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Luzlexis Arias-Di Donato
Museo del Instituto de Zoología Agrícola, Facultad de Agronomía, Universidad Central de Venezuela, Aragua, Venezuela.
luzdv.arias@gmail.com

Jonathan Liria
A) Laboratorio Museo de Zoología, Facultad Experimental de Ciencias y Tecnología, Universidad de Carabobo, Carabobo, Venezuela.
B) Universidad Regional Amazónica IKIAM, km 7 vía Muyuna. Napo, Ecuador.
Jonathan.liria@gmail.com

Correspondence
Luzlexis Arias-Di Donato
Museo del Instituto de Zoología Agrícola, Facultad de Agronomía, Universidad Central de Venezuela, Aragua, Venezuela.
luzdv.arias@gmail.com

Vital Statistics of *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) under Different diets from Venezuela

Luzlexis Arias-Di Donato, Jonathan Liria

Abstract
The life history of an important forensic blowfly, *Chrysomya megacephala* (Fabricius) from Venezuela, was studied at 28 °C, 47% RH and 12 h photoperiod in laboratory under two protein substrates: beef liver and sardine. The data were analyzed using the age-stage life table method with TWO-SEX computer program that considers the development rate among individuals and between sexes. The development time was: 8 h from egg-1st larvae, 20 h 1st-2nd larvae, 28 h 2nd-3rd larvae, 56 h 3rd-pupa and 83 h pupa-adult. The total development time was 200 h and 198 h, with liver and sardine respectively. Were found significant differences (Kruskal-Wallis test), between instars duration and protein substrates, with exception of egg-1st larvae and 3rd larvae-pupa. The specimens under liver showed high fecundity and low generation time; however under sardine, the life expectancy and survival rate were high, in contrast to low mortality and long generational time. Our study represents the first investigation in Venezuela that determines the vital statistics in blowfly species.

Keywords: Chrysominae, life table, mortality, survivorship, forensic entomology

1. Introduction

The human or animal carcasses decomposition is attributed to biological, chemical and physics process that emitted compounds that attract several arthropods; some species in the Order Diptera occurs in these decomposition substrates and the immature staged feed directly on the carcasses [1]. The Calliphoridae are the most important flies associated to forensic studies, because the immature stages development are used to estimate the length of time (Post Mortem Interval) between death and corpse discovery [2, 3]. Determination of the Post Mortem Interval is a crucial and fundamental step in any death scene investigation when a death is not witnessed [3]. Zied *et al*., [4] stated that the life table of a population gives the most comprehensive description on the growth, survival and fecundity. Therefore, a basic demographic study (instars duration, mortality, fecundity, etc.) in blowflies and other insects of forensic importance, is a fundamental and crucial aspect to support medico legal death investigations. Some investigations focused on the life-cycle, colonization, reproductive and population parameters of Calliphoridae species has been carried out. Zied *et al*., [4] and Gabre *et al*., [5] from specimens collected in Egypt, estimated the life table of *Lucilia cuprina* (Wiedemann) and *C. megacephala* (Fabricius), respectively. Later, Rueda *et al*., [6] studied the vital parameters of *Lucilia sericata* (Meigen) from Colombia reared under two artificial diets. Recently, Pinilla *et al*., [7] determined in Colombia, the life-cycle, reproductive and population parameters of *Sarconepsis magallanica* (Le Guillou) under different diets, and Saleh *et al*., [8] estimated the life table of *Lucilia sericata* collected in Iran. Finally Sanei-Dehkordi *et al*., [9] determined in Iran, the experimental colonization and life table of *Calliphora vicina* (Robineau-Desvoidy). *Chrysomya megacephala* is a common blowfly species in Venezuela [10, 11], with medical and forensic importance [12, 13]. Due to this, the main purpose of this work was to establish under laboratory conditions a colony of *C. megacephala*, from samples of adult specimens collected in Venezuela, to build life tables and to evaluate two protein substrates.

2. Materials and Methods

2.1. Sampling specimens: The laboratory colony of *C. megacephala* used in this study was initially established in March 2012, from adult collections in the surroundings of the Departamento de Biología at the Universidad de Carabobo, Valencia – Venezuela.

2.2. Maintenance of blowflies in the laboratory: The adults were kept in cages (25 x 35 x 25 cm) with white cloth "doppio velo" type; each cage contain 10 specimens (eight females and two males) at 28 ºC ±1, 47% RH and 12 h photoperiod.

2.3. Diets and life cycle: Adults were supplied daily with granulated sugar, water *ad libitum* supplied in a petri dish with cotton; another petri dish containing the protein substrate: one cage with 20 gram of beef liver, and other with 20 gram of sardines. From each cage/diet were taken 100 eggs, and subsequently placed individually to with 5 gram of protein source (liver or sardine) in bottles covered with doppio velo and secured with a rubber band. Each hour the individuals were revised, and the stage development and mortality were registered. At the prepupal stage, were used dry paper napkins as medium for pupation. In the adult emergence, females and males were transferred to cages with 5 gram of protein substrate and a petri dish with water; finally, for fecundity evaluation, were counted the eggs hatch until the last female die.

2.4. Life table and data analysis: For the life table study, the raw data from 200 specimens (100 for each diet and two replicates) were analyzed using the age-stage, two sex life table method [14, 15] with the TWO-SEX computer program. The differences between instar development time and protein substrates were analyzed with a Kruskal-Wallis test, in the PAST statistical computer program [16].

3. Results and discussion

Table 1 shows *C. megacephala* development time for each instars and protein substrates (beef liver or sardine): egg to 1st larvae (8 h with liver; 9 h with sardine), 1st to 2nd larvae (21 h; 20 h), 2nd to 3rd larvae (28 h; 30 h), 3rd to pupa (56 h; 56 h) and pupa to adult (87 h; 83 h); the total development time was 200 h and 198 h, with liver and sardine respectively. The instars development time and protein substrates showed significant differences (Kruskal-Wallis X^2=1674; p<0.001), with exception of egg to 1st larvae and 3rd larvae to pupa. Our findings were different to others studies, Goodbrod & Goff [17] studying the effect of larval densities in the development at 23.5 °C of *C. megacephala* and *C. rufifacies* (Macquart), found for 2 larvae/g of beef liver a duration of 150 h. Later, Sukontason *et al*., [18] reported 108 h from 1st larvae to adult, from a cohort of *C. megacephala* growth in 28 °C and using pork liver. Recently, Aguirre-Gil *et al*., [19] studying the larval development of *C. megacephala* under different diets and larval densities, found for 1 larvae/g of beef liver, a duration of 7.65 days (or 186.6 h) at 25 °C.

In relation with the life table parameters, the specimens under beef liver substrate, obtained high values of intrinsic rate of increase (r=0.41) and finite rate of increase (λ=1.51), short generation time (T=15 days) and low net reproductive rate (Ro=264), in contrast those specimens under sardine diet obtained low values of r=0.37, and λ=1.45, long T=17 days, and high Ro=558. The longevity obtained was different between both protein substrates, 47 days under beef liver diet and 57 days for sardine. Later, the fecundity (eggs/female) was high under beef liver (82.02) and low in sardine (67.06). The age-stage mortality in females was high at 40 days in beef liver and 56 days with sardine; the high mortality was obtained under beef liver at 11 days (62.5%) in the larval stages, and 63.5% in sardine at the same time, but in the pupa stage. The age-stage survival rate (Figure 1) showed a low value in female specimens under beef liver (41 days), in contrast those in sardine (57 days); the age-stage expectancy life (Figure 2) in female showed 58 days and 42 days, under sardine and beef liver respectively. These results differ from Gabre *et al*., [5], those report 32 days longevity in *C. megacephala* females at 26 °C under beef liver, 41 to 43 days for male and female survival rate respectively, and fecundity 48 eggs/female. The age-stage reproductive value under beef liver (Figure 3) was high (149.3) in flies with 17 days in comparison with the diet under sardine; similar results correspond to Gabre *et al*., [5] with 161.2 in 19 days. On the other hand, the reproductive value was lower than reported by these authors. This can be explained because the 17 days females, compared to other age groups, offer a high physiological potential that contributed to the population.

Carvalho & Von Zuben [20] estimated demographic aspects of *C. megacephala* maintained under laboratory conditions, with different larval densities (100 to 800) in temperature-controlled chambers at 25 °C. They found variations in the life expectancy from 49.5 days (in 100 larvae density), 61.83 days (in 200 larvae), 51.02 (in 400 larvae) 39.6 days (in 800 larvae); in relation to fecundity, these author reports differences in the total fecundity and net fecundity, the greatest values obtained were found at density 100, followed by 200 larvae, while the smallest values were found on 800 larvae. The main differences between vital parameters among these studies, could be attributed to the life table estimation; in our investigation were used the two-sex life table method [14, 21]. According to Zied *et al*., [4] and Gabre *et al*., [5], the traditional age-specific life table, ignored the male population and the variable developmental rates among individuals. Furthermore, because only the age was taken into consideration, the age-specific life table cannot describe the stage differentiation of insect population. Finally, following to Gabre *et al*., [5] for a detailed understanding of the population dynamics of blowflies species, data appropriate for life table studies must be collected on different diets under both laboratory and field conditions. And this information can be useful in determining the Post Mortem Interval, especially if the stage structure of Calliphoridae population on the corpse is recorded in a death investigation. Our study represents the first investigation in the country, that determine vital statistics in blowfly species using life table methods. However are necessary studies in other important forensic species, for example *C. albiceps* (Wiedemann), *Lucilia eximia* (Wiedemann), *L. cuprina*, among others, that consider different protein sources and a wide temperature range.

Table 1: Development time (in hours) mean and standard deviation of *Chrysomya megacephala,* from egg to adult under two protein substrates (beef liver or sardine)

Stage	Liver (n=200)	Sardine (n=200)
Egg - 1st Larvae	8 ± 0.62*	9 ± 0.74*
1st Larvae - 2nd Larvae	21 ± 3.39	20 ± 1.93
2nd Larvae - 3rd Larvae	28 ± 5.63	30 ± 2.67
3rd Larvae - Pupa	56 ± 7.75*	56 ± 4.30*
Pupa-Adult	87 ± 10.14	83 ± 4.35

(*) Indicated non-significant differences with Kruskal-Wallis test.

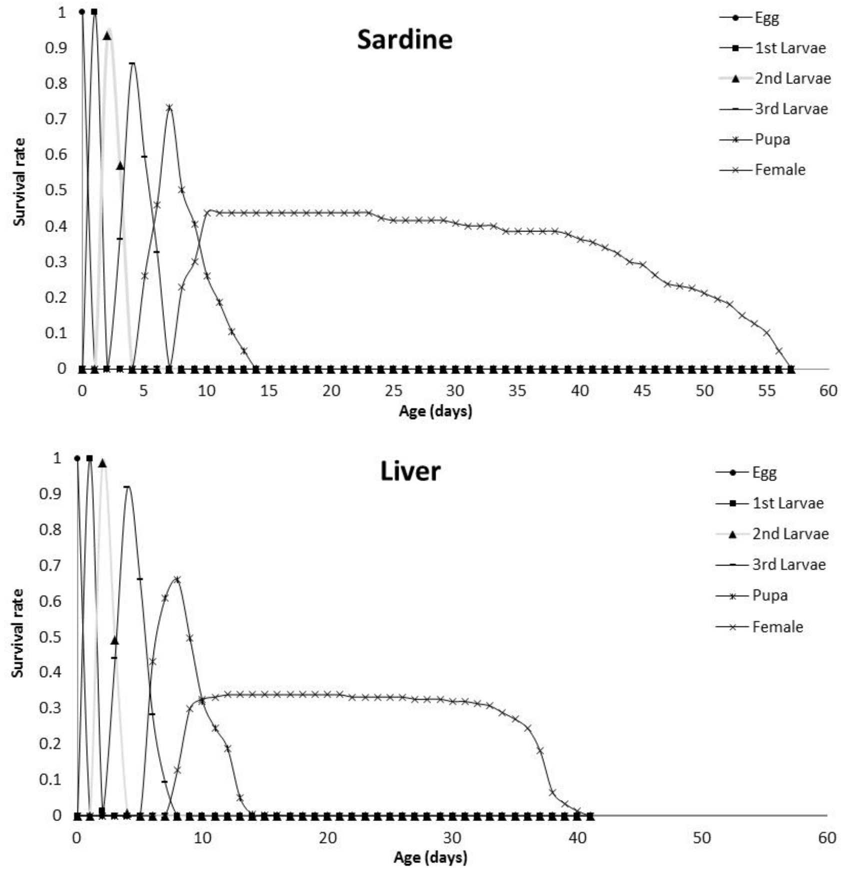

Fig 1: The age-stage specific survival rate of *Chrysomya megacephala,* from egg to female under two protein substrates (sardine or beef liver).

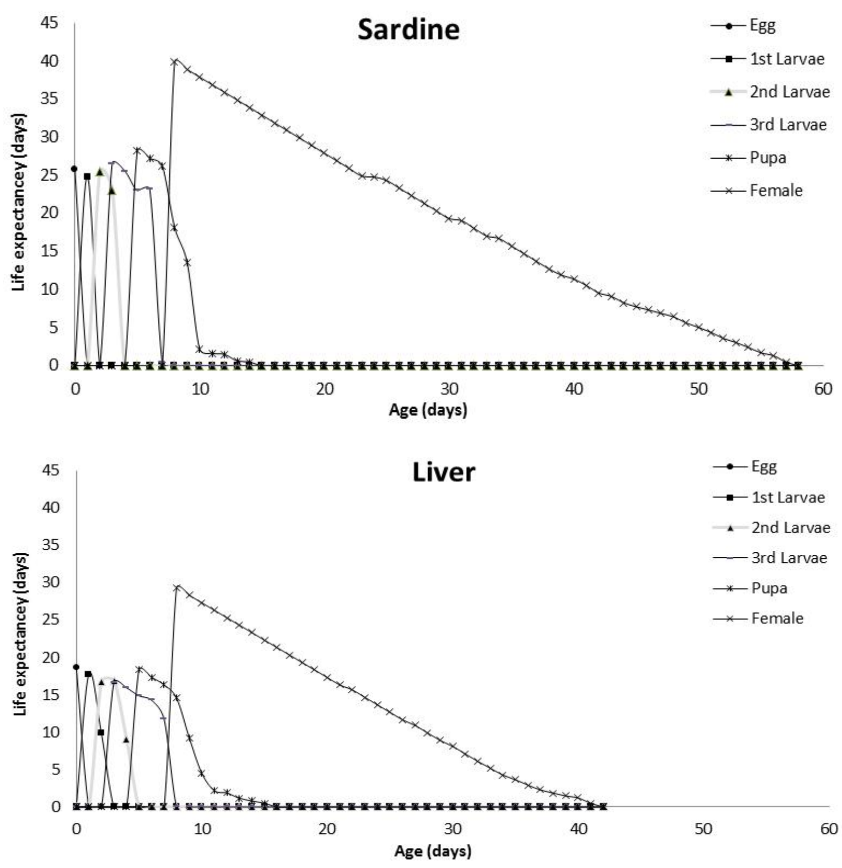

Fig 2. Life expectancy of each age-stage group of *Chrysomya megacephala, f*rom egg to female under two protein substrates (sardine or beef liver).

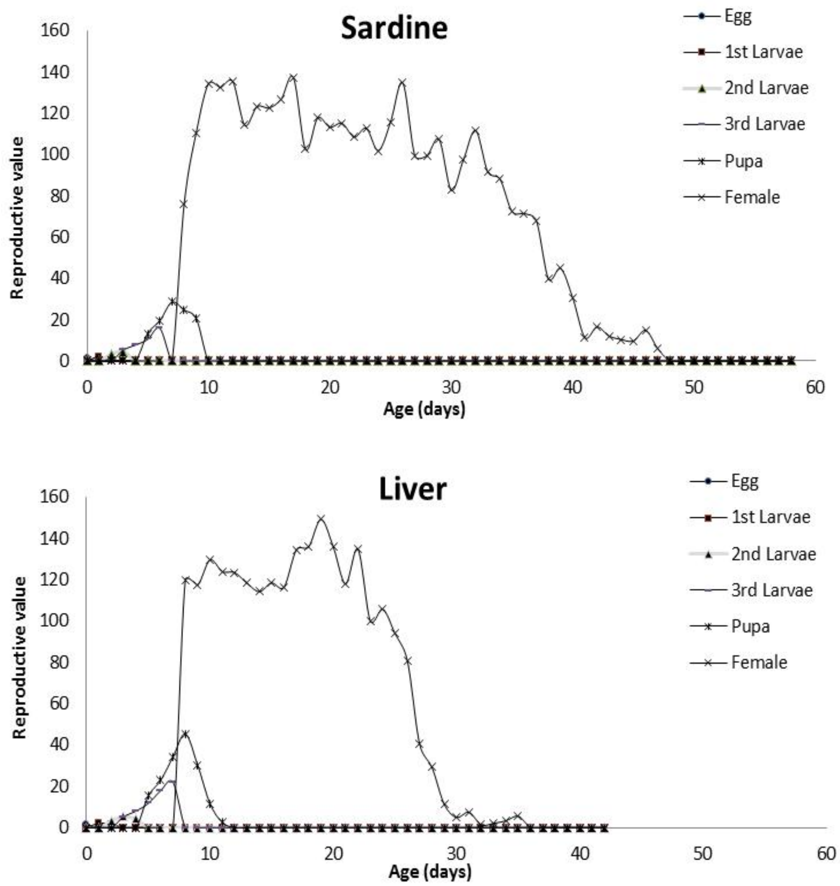

Fig 3: Reproductive value of each age-stage group of *Chrysomya megacephala, f*rom egg to female under two protein substrates (sardine or beef liver).

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