

BIOLOGICAL PARAMETERS OF *Sarcophaga carnaria* L. (DIPTERA: SARCOPHAGIDAE) NECROPHAGOUS FLY BREEDING ON TWO PIG SUBSTRATES (*Sus scrofa domesticus* L.) AT THE NATIONAL FLORISTIC CENTER, ABIDJAN- IVORY COAST

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**Abstract**

The need to elucidate the crimes, led us to study the necrophagous insects including *Sarcophaga carnaria*, a larviparous fly. These develop while consuming the decaying corpse. This gives them great importance in forensic entomology. The aim of our study is to determine the biological parameters of *S. carnaria*. The work took place at the national floristic center, located at the Félix Houphouët Boigny University. Follow-up was done from 3 september 2015 to 11 november 2015. The total development time of *S. carnaria* was  $16.07 \pm 0.19$  days on the liver at an average temperature of  $26.92 \pm 1.9^{\circ}\text{C}$  and an average relative

humidity of  $85.08 \pm 3.1$  % whereas it was  $17.12 \pm 32$  days on the striated muscle at the same temperature and relative humidity. The rate of emergence on the liver was  $77.55 \pm 3.40$  % on the liver whereas on the striated muscle it was  $63.84 \pm 3.68$  %. The sex-ratio of *S. carnaria* on pig liver was  $0.80 \pm 0.03$  and on striated muscle was  $0.95 \pm 0.06$ . The life expectancy of males and females gave  $16.76 \pm 0.84$  days and  $20.23 \pm 0.77$  days on the liver, whereas on striated muscle, Was  $14.33 \pm 0.21$  days and  $17.03 \pm 0.36$  days at an average temperature of  $26.15 \pm 0.71^\circ\text{C}$  and an average relative humidity of  $86.23 \pm 1.3$  %.

**Keywords:** *Sarcophaga carnaria*, substrate, lifetime, cycle duration, emergence rate, temperature

## INTRODUCTION

The criminal justice system of some African countries lacks modern means of investigation, which has an impact on sentencing rates (United Nations, 2005). It is necessary to strengthen this system for an effective fight against crime through modern means of investigation, including forensic entomology (Gennard, 2007). After the death of a person, forensic medicine is able to determine the date and time of death within 72 hours (Charabidze, 2008). Beyond this threshold, she no longer finds her traditional means of determining the date of death (Charabidze, 2008). The study of the insects present on the corpse appears to be one of the most relevant solutions for estimating the post mortem interval (MPI) (Benecke, 2004). The norms used in medico-legal entomology are those set up by Mégnin (1894) for temperate countries. The literature does not mention any data on the biology of necrophagous insects in Ivory Coast. To overcome this deficiency, we proposed to determine some biological parameters of *S. carnaria* breeding on two pig substrates for use in forensic entomology expertise.

## 1. MATERIALS AND METHODS

### 1.1. Study site

The study was carried out at the National Floristic Center (CNF), located at the Félix Houphouët Boigny University ( $05^\circ 20' \text{N} - 03^\circ 65' \text{W}$ ). The soil saturation rate is less than 20 %. The organic matter content is quite low (2-3%). On a pedological level, the humid horizon is not very thick. The CNF is a completely planted forest environment which includes a botanical garden made up partly of fallow and an arboretum (Kouakou, 2009). The

temperature varied between 23 and 29°C. The relative humidity oscillated between 82 % and 88 %. The follow-up was carried out from 03 september 2015 to 11 november 2015.

## 1.2. Equipment

The study required the use of portions of striated muscle of pig (*S. scrofa domesticus* L.) and liver of mass 200g each. These were used as substrate for the larvae of *Sarcophaga carnaria*. The transfer of adult insects from one cage to another was made possible by a vacuum of diptera and a transfer box. A soft metal clip was used to remove the larvae from the substrates. A thermo-hygrometer was used for the measurement of temperature and relative humidity. The protective equipment consisted of pairs of gloves, a white coat and a nose cover. The identification material used consisted of hand-held magnifiers, a binocular lens, and fly-testing guides Scholtz and Holm (1996); Bourel (2006); Whitworth (2010) and (Szpila, 2014).

## 1.3. Methods

### 1.3.1. Mass breeding of flies

A kilogram of pig or striated muscle liver was arranged each in a plexigrass tray in the open air. After 8 hours of exposure, the tray containing substrates infested eggs or first instar larvae of flies, have been removed, then the substrates were placed in breeding cages. The follow-up was done until the emergence of new flies. After emergence, obtained flies have been identified in order to obtain the species, *Sarcophaga carnaria* for the realization of specific breeding.

### 1.3.2. Specific breeding of *Sarcophaga carnaria*

Three pieces of striated muscle and 3 pieces of pig liver, each weighing 200 g, were used as breeding substrate. The different substrates were placed in breeding cages containing the sand sterilised in the autoclave at a temperature of 121°C and a pressure of 1.5 bar. Thirty pairs of *S. carnaria* have been placed, each in a breeding cage. To release the vitellogenesis in females, 50 ml of pig blood (protein source) has been available to them (Alonso et al., 2015). Twelve hours later the blood was removed, then a piece of striated muscle or liver of pig weighing 200 g was placed in each cage as a spawning substrate. The time of the first larvae laying were noted. The insects were removed 12 hours later. Then, the laying larvae were followed until the emergence of the adult.

### 1.3.3. Determination of larval development the rate

Thirty larvae laid freshly took in a female and placed in a cage of breeding containing pig's liver, and thirty other larvae taken the same female that previously were placed in a cage containing striated muscle. The experience was repeated three times for each substrate. After the contact of breeding larvae with the substrate, three larvae were every day taken in each cage. These were scalded for 30 seconds and then measured with graph paper.

#### 1.3.4. Duration of the development cycle

The study of the length of the development cycle was to follow lots of 10 larvae freshly laid by each of the 30 females at the level of each cage containing striated muscle or liver. The different larval stages have been determined by the appearance of stigmata respiratory. As soon as the last-stage larvae reach the stage of pupation, nymphs are transferred to some other cages called cages of emergences, all devoid of feeder substrate, saw that the pupae do not feed. Larval development time is the time between the moment of laying larvae and the obtaining of larvae of stage 3. This period is the sum of the time of passage of L1 to L2 and that one of L2 to L3 (tl) (DL).

$$\checkmark DL = \frac{\sum d_{iki}}{\sum k_i}$$

$d_i = t_l$ ;  $k_i$ : staff of larva of stage 1

The time between the moment of obtaining the larvae of stage 3 (JL) of the pupa (Jp) is the post-feeding instar noted (P).

$$\checkmark P = \frac{\sum f_{isi}}{\sum s_i}$$

$f_i = J_p - J_L$ ;  $I_f$ : number of larvae L3

The duration of the pupa, which is the time between pupation (P) of the emergence of the adult (Ea). The developmental time of the pupa is the period between obtaining pupa and the adult stage noted  $D_p$ .

$$\checkmark D_p = \frac{\sum g_{izi}}{\sum z_i}$$

$g_i = E_a - P$

$z_i$ : number of pupa

The calculation of the emergence rate (Te) was conducted by the following relationship:

$$\checkmark \quad Te = \frac{Ne}{NtL} \times 100$$

Te: Emergence percentage rate, Ne: number of imagos emerging, NtL: total number of larvae  
Total development duration is the period between the laying of the larvae of the adult stage  
DC.

$$\checkmark \quad DC = Pi + DL + P + Dp$$

### 1.3.5. Determination of the effect of temperature on development time

The study of the effect of temperature on the development of the life cycle of *S. carnaria* time has been determined. To do this, 30 larvae have been introduced each in a cage of breeding at five day intervals. The experiment was repeated three times. From the exposure of substrates to the larvae, the temperature has been taken every morning at 6 hours, at noon and in the evening at 6 pm. These three temperatures are reduced to a daily average temperature. The observations were made until the emergence of the adult. When there is emerging in a cage, temperatures taken since the beginning are reduced to the average temperature of the cycle.

### 1.3.6. Lifecycle of the imagos and the sex-ratio

For the study of the life of the adult, they were relocated in a completely covered cage of chiffon. These imagos of *S. carnaria* were fed with portions of striated muscle or liver and sugar water. Every two days, substrates were removed and replaced by another until the death of the last individual. The number of dead imagos noted every day and the sex is determined. The average duration of life ( $D_v$ ) of imagos, expressed in days, has been determined from the results achieved by establishing the difference of the sum of the products of the different lengths of life ( $x_i$ ) by the number of insects dead ( $n_i$ ) to the sum of the total of dead insects.

$$D_v = \frac{\sum x_i n_i}{\sum n_i}$$

$x_i$ : individual life expectancy;  $n_i$ : number of insects

$$\text{Sex-ratio} = \frac{\text{Number of males}}{\text{Number of females}}$$

### 1.3.7. Data processing

The statistical analysis of the data was carried out using the software Statistica version 7.1. An analysis of two-factor variance (ANOVA) followed by the Newman-Keuls test at the 5 %

threshold allowed us to assess the homogeneity of the samples. Correlations between temperatures and durations of the development cycle were established using the Pearson test.

## 2. RESULTS AND DISCUSSION

### 2.1. Results

#### 2.1.1. Average life cycle

The development cycle of *Sarcophaga carnaria* occurred in 3 larval stages and a pupal stage. In *S. carnaria*, passage from first instar larva to third instar larva occurred from six to eight days, an average duration of  $6.47 \pm 0.14$  days on pigs' liver whereas on the striated muscle, the larval development took place for five to eight days, whether an average duration of  $7.20 \pm 0.18$  days at an average temperature of  $25.90 \pm 0.18^\circ\text{C}$  and  $85.45 \pm 3.2\%$ . The ANOVA followed by the Newman Keuls test at the 5% threshold ( $F = 10.112$ ;  $ddl = 1$ ;  $P = 0.00236$ ) indicated a significant difference between larval development times on the liver and striated muscle (Table I). In the post-feeding time, the stage 3 (L3) larvae leave the substrate to bury into the sterilized sand. At this time, the larva's cuticle contracts and then sclerifies, turning brown to form the pupa (rigid envelope protecting the nymph). Post-feeding time (Pa), time spent by the third stage larvae to become a pupa, was varied from 1.5 to 2 days with an average of  $1.74 \pm 0.11$  days on the liver. This duration varied from 1.3 to 1.8 days, whether an average of  $1.45 \pm 0.12$  days on the striated muscle (Table 1). The ANOVA test followed by the Newman Keuls test at the 5% threshold: ( $F = 3.1601$ ,  $ddl = 1$ ,  $P = 0.080701$ ) revealed that there was no significant difference between the pupation times of this phase in both substrates. The pupal duration occurred between 6-10 days on pig liver with an average duration of  $8.17 \pm 0.15$  days. This development took place from 6 to 12 days, whether an average duration of  $8.37 \pm 0.28$  days on the striated muscle at an average temperature of  $26.06 \pm 1.5^\circ\text{C}$  and an average relative humidity of  $84.72 \pm 4.1\%$ . The ANOVA followed by the Newman Keuls test at the 5% threshold ( $F = 0.399$ ,  $ddl = 1$ ,  $P = 0.5298$ ) revealed no significant difference between the duration of pupal development on the striated muscle and that of the liver (Table I). However, the pupal duration on the striated muscle was greater than that obtained on the liver. The beginning of the emergence was marked by the opening of the puparium by the insect. Once the head came out, he leaned on the latter to have the rest of the body expelled. Just after the emergence, the insect's body increased in volume and its dull color became more radiant. The time taken by the wings of the insect emerged to unfold was

23 ± 2 minutes. The duration of the development cycle (Dc) of *S. carnaria* was 16.07 ± 0.19 days on pig liver at an average temperature of 26.92 ± 1.9°C and at an average relative humidity of 85.08 ± 3.1 % (Figure 3). It was 17.12 ± 0.32 days on the striated muscle under the same ambient conditions (Table 1). The ANOVA test followed by the Newman Keuls test at the 5% threshold (F = 7.897, ddl = 1, P = 0.00674) revealed a significant difference between the developmental period (Dc) of *S. carnaria* on the striated muscle and on the liver. The linear regression between the cycle development time (Larvae of instar1 to adult) and the ambient temperature of the study site showed that this duration decreased when the temperature increased in the liver (r = -0.889, P <0.001) and on the striated muscle (r = -0.841, P <0.001) (Figure 1 and Figure 2).

.Table 1: Average life cycle (day)

Substrate	Larval duration	Post-feeding time	Pupal duration	Cycle duration
Liver	6.47 ± 0.14 <sup>b</sup>	1.74 ± 0.11 <sup>a</sup>	8.43 ± 0.19 <sup>b</sup>	16.07 ± 0.19 <sup>b</sup>
Striated muscle	7.20 ± 0.18 <sup>a</sup>	1.45 ± 0.12 <sup>b</sup>	8.50 ± 0.28 <sup>a</sup>	17.12 ± 0.32 <sup>a</sup>
<i>P-value</i>	0.002364	0.080701	0.846948	0.00674
<i>ddl</i>	1	1	1	1
<i>F</i>	10.112	3.1601	0.038	7.897

ANOVA followed by the Newman Keuls test at the 5 % threshold; N = 30;

NB: Figures followed by the same letters in the same column are not significantly different at the 5 % threshold according to the Newman Keuls test

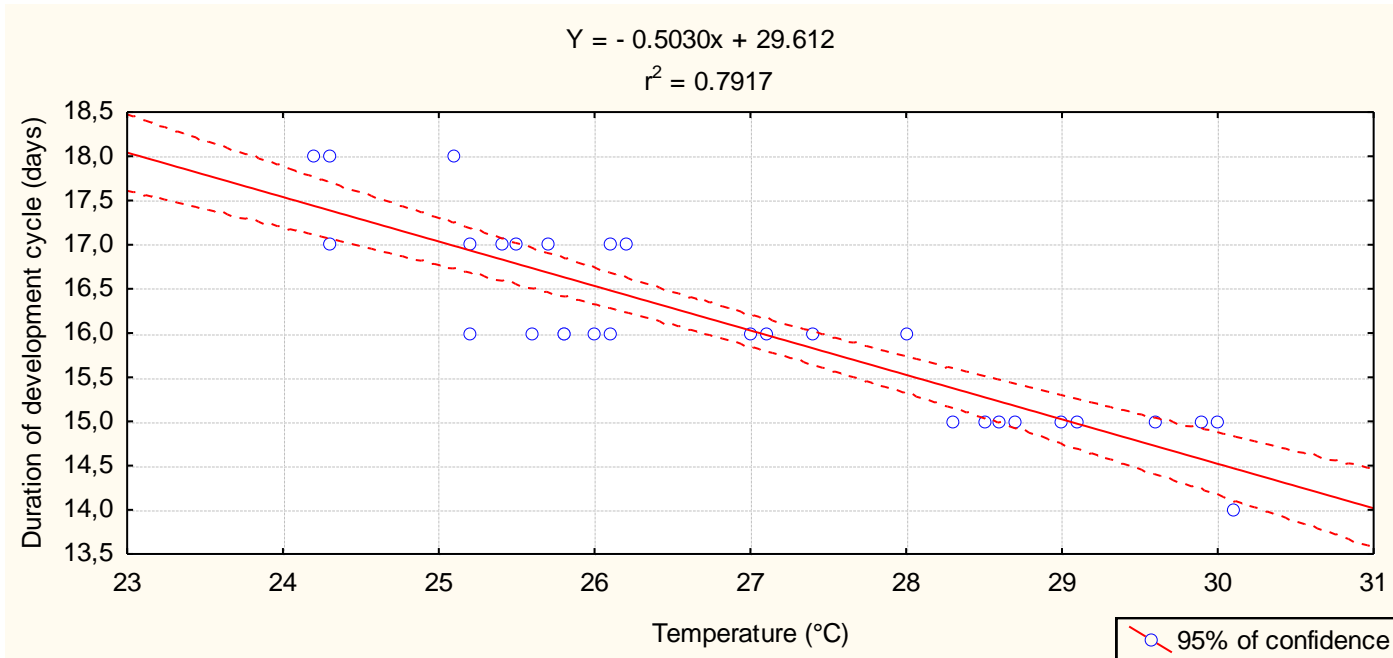


Figure 1: Relationship between temperatures and *S. carnaria* cycle duration on liver

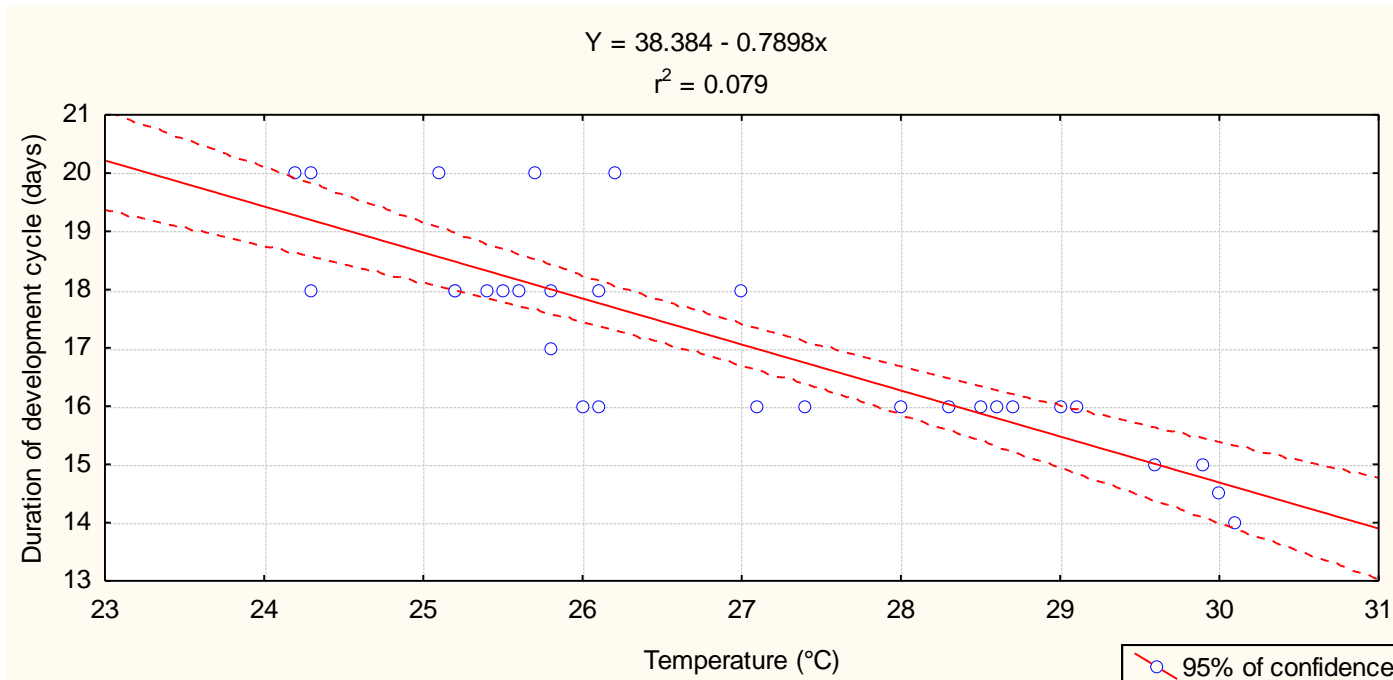


Figure 2: Relationship between temperatures and *S. carnaria* cycle duration on striated muscle



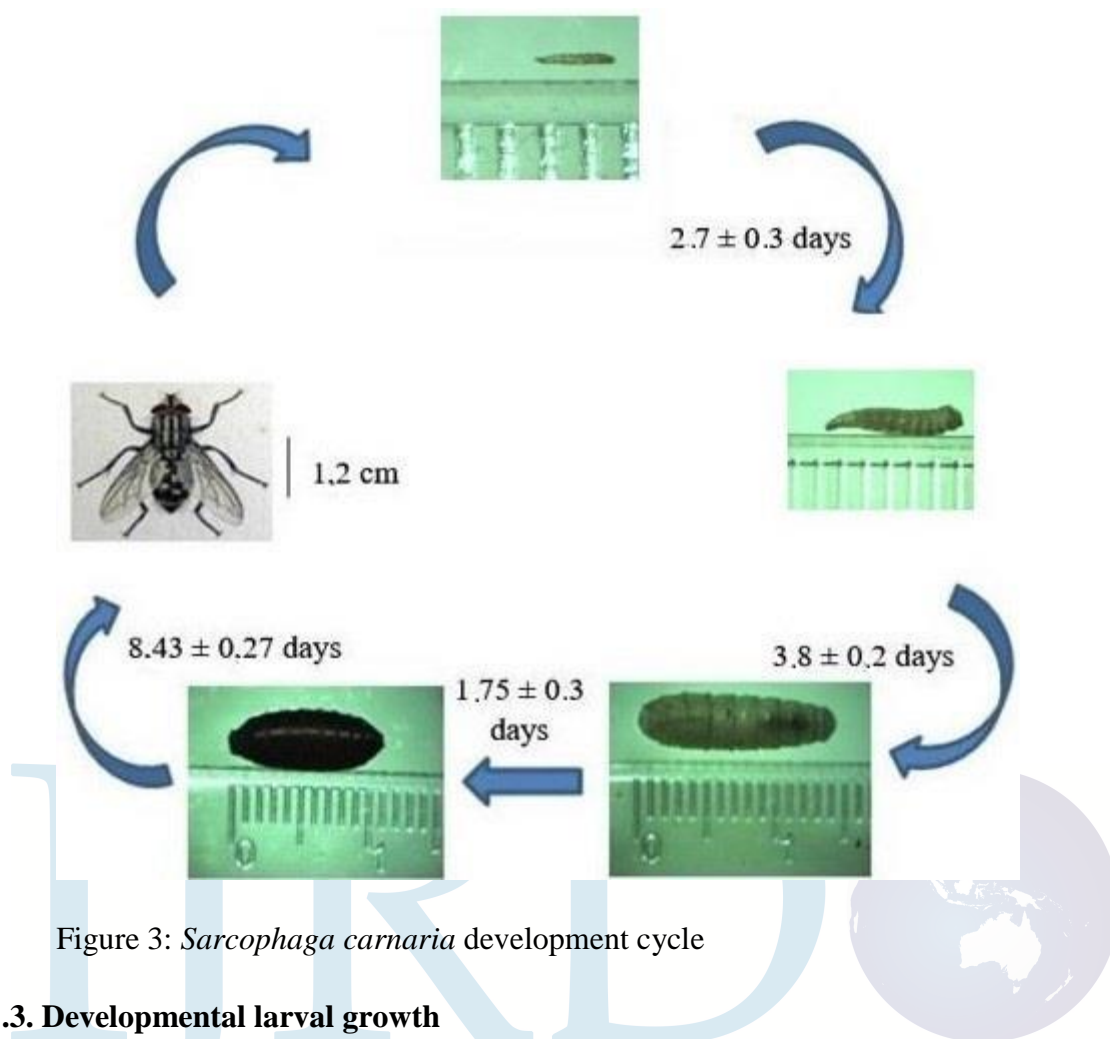


Figure 3: *Sarcophaga carnaria* development cycle

### 2.1.3. Developmental larval growth

The experiment with the larval batches on the striated pig muscle resulted in an average pupation of  $7.20 \pm 0.18$  days. The size measured per day was used to calculate the larval development rate. The calculated development rate was  $2.48 \pm 0.09$  mm / day. On the pig liver, the larvae were contacted with the substrate and the size of these was measured from stage 1 (L1) to the post-feeding phase. These studies made it possible to obtain an average pupation at  $6.47 \pm 0.14$  days. The calculated development rate was  $3.21 \pm 0.05$  mm / day  $25.90 \pm 0.18^\circ\text{C}$  and  $85.45 \pm 3.2$  %. The ANOVA test followed by the Newman Keuls test at the 5 % threshold ( $F = 10,112$ ,  $ddl = 1$ ,  $P = 0.00236$ ) indicated a significant difference between the larval development rates of the liver and larvae on the striated muscle (Table 2).

Table 2: Larval duration and larval development rate

Substrate	Larval duration (jour)	larval developmental velocity (mm/jour)
Liver	$6.47 \pm 0.14^b$	$3.21 \pm 0.05^a$

Striated muscle	$7.20 \pm 0.18^a$	$2.48 \pm 0.09^b$
<i>P-value</i>	0.00236	0.02314
<i>ddl</i>	1	1
<i>F</i>	10.112	4.613

ANOVA followed by the Newman Keuls test at the 5 % threshold; N = 30;

NB: Figures followed by the same letters in the same column are not significantly different at the 5 % threshold according to the Newman Keuls test

#### 2.1.4. Rate of emergence and sex-ratio

The mean emergence rate of *Sarcophaga carnaria* on pig liver (*S. scrofa domesticus* L.) was  $77.55 \pm 3.40\%$ ; whereas on the striated muscle it was  $63.84 \pm 3.68 \%$  at an average temperature of  $25.98 \pm 1.9^\circ \text{C}$  and an average relative humidity of  $85.08 \pm 3.1 \%$ . The test ANOVA followed by the Newman Keuls test at the 5 % level; ( $F = 7.483$ ,  $ddl = 1$ ,  $P = 0.00825$ ) revealed a significant difference in the emergence rates of *S. carnaria* on striated muscle and on pig liver (Table 3). The sex ratio of *S. carnaria* on pig liver was  $0.80 \pm 0.03$  and was  $0.95 \pm 0.06$  on striated muscle (Table III). The ANOVA followed by the Newman Keuls test at the 5 % threshold ( $F = 4.4141$ ,  $ddl = 1$ ,  $P = 0.040$ ) revealed a significant difference between the sex-ratio of *S. carnaria* raised on the liver and that of the striated pig muscle.

Table 3: Emergence rate and Sex ratio

Substrate	Emergence rate (%)	Sex-ratio (%)
Liver	$77.55 \pm 3.40^a$	$0.80 \pm 0.03^b$
Striated muscle	$63.84 \pm 3.68^b$	$0.95 \pm 0.06^a$
<i>P-value</i>	0.008250	0.040
<i>ddl</i>	1	1
<i>F</i>	7.483	4.4141

ANOVA followed by the Newman Keuls test at the 5 % threshold; N = 30

NB: Figures followed by the same letters in the same column are not significantly different at the 5 % threshold according to the Newman Keuls test.

### 2.1.5. Average lifetime of imago de *Sarcophaga carnaria*

The lifetimes of males and females were  $16.76 \pm 0.84$  days and  $20.23 \pm 0.77$  days on the liver (Figure 4A). The ANOVA test followed by the Newman Keuls test at the 5 % threshold ( $F = 9.296$ ,  $ddl = 1$ ,  $P = 0.00345$ ) revealed a significant difference between the life span of males and that of females. The life span of the males on the striated muscle was  $14.33 \pm 0.21$  days. It was in females  $17.03 \pm 0.36$  days (Figure 4B) at an average temperature of  $26.15 \pm 0.71^\circ\text{C}$  and at an average relative humidity of  $86.23 \pm 1.3\%$ . The ANOVA followed by the Newman Keuls test at the 5 % threshold ( $F = 45.698$ ,  $ddl = 1$ ,  $P = 0.0000$ ) revealed a significant difference between the life expectancy of males and females. On both substrates, on average, the date of death of the largest number of females was the 17<sup>th</sup> day. The largest number of males, died on the 15<sup>th</sup> day on the liver while on the striated muscle, this number was the 14<sup>th</sup> day. In females, the greatest number died on the 22<sup>nd</sup> day on the liver whereas on the striated muscle, the greatest number died on the 18<sup>th</sup> day (Figure 5).

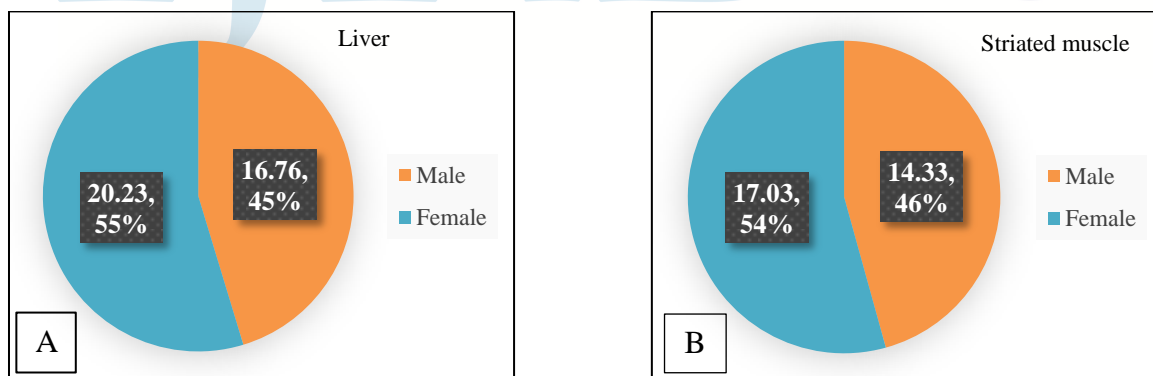


Figure 4: Longevity of adult male and female *Sarcophaga carnaria* on the liver and striated muscle

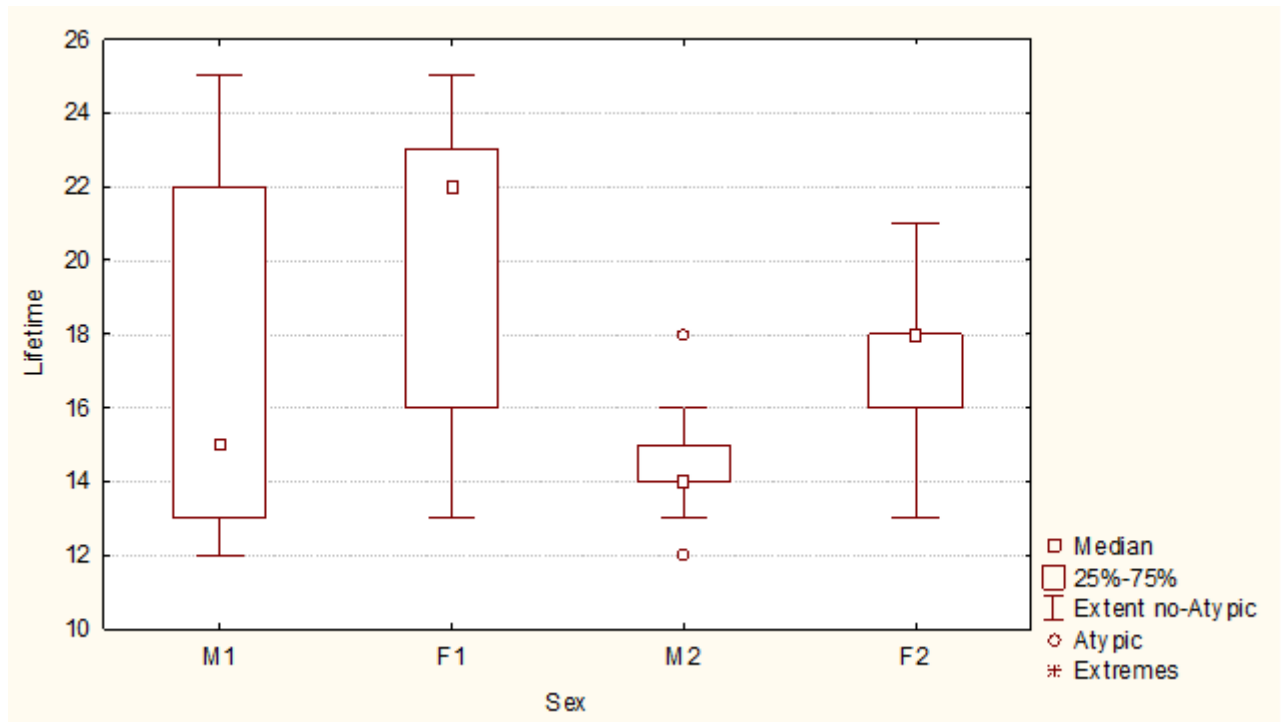


Figure 5: Specification of the period of death of adults of *Sarcophaga carnaria*

M1: Male; F1: Female: (liver)

M2: Male; F2: Female (striated muscle)

ANOVA followed by the Newman Keuls test at the 5% threshold;  $F = 15.967$ ;  $dd1 = 3$ ;  $P = 0.0000$

## 2.2. Discussion

The developmental cycle of *Sarcophaga carnaria* was longer on the striated muscle than on the liver. This would be due to the chemical composition and the speed of putrefaction of the liver which releases more nutrients necessary for the good development of the larvae. In fact, studies carried out on different types of meat by Day and Wallman (2006) have shown that not all animal tissues have the same nutritive value for Diptera larvae. Studies on the larval development of *Calliphora vicina* larvae on pig brain and pig hearts revealed that larvae developed more rapidly on the brain (Kaneshrajah and Turner, 2004). This difference is due to the fact that the brain decomposes faster thus releasing more quickly the nutrients needed for larval development (Day and Wallman, 2006). The growth rate of the larvae was greater on the liver than on the striated muscle. This difference in developmental velocity can be explained by the tissue architecture of the liver, which has a smooth and soft appearance,

favoring an intense activity of the larvae and also by its richness in nutrients. According to Tarone and Foran (2006), and Nability (2007), pig liver, by its rapid putrefaction releases nutrients necessary for larval growth. Significant differences were found in the mean duration of each stage of the developmental cycle (larval development, pupation and pupal development) on the liver and striated muscle. The study of the effect of temperature on the duration of the development cycle revealed that when the temperature increases, the duration of the development cycle decreases on both substrate types. The increased temperature would be responsible for the increased metabolic reactions of *S. carnaria* larvae. These reactions would be responsible for increasing the fly's development rate. These observations coincide with those of Charabidze, (2008) who, during his work, observed that the aggregation of the larvae of Diptera produced an increase in local temperature, which would be at the origin of the rapid growth of the larvae. The emergence rate was higher on the pig liver than on the striated pig muscle. This difference is due to the fact that the pig liver is more favorable to the development of *S. carnaria*, in view of its richness in nutrients, in particular in ammonia, Hobson (1932a, 1932b). According to this author, the alkalization of the breeding substrate by the ammoniacal substances would facilitate the digestion of substances ingested by the larvae. The sex-ratios ( $0.80 \pm 0.03$  on the liver and  $0.95 \pm 0.06$  on the striated muscle) obtained were in favor of the females. The fact that the sex-ratio was in favor of the females seems to increase the reproductive potential of the species (Tano et al., 2011). The analysis carried out revealed that females emerged more than males within the two breeding substrates. The lifetimes of the males of this species were shorter than those of the females, both on the pig liver and on the striated pig muscle. These results corroborate the results of Tarone et al. (2011) who carried out studies on different pig substrates. The short life of males could be attributed to the energy released during mating. This argument is consistent with that of Rueda et al. (2010) studying the table of life on two artificial breeding grounds of Diptera. Reproductive activity contributes to a sharp decrease in the life span of males than in females. Williams (1984) described this phenomenon as "cost of reproduction", a concept that links reproductive effort to other functions of the insect.

## CONCLUSION

The study of biological parameters showed that *Sarcophaga carnaria* is a holometabol whose development cycle comprises three larval stages and one pupal stage. The total cycle time of this necrophagous insect was  $17.12 \pm 0.32$  days on the striated muscle and  $16.07 \pm 0.19$  days on the liver at an average temperature of  $26.92 \pm 1.9^\circ\text{C}$  and an average relative humidity of

85.08 ± 3.1 %. The emergence rate was 1.21 times higher on the pig's liver than on the striated muscle. Pig liver appears to be the best substrate for a rapid development of *S. carnaria*. The sex ratio was in favor of females on both breeding substrates. The lifetimes of the male flies on the two substrates were respectively 16.76 ± 0.84 days on the liver and 14.33 ± 0.21 days on the striated muscle. It was 20.23 ± 0.77 on the liver and 17.03 ± 0.36 on the striated muscle for females at an average temperature of 26.15 ± 0.71°C and at a relative humidity of 86.23 ± 1.3 %. The study of the biological parameters of *S. carnaria* and those of other necrophagous insects in the different climatic zones of Ivory Coast should elucidate many murders in the perspective of social justice. Therefore, the determination of the biogenetic strains of cadaver insects would make it possible to identify them better.

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