5% threshold indicated a significant difference between the average duration of the prepupal phase at the level of the rearing substrates ($F = 3.798$, $df = 4$, P-value < 0, 05) (Figure 3F). Under these conditions, it seems that the quantity of substrate has influenced the duration of this phase.

Pupal Phase

The duration of the pupal phase was 4.20 ± 0.12, 4.50 ± 0.10, 5.07 ± 0.16, 6.10 ± 0.15 and 6.17 ± 0.14 days, for the larvae respectively raised on the substrates of 50, 100, 150, 200 and 250 g. The analysis of variance and the Newman

Keuls test at the 5% threshold revealed a highly significant difference between the average pupal development times ($F = 43.643$, $df = 4$, $P < 0.05$) (Figure 3G). It therefore, seems that the amount of substrate has strongly influenced the pupal development time.

To feed at all stages, the larvae formed groups on the different substrates on which they were grown. From stages 2 and 3, an intra-specific predation phenomenon was observed, when the quantity of nutrient substrate decreased (Figure 3H). This phenomenon was more accentuated on substrates of 50 and 100 g. On the 50 g substrate, this phenomenon was observed at the time interval between 55.68 and 76.8 hours. On the 100 g, it was observed between 70.8 and 82.32 hours. This intra-specific predation caused a decrease in the number of pupae.

Total Duration of the Complete Development Cycle of *Chrysomya Albiceps*

The total development time of *C. albiceps* was the sum of incubation, larval development, prepupal development, and pupal development. The total development times of *C. albiceps* were 9.30 ± 0.11, 9.93 ± 0.11, 10.77 ± 0.13, 11.93 ± 0.13 and 12.17 ± 0, 14 days, respectively on the substrates of 50, 100, 150, 200 and 250 g. Analysis of variance and the Newman Keuls test at the 5% threshold revealed a highly significant difference between the total development times of larvae grown on substrates of different masses ($F = 104.64$, $df = 4$; P-value < 0.001) (Table 1). The quantity of the substrate appeared to influence the total development time of *C. albiceps*.

Pupation Rate

Mean pupation rates of *Chrysomya albiceps* were 28.83 ± 0.62, 38.77 ± 0.65, 50.57 ± 0.66, 74.35 ± 0.65 and 84.15 ± 0.71 %, respectively, on substrates of 50, 100, 150, 200 and 250 g. The analysis of variance and the Newman Keuls test at the 5% threshold revealed a highly significant difference between the average pupation rates of *C. albiceps* on the different substrates ($F = 1256.94$, $df = 4$, P-value < 0.0000) (Table 2). The quantity of substrate influenced the pupation rate of the larvae.

Emergency Rate

Mean adult emergence rates of *Chrysomya albiceps* were 28.43 ± 0.82, 38.37 ± 1.03, 51.23 ± 1.08, 67.13 ± 1.08 and 68.40 ± 1.09%, respectively for larvae raised on 50, 100, 150, 200 and 250 g of pig liver. The analysis of variance and the Newman Keuls test at the 5% threshold revealed a highly significant difference between *C. albiceps* emergence rates, the larvae of which were raised on the different substrate quantities ($F = 286, 78$, $df = 4$, P-value < 0.05) (Table 2). The amount of substrate significantly influenced the emergence rate of adults of *Chrysomya albiceps.*

Sex-Ratio

Adult sex ratios of *Chrysomya albiceps*, emerged from larvae reared on the different substrate quantities, were 0.92 ± 0.22, 1.10 ± 0.06, 0.94 ± 0.07, 1, 00 ± 0.07 and 1.02 ± 0.07, respectively for substrates of 50, 100, 150, 200 and 250 g. The analysis of variance and the Newman Keuls test at the 5% threshold revealed no significant difference between the sex ratios of *C. albiceps* emerged from substrates of different masses ($F = 0.3771$, $df = 4$, P-value > 0.05) (Table 2).

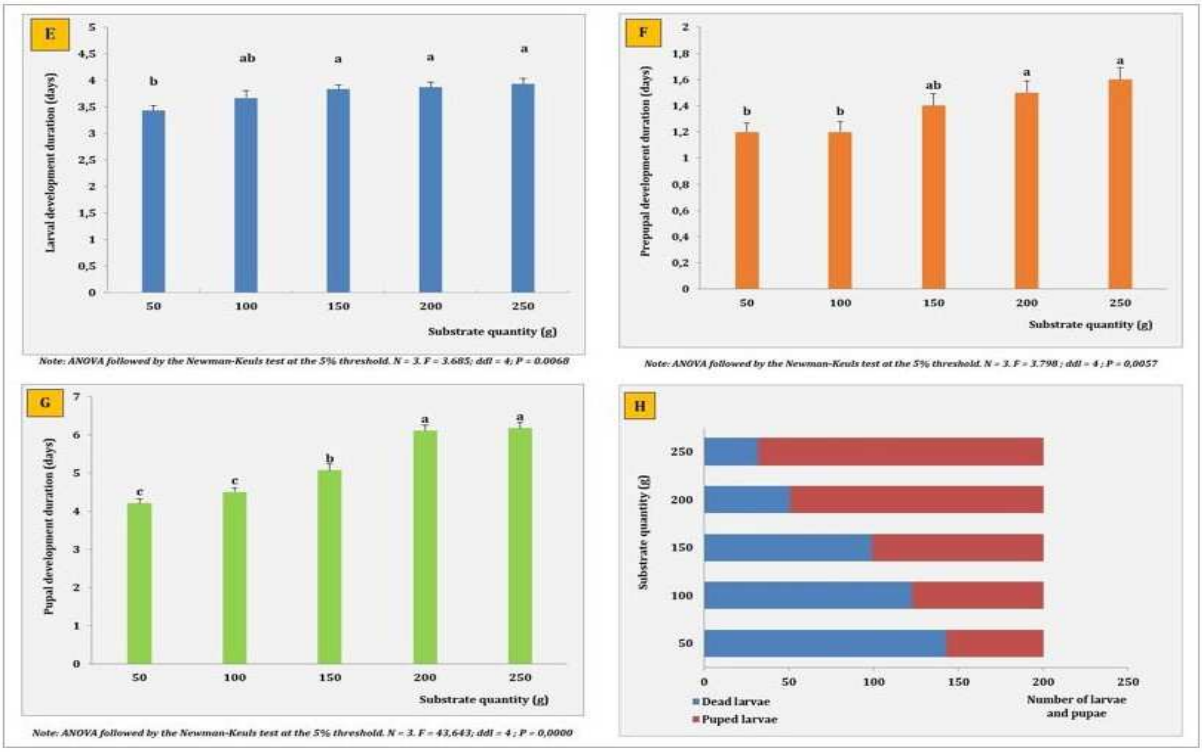


Figure 3: E - Development Duration of *Chrysomya Albiceps* Larvae, Depending on the Quantity of Nutrient Substrate. F - Prepupal Development Time as a Function of the Quantity of Nutrient Substrate. G - Duration of Pupal Development as a Function of the Amount of Nutrient Substrate. H - Effect of Intraspecific Predationon Pupal Population

Table 1: Effects of the Nourishing Substrate Quantity of Chrysomya Albiceps Larvae, on the different Stages of Development

Substrate quantity (g)	D_L (days)	D_{PR} (days)	D_P (days)	D_T (days)
50	3.43 ± 0.09^{b}	1.20 ± 0.07^{b}	4.20 ± 0.12^{c}	9.30 ± 0.11^{d}
100	3.67 ± 0.13^{ab}	1.20 ± 0.08^{b}	4.50 ± 0.10^{c}	9.93 ± 0.11^{c}
150	3.83 ± 0.09^{a}	1.40 ± 0.09^{ab}	5.07 ± 0.16^{b}	10.77 ± 0.13^{b}
200	3.87 ± 0.09^{a}	1.50 ± 0.09^{a}	6.10 ± 0.15^{a}	11.93 ± 0.13^{a}
250	3.93 ± 0.11^{a}	1.60 ± 0.09^{a}	6.17 ± 0.14^{a}	12.17 ± 0.14^{a}

Note : D_L = Larval Development (F = 3.685 ; ddl = 4; P = 0.0068), D_{PR} = Prepupal Development (F = 3.798 ; ddl = 4 ; P = 0.0000), D_P = Pupal Development (F = 43.643 ; ddl = 4 ; P = 0.0000), D_T = Total Development (F = 104.64 ; ddl = 4 ; P = 0.0000. The numbers followed by the same letters in the same column are not significantly different, at the 5% threshold according to the Newman Keuls test.

Life Duration of Images

The mean adult lifetime of *Chrysomya albiceps* was 15.98 ± 0.30, 17.20 ± 0.30, 18.30 ± 0.29, 22.17 ± 0.44 and 24.05 ± 0.45 days, respectively, for substrates of 50, 100, 150, 200 and 250 g. Analysis of variance and the Newman Keuls test at the 5% threshold revealed a significant difference between the lifespan of adults emerged from substrates of different masses (F = 89.14, df = 4, P-value < 0.05) (Table 2).

Table 2: Effects of the Quantity of Feeder Substrate of *Chrysomya Albiceps* Larvae, on Pupation, Emergence of Adults, Imaginal Lifetime and Sex Ratio

Quantity of substrate (g)	Average imaginal lifetime (days)	Average pupation rate (%)	Average emergence rate (%)	Sex-ratio
50	15.98 ± 0.30^{e}	28.83 ± 0,62^{e}	28.43 ± 0.82^{c}	0.92 ± 0.22^{a}
100	17.20 ± 0.30^{d}	38.77 ± 0.65^{d}	36.17 ± 1.02^{d}	1.10 ± 0.06^{a}
150	18.30 ± 0.29^{c}	50.57 ± 0.66^{c}	51.67 ± 1.08^{c}	0.94 ± 0.07^{a}
200	22.17 ± 0.44^{b}	74.35 ± 0.65^{b}	81.20 ± 1.21^{b}	1.00 ± 0.07^{a}
250	24.05 ± 0.45^{a}	84.15 ± 0.71^{a}	96.73 ± 1.21^{a}	1.02 ± 0.07^{a}

Note: ANOVA followed by Newman Keuls test at 5%; N = 30. Average lifetime (F = 89.14, ddl = 4, P = 0.0000); Pupation rate (F = 1256.94, ddl = 4, P = 0.0000); Emergence rate (F = 734.45, ddl = 4, P = 0.0000); Sex ratio (F = 0.3771, ddl = 4, P = 0.0000). The numbers followed by the same letters in the same column are not significantly different at the 5% threshold according to the Newman Keuls test.

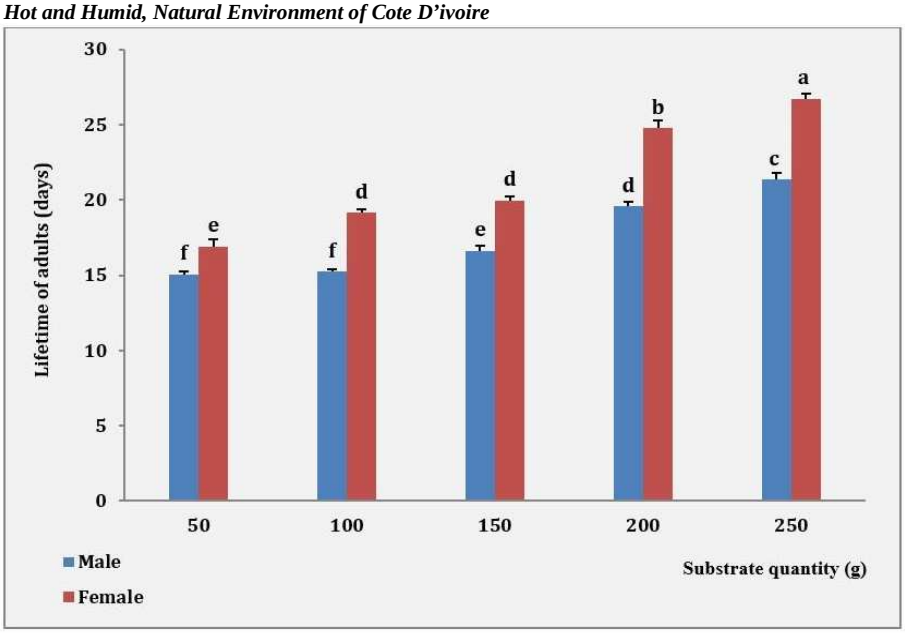
Imaginal Lifetime According to Sex

Considering the average lifetimes according to sex, which of the females derived from the substrate of 50 g, was 16.90 ± 0.51 days. Those raised on substrates of 100, 150, 200 and 250 g, respectively, had an average life duration of 19.17 ± 0.23, 19.97 ± 0.24, 24.77 ± 0.49 and 26.73 ± 0.34 days. Mean male lifetime was 15.07 ± 0.20, 15.23 ± 0.19, 16.63 ± 0.31, 19.57 ± 0.28 and 21.37 ± 0.46 days, respectively for substrates of 50, 100, 150, 200 and 250 g. The analysis of variance and the Newman Keuls test at the 5% threshold revealed a significant difference between the mean lifetime of males and females of *C. albiceps*, reared on different substrates quantities ($F = 126.86$, $df = 9$, P-value < 0.001) (Figure 4).

Indeed, in general, whatever the amount of feeder substrate, on which the larvae of *Chrysomya albiceps* were raised, the average life duration of emerged female adults is still significantly greater than that of adult males. At the same time, we observed that in females, the average longevity of adults was significantly influenced by the different nutrient substrate quantities on which the larvae were raised (Figure 5). The same is true for adult males (Figure 6).

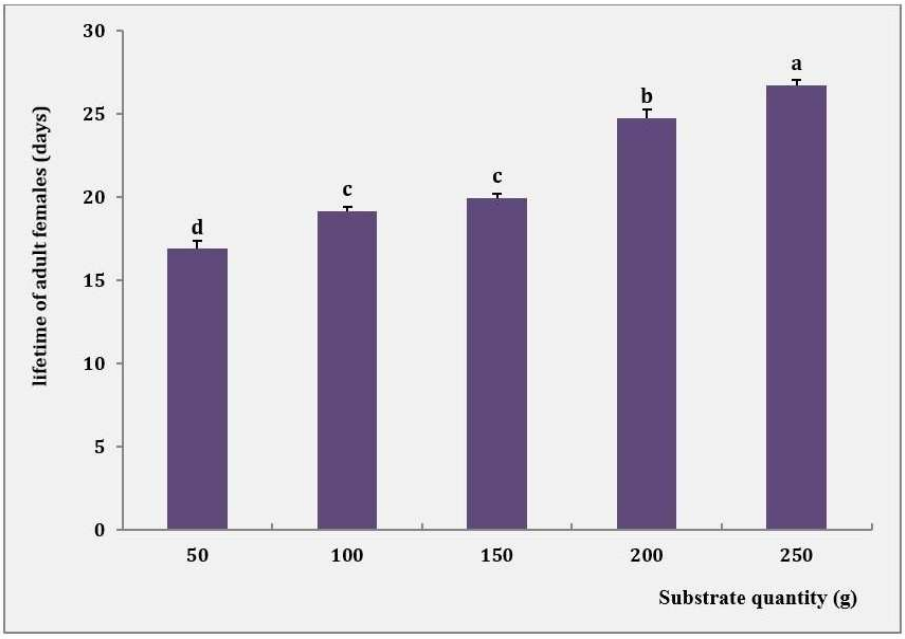
Effects of Larval Density on the Rate of Mass Loss of Nutrient Substrate

The experiment carried out made it possible to highlight the effect of the larval density on the rate of mass loss of the nourishing substrate. The lowest mass loss rates were obtained with densities of 100 and 150 larvae, that to say, respectively, 0.02 and 0.08%. The highest was obtained with densities of 200, 250 and 300 larvae, that to say, respectively, 0.13, 0.19 and 0.25%. The analysis of variance and the Newman Keuls test at the 5% threshold revealed a significant difference between the mass loss rates caused by the different larval densities ($F = 11688.4$, $df = 4$, P-value < 0.001) (Table 3).



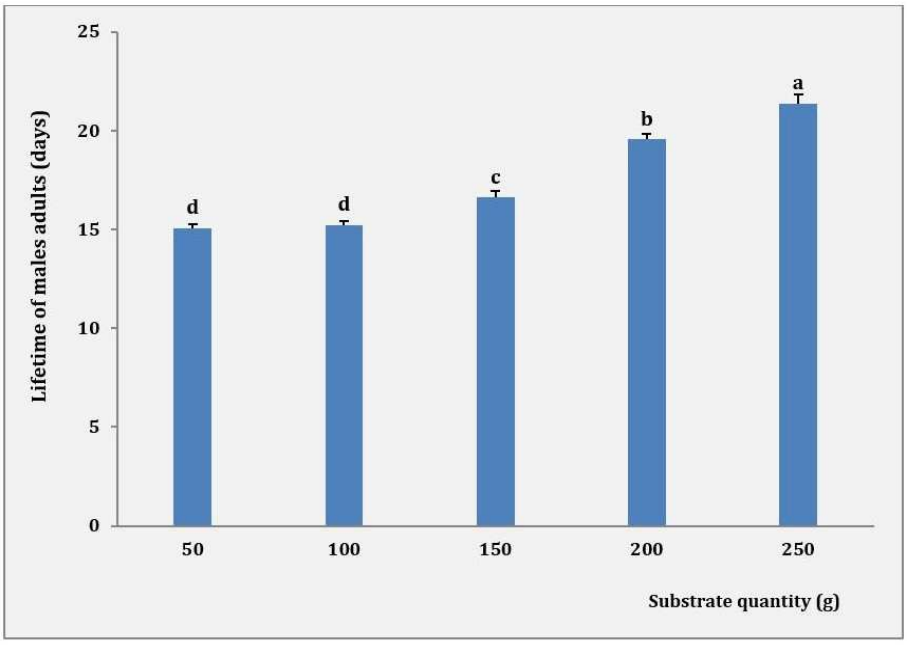
Note: ANOVA followed by Newman Keuls test at the 5% threshold. N = 30; F = 89.14; ddl = 4; P = 0.0000; (M = 50 g, F = 11.053, ddl = 1, P = 0.0015); (M = 100 g, F = 173.6, ddl = 1, P = 0.0000); (M = 150 g, F = 72.175, ddl = 1, P = 0.0000); (M = 200 g, F = 83.798, ddl = 1, P = 0.0000); (M = 250 g, F = 87.971, ddl = 1, P = 0.0000)

Figure 4: Compared Longevity of Adult Male and Female *Chrysomya Albiceps*, Reared on different Quantity of Nutrient Substrate



Note: ANOVA followed by Newman Keuls test at the 5% threshold. N = 30 F = 114.04; ddl = 4; P = 0.0000.

Figure 5: Compared Longevity of Female Adults of *Chrysomya Albiceps*, Reared on Different Quantities of Nutrient Substrate



Note: ANOVA followed by Newman Keuls test at the 5% threshold. N = 30. F = 83.77; ddl = 4; P = 0.0000.

Figure 6: Compared Longevity of Male Adults of *Chrysomya Albiceps*, Reared on Different Quantities of Nutrient Substrate

Table 3: Effects of Larval Density on the Loss Rate Mass of the Nutrient Substrate

Larval density (or Number of larvae)	Loss rate mass of the substrate (%)
100	0.02 ± 0.001^{e}
150	0.08 ± 0.001^{d}
200	0.13 ± 0.001^{c}
250	0.19 ± 0.001^{b}
300	0.25 ± 0.001^{a}

Note: ANOVA followed by Newman Keuls test at the 5% threshold. N = 30. F = 11688.4; ddl = 4; P = 0.0000.

DISCUSSIONS

Chrysomya albiceps is one of the earliest species of Diptera Calliphoridae that colonizes a corpse exposed in the open air, in the southern forest zone of Côte d'Ivoire, only a few hours after death (Koffi *et al.*, 2017a). In this species, as in most necrophagous Calliphoridae, the early appearance of females on an exposed corpse is motivated by egg-laying. In our experimental conditions, the average number of eggs laid per female was 258. Our results are a few superior to those of Wall (1993), who performed his work, in an open temperate environment, on *Lucilia sericata*, a species of necrophagous Calliphoridae, very close to *Chrysomya albiceps*. This difference in the results could be explained by the higher temperature of the hot and humid subequatorial climate, which favored, in our experimental conditions, the spawning of *C. albiceps* females.

After hatching, larvae reared on large portions of the feeder substrate had a longer developmental life duration than those grown on small portions of pig liver. The pupae obtained after the larval phase, have respectively undergone a similar development time. Our results are similar to those of Yapo *et al*. (2017), who studied some aspects of the biology of *Lucilia sericata*, in the natural environment, in the southern forest zone of Côte d'Ivoire. The fact that the quantity of substrate has significantly influenced the duration of larval development and pupal development, could be explained by a greater availability of nutrients in the larger portions. On these, the larvae had time to move from one stage to another, in contrast to small portions of the substrate, on which the duration of larval stages was shortened. Indeed, according to Denno and Cothran (1975), the size of the substrate can limit the availability of food, which in turn can influence the evolution of larval populations. In addition, stage 2 and 3 larvae exhibited intraspecific predation when their food source became insufficient. This had the effect of reducing their numbers.

Pupal development time was the longest phase of all stages of *C. albiceps* development. This phenomenon was also observed by Greenberg (1971). He noted that time spent in the prepupal and pupal stages can represent up to 75% of the total development time. This would be due to the fact that, the differentiation of the organs of the adult insect, starts from this stage.

The low pupation rates observed on the different portions of pork liver were also obtained by Faria *et al*. (1999). These researchers observed that, after the total disappearance of the breeding substrate of *Chrysomya albiceps* larvae, the competition is transformed into intra-specific predation. Thus, the low pupation rate obtained with small substrates is due to the death of certain larvae, victims of intra-specific predation. This result joins those of Putman (1977) and Smith & Wall (1997), according to which competition appears to be the main factor limiting the larval populations that develop on the body, mainly in the case of small cadavers.

Concerning the effect of larval density, on the rate of mass loss of the nutrient substrate, the results showed that the rate of mass loss increases with density. These results are consistent with those of Fabre (1923), Hobson (1932b), Putman (1977) and Slone & Gruner (2007). These authors observed that gregorian , formed by a high density of larvae, produces a local rise in temperature that can reach surprising proportions. Thus, the rapid loss of mass of the liver portion, with a high larval density, could be explained by an easiness of feeding the larvae. This ease of feeding would be due to the local liquefaction of the tissues, by the joint action of the salivary enzymes and the movements of the hooks of the larvae, caused by the elevation of the temperature.

Regarding the emergence rate, it was higher on large substrates. The lowest emergence rates were obtained with small portions of pig liver. The significant influence of substrate size on adult emergence rates could be explained by the fact that large substrates had sufficient nutrients for the development of *Chrysomya albiceps* larvae while avoiding the intraspecific predation phenomenon.

As for the sex ratio, it showed no significant difference on the different substrates. This observation is consistent with Yapo *et al*. (2017), who carried out their work on *Lucilia sericata*, a species close to *Chrysomya albiceps*.

The study of the life duration of adults recorded a significant difference in different substrates. Adults emerged from larvae reared on large substrates and lasted longer than those that emerged from larvae grown on small substrates. These results are consistent with those of Higley *et al*. (1986), Joplin and Moore (1999) and Nabity*et al.* (2007). According to these authors, the availability of nutrients (proteins, lipids) and mineral elements (ammonia, iron, zinc) influences the development of insects. Thus, this difference in adult longevity would be due to the nutrient availability of the substrates.

In fact, the larvae, which have been sufficiently nourished, have more protein energy, which is necessary to ensure the rest of their imaginal life.

Regarding the average imaginal life, according to sex, it was longer in females than in males, on all substrates. Our results are similar to those of Rueda *et al*. (2010). The short lifetime of males could be explained by the fact that they release during the mating, a significant quantity of energy. This argument was also made by Rueda *et al*. (2010), studying the table of life of *Lucilia sericata*. Thus, the reproductive activity would contribute to a sharp decrease in the longevity of males and females. This is what Williams (1984) called "cost of reproduction", a concept that links the effort of reproduction to the other functions of the insect.

CONCLUSIONS

The few aspects of the *Chrysomya albiceps* biology, studied in the natural environment, subequatorial hot and humid of Côte d'Ivoire, made it possible to note that it is an oviparous insect, with holometabolic development. The average number of eggs laid by a *C. albiceps* female was 258.03 ± 91 at an average temperature of 28.24 ± 0.13°C and a mean relative humidity of 84.28 ± 0.39%.

Its complete development cycle has highlighted, three larval stages and a pupal stage. In stages 2 and 3, larvae of *C. albiceps* practice intra-specific predation, when the food source is insufficient. In our experimental conditions, the mean total development time of *C. albiceps* increased with the size or quantity of the larval nutrient substrate. Females lived longer than males on different substrates. Pupation and emergence rates were significantly influenced by the amount of feeder substrate on the one hand, and intraspecific predation on the other. As for the sex ratio of emerged adults, it was in no way influenced by the different amounts of substrate. The rate of mass loss of the substrate has evolved with the density of the larvae.

In the context of an entomological expertise to date a death, the information gathered through this study, are essential, in so far as, the discovery of a corpse, may as well concern, a small body like that of a child of 8 kg, that of an adult person of 90 kg for example. Under these conditions, one could imagine that the larvae of the same species of necrophagous Diptera, could present different development cycles, according to the important difference in size of the body. Moreover, the results obtained during this study could allow establishing a short or medium postmortem interval, in the context of a realizable judicial investigation in Côte d'Ivoire.

DECLARATION OF INTEREST

The authors declare that they have no conflicts of interest in relation to this article.

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