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Rearing five species of Diptera (Calliphoridae) of forensic importance in Colombia in semicontrolled field conditions

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ABSTRACT

The family Calliphoridae is widely known to lead the colonization of corpses and their development rates are frequently used to estimate the postmortem interval. This study presents the larval growth of five forensically important species of Calliphoridae in Colombia. Rearing took place in semicontrolled field conditions where the egg masses were collected. We show curves of larval growth, larval length and time intervals to reach all immature stages for Lucilia eximia *and* Cochliomyia macellaria *at two sites with different climatic conditions and for* Chrysomya albiceps, Chrysomya megacephala *and* Calliphora nigribasis *at one site. Overall, high temperatures speeded up the development of the species reared at two different sites, whereas low temperatures for* C. nigribasis, *lengthened the total development time. Differences between this study and others can be explained by the experimental conditions in the field without the possibility of strict laboratory rearing controls.*

Keywords: *Lucilia eximia, Cochliomyia macellaria, Chrysomya* spp., *Calliphora nigribasis,* larval growth.

INTRODUCTION

The estimation of postmortem interval is the main application of forensic entomology. Therefore, major knowledge requirements are the species associated with decomposing corpses in different biogeoclimatic zones, their development rates with emphasis on development times of each immature stage (Turchetto *et al.,* 2001) as well as lengths and weights of the different larval stages (Wells & Lamotte, 2001).

The laboratory rearing of insects collected from a death scene is an integral component of the analysis of entomological evidence. Rearing allows the entomologist to make more positive species identifications, particularly with fly larvae as definitive species determination cannot be made given the lack of distinct morphological differences. It also allows a clearer determination of the postmortem interval (PMI) since rearing of subsequent life stages provides a better approximation of the development stage of the insects at the time of collection (Byrd, 2001).

Larval lengths or weights can be used to estimate PMI under two approaches. The first is to measure the accumulated amount of days or hours needed to reach a particular stage of development (Ames & Turner, 2003) and the second is the use of an iso-

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megalen diagram, i.e. matching the size of the larvae found on the corpse with the development rates for the same blowfly larvae experimentally reared at the seasonal average temperature in which the body was found (Introna *et al.,* 1989). However, field temperatures are rarely constant and it has been shown that fluctuating temperature has a great effect on larval development (Introna *et al.,* 1989).

The family Calliphoridae is known as the initial colonizer in the faunal succession on human cadavers (Smith, 1986). Therefore, they are the primary and most accurate forensic indicators of time of death and their development rates are needed to allow more precise PMI estimates (Grassberger & Reiter, 2001). Thus, it is necessary that the development rates for the species involved in decomposition be known for the same conditions of temperature at the possible crime scene (Introna *et al.,* 1989).

The main purpose of this study was the rearing *in situ* of the first species to colonize pig corpses in decomposition in four different bioclimatic zones in Colombia. This is the first approach to the application of larval growth as a tool in PMI estimates.

MATERIAL AND METHODS

All of the eggs were collected on pigs (by permission of the Ethical Committee of Universidad de Antioquia, Medellín) in the early stages of decomposition and larvae were reared at the same site as the corpses.

The study was carried out at four sites: Tintipán Island, municipality of Gomez Plata, city of Medellín and Chingaza National Park.

Tintipán Island (beach environment) is located off the Caribbean coast within the San Bernardo archipelago. The area is designated as tropical very dry forest (bms-T) (Holdridge, 1987), with mangroves as the predominant flora, it has an altitude of 2 m above sea level, an average rainfall of 1000 mm and an average temperature of 27°C.

Gomez Plata (rural environment) is located at 75 km from Medellín. It is designated as tropical premontane wet forest (bmh-P) (Espinal, 1985). The site is open pasture used by cattle, at 1080 m above sea level with an average temperature of 25°C, relative humidity of 80% and an average rainfall of 1800 mm.

The city of Medellín (urban environment) is designated tropical premontane moist forest (bh-P) (Holdridge, 1987). It is located at 1450 m above sea level with an average temperature of 24°C and an average rainfall of 1409 mm.

Chingaza National Park (paramo zone) is at 3035 m above sea level, it has an average temperature of 10°C and relative humidity superior to 80% (INDERENA, 1986).

The egg masses of approximately 100 to 130 individuals were placed in plastic jars with 150 g of beef liver. The jars were placed in containers of polystyrene to avoid contamination with other insects, to minimize drastic changes in temperature and to eliminate the effects of sun exposure. The larvae did not have a light source within the containers.

Each plastic jar was sampled as follows: the time was noted at the moment of egg collection (we assumed this moment to be oviposition). During the first 36 hours, we observed the moment of eclosion and removed samples every three hours between 7 am and 7 pm in order to get sufficient larvae at the first stage of development. These larvae growth faster and observations need to be made at shorter intervals (Krüger *et al.,* 2003). After the first 36 hours, sampling continued every six hours. The samples consisted of ten larvae per plastic jar at all of the evaluated sites, except Medellín, where each sample consisted of four larvae. When the larvae reached the postfeeding stage, we placed sawdust in the jars (Dale & Prudot, 1986) and made observations every 12 hours to verify the presence of pupae and the emergence of adults. During the postfeeding stage we stopped the sampling to prevent handling just before pupation at which time development can be delayed or blocked (Anderson, 2000). The container and environmental temperatures were measured at each sampling time and the average temperature of rearing was calculated from these data.

All of the samples were fixed in 80% ethanol and examined under a binocular stereoscope to identify the species with the use of different keys (Shewell, 1981; Dear, 1985; Smith, 1986; Wells *et al.,* 1999).

All larvae were measured (total body lengths) and the accumulated time of development was calculated for all the instars for all the sample jars. Line graphs of larval growth, with intervals for the ages (in hours) and the lengths of each immature stage were plotted using JMP 3.2.2 (SAS Institute Inc., 1997). All insect material was deposited in the Entomological Collection of the Universidad de Antioquia in Medellín.

RESULTS

During this study, five species belonging to Calliphoridae were reared: *Lucilia eximia* (Wiedemann, 1819), *Cochliomyia macellaria* (Fabricius, 1775),

Chrysomya albiceps (Wiedemann, 1819), *Chrysomya megacephala* (Fabricius, 1794) and *Calliphora nigribasis* Macquart, 1851. *L. eximia* and *C. macellaria* were reared at two different sites with different temperature regimes (*L. eximia* at 23.13 ± 2.45°C, in Medellín and 25.30 ± 3.26°C, in Gómez Plata; *C. macellaria* at 25.30 ± 3.26°C, in Gómez Plata and 30.74 ± 0.71°C, in Tintipán). *C. albiceps, C. megacephala* and *C. nigribasis,* were reared at 25.30 ± 3.26°C, in Gomez Plata, 23.13 ± 2.45°C, in Medellín and 10.62 ± 2.51°C, in Chingaza, respectively.

In all cases, development time for the eggs and the moment of eclosion was obtained (Table 1). For *L. eximia, C. macellaria, C. albiceps* and *C. megacephala,* the eclosion was near to 15 hours subsequent to the oviposition, whereas *C. nigribasis* needed 64 hours.

The larval growth represented by the progressive increase in length is showed in larval growth curves (Figs. 1-7). The average and standard deviations of the lengths and the development times guarantee the fact of find a larva in a particular stage (Table 2 and 3). *L. eximia* reached higher lengths when it was reared at 23.13°C in urban environment (Fig. 1) in comparison with its rearing at 25.30°C in rural environment (Fig. 2), whereas the lengths of third instar larvae of *C. macellaria* showed overlapped intervals between beach environment at 30.74°C and rural environment at 25.30°C (Fig. 3, 4 and Table 2). Instead, the postfeeding larvae characterized by a decrease in size (Greenberg & Kunich, 2002) (Figs. 3 and 4), showed narrower and not overlapped intervals for the length (Table 2).

With regard to the accumulated time of development for immature individuals to reach all instars (Table 3), we observed that *L. eximia* exhibited successful growth for all larval instars in both rearings, according to the larval growth curves (Figs. 1 and 2). However, we did not obtain pupae or adults. At

TABLE 1: Time of eclosion (in hours) of the species at all development temperatures.

Species	Temperature (°C)	Egg duration (h)
Lucilia eximia	23.13±2.45	15.16±0.77
Lucilia eximia	25.30±3.26	16.42±2.31
Cochliomyia macellaria	30.74±0.71	15.80±2.91
Cochliomyia macellaria	25.30±3.26	15.82±1.50
Chrysomya albiceps	25.30±3.26	15.00±1.73
Chrysomya megacephala	23.13±2.45	15.00±0.71
Calliphora nigribasis	10.62±2.51	64.02±1.79

TABLE 2: Length (in mm) for the immature stages of the species at all development temperatures.

Species	Temperature (°C)	Larval length (mean±SD)			
		L1	L2	L3	PFL
Lucilia eximia	23.13±2.45	2.24±0.54	4.76±1.02	11.16±2.07	10.52±1.39
Lucilia eximia	25.30±3.26	2.20±0.54	3.98±0.85	9.74±1.64	10.29±0.86
Cochlyomyia macellaria	30.74±0.71	2.58±0.36	4.64±0.85	10.05±1.65	8.36±1.63
Cochlyomyia macellaria	25.30±3.26	2.10±0.50	4.27±1.10	9.85±2.09	11.76±1.86
Chrysomya albiceps	25.30±3.26	2.12±0.17	5.16±1.17	10.75±2.70	
Chrysomya megacephala	23.13±2.45	2.05±0.24	5.88±1.11	11.81±2.36	12.95±1.29
Calliphora nigribasis	10.62±2.51	2.81±0.63	7.67±1.77	18.37±2.72	17.82±2.22

L1, first larval instar; L2, second larval instar; L3, third larval instar; PFL, postfeeding larvae.

TABLE 3: Time to reach the immature stages (in h) for the species at all temperatures.

Species	Temp. (°C)	Time to reach the immature stage (mean ± DS)					
		L1	L2	L3	PP	P	A
Lucilia eximia	23.13±2.45	20.69±4.93	41.82±8.84	57.88±10.09	101.45±19.23	†[a]	†[a]
Lucilia eximia	25.30±3.26	20.31±4.14	30.93±5.23	55.77±9.77	90.09±9.83	†[a]	†[a]
Cochliomyia macellaria	30.74±0.71	17.19±3.16	24.69±3.35	54.20±11.08	95.37±11.94	86–120 103±24.04	158–167 162.5±6.36
Cochliomyia macellaria	25.30±3.26	22.24±5.86	44.48±8.87	78.03±11.61	111.25±12.31	116–206 161±63.64	257-332 294.5±53.03
Chrysomya albiceps	25.30±3.26	19.38±4.26	48.88±8.34	89.75±19.42		192–239 215.5±33.23	332–352 342±14.14
Chrysomya megacephala	23.13±2.45	18.60±3.44	45.04±9.23	65.58±12.22	107.26±13.68	164–309 236.5±102.53	281–357 319±53.74
Calliphora nigribasis	10.62±2.51	111.54±31.41	205.47±24.32	318.11±42.12	551.79±95.00	1324[b]	2664[b]

[a] Pupae and adults did not appear for this species.

[b] The data were obtained from other observations of the same species in the same conditions but not part of the present study.

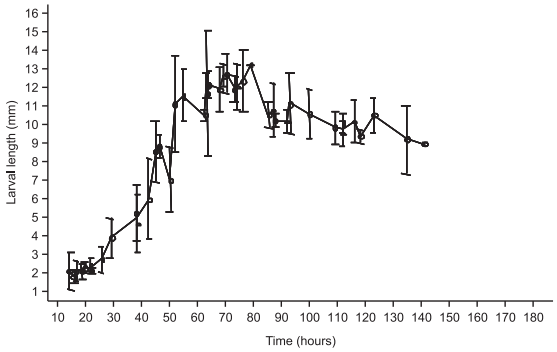

FIGURE 1: Larval growth of *Lucilia eximia* at 23.13 ± 2.45°C.

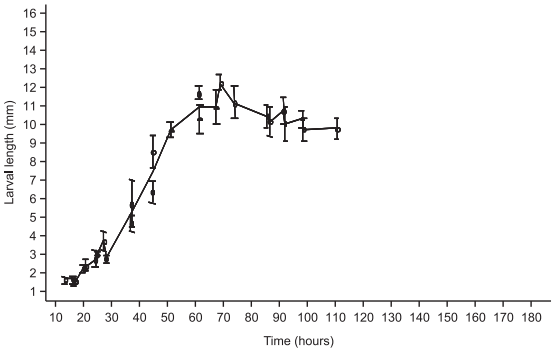

FIGURE 2: Larval growth of *Lucilia eximia* at 25.30 ± 3.26°C.

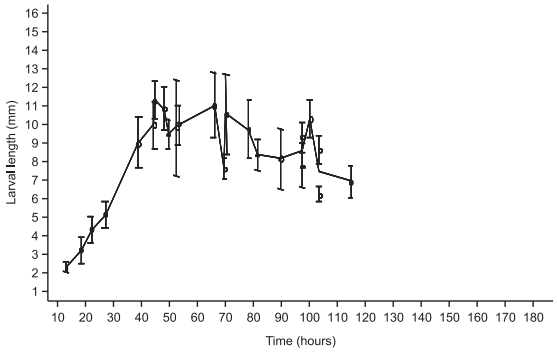

FIGURE 3: Larval growth of *Cochliomyia macellaria* at 30.74 ± 0.71°C.

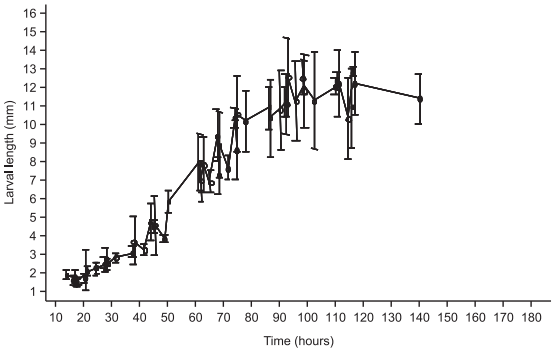

FIGURE 4: Larval growth of *Cochliomyia macellaria* at 25.30 ± 3.26°C.

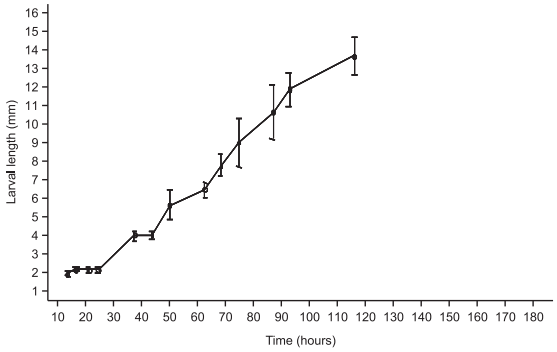

FIGURE 5: Larval growth of *Chrysomya albiceps* at 25.30 ± 3.26°C.

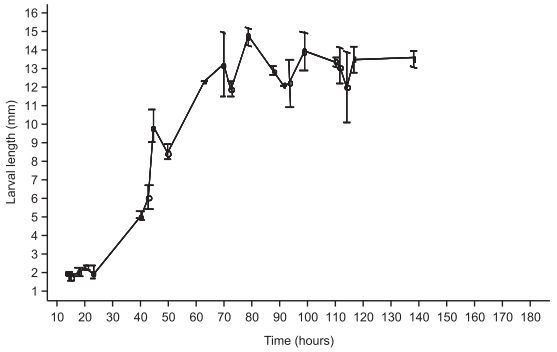

FIGURE 6: Larval growth of *Chrysomya megacephala* at 23.13 ± 2.45°C.

25.30°C, growth took less time (90.09 ± 9.83 hours) than at 23.13°C (101.45 ± 19.23 hours) (Table 3). *C. macellaria* showed successful development to the emergence of the adults at the two different temperatures (Figs. 3 and 4). At 30.74°C, development was faster, concluding after 162.5 hours. Also, greater synchrony was shown for the accumulated time intervals of each stage (Table 3). At 25.30°C the time for total development was 294.5 hours. For this species, a difference of 5°C can alter development velocity by 132 hours, according to the present study. *C. albiceps* completed its development to the adult stage in 332 hours in rural environment (Table 3). The postfeeding larvae are not appreciable in the curve, as there was no decrease in its length at the end of the larval growth (Fig. 5 and Table 2). *C. megacephala* reached the adult stage at 23.13°C in urban environment. Appearance of pupae took place in a wide interval (between 164 and 309 hours); therefore we assumed that the pupation was not very synchronic (Table 3). The opposite occurred with the adults, appearing over a narrower interval (between 281 and 357

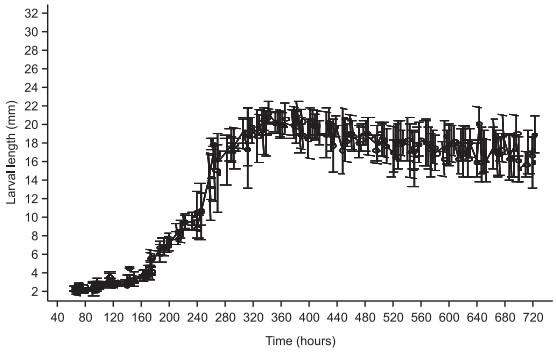

FIGURE 7: Larval growth of *Calliphora nigribasis* at 10.62 ± 2.51°C.

hours). All larval instars were identified, including the postfeeding larvae that are visible in the larval growth curve (Fig. 6). Finally, *C. nigribasis* showed a different behavior, given that it is a typical species from the Andean zone, in high mountains and low temperatures; hence the development was successful at 10.62°C (Fig. 7). Nevertheless, the colony providing the larval growth curve died after several days in a postfeeding larval stage, possibly because they entered diapause after missing the main conditions for pupation (Krüger *et al.,* 2003). The data presented here (Table 3) was taken from another group of larvae that were not sampled but were reared under the same conditions.

DISCUSSION

This study is the first report for Colombia on the larval growth of the principal species to arrive at a corpse (Wolff *et al.,* 2001; Perez *et al.,* 2005; Martinez *et al.,* 2007) in four different bioclimatic zones: *L. eximia* at 23.13°C and 25.30°C in Medellín and Gomez Plata, respectively; *C. macellaria* at 25.30°C and 30.74°C in Gomez Plata and Tintipán, respectively; *C. albiceps* at 25.30°C in Gomez Plata; *C. megacephala* at 23.13°C in Medellín and *C. nigribasis* at 10.62°C in Chingaza. *L. eximia* seems to be very sensitive to stress and in the present study this could have been caused by the lack of a successful migration event and posterior burial. This species, as others of the subfamily Calliphorinae (Greenberg, 1990), has been characterized by displaying strong intentions to escape from sample jars, showing their need for migration away from the food source and thus explaining the mass movement of larvae during the postfeeding stage in the jars (Anderson, 2000; Gomes *et al,* 2003; Gomes & Zuben, 2004). The absence of this migration requirement given by the small space, and the absence of burial given by the kind of substrate to pupate (sawdust instead sand), could explain the failure to reach pupation and the adult stage. For this species, the time taken for maximum larval growth at 25.30°C was similar to the results obtained for *Lucilia sericata* (a closely related species), reported by Grassberger & Reiter (2001). The times obtained for its rearing at 23.13°C agree with Anderson (2000) for the first and second instar of development. However, *L. eximia* is different from *L. sericata,* because the first, prefers tropical and warm environments, becoming an indicator species of urban and rural premontane biotic zones.

The development of *C. macellaria* demonstrated how the total duration of development decreases with increasing temperature within the optimal range (Chapman, 1982). These results are also in agreement with other studies where an increase in temperature within the optimal range can produce accelerated development (Byrd & Allen, 2001; Grassberger & Reiter, 2002a; Grassberger *et al.,* 2003; Krüger *et al.,* 2003). Considering that *Cochliomyia* is a neotropical genus with a wide distribution (Dear, 1985), that exhibits preferences for warm and moist environments and it is dominant in rural areas (Prudot & Dale, 1987) it is expected to adapt to the different temperature regimes produced by the fluctuations in rural and beach environments like Gomez Plata and Tintipan, respectively.

The times for pupation and emergence of the adults of *C. albiceps* (8-10 days and 13-14 days, respectively) are similar to that obtained by Grassberger *et al.* (2003) at 25°C. They show times from oviposition to pupation and emergence of the adults of 8 days and 13 days, respectively. Baumgartner & Greenberg (1985) showed the presence of this species since premontane to montane biotic zones. This information encloses the area where we found it (Gomez Plata at 1080 m) and reveals an altitudinal adaptation where the temperature is the main factor that influences the rate of development.

The rearing of *C. megacephala* showed similarities with Wells & Kurahashi (1994), with regard to the moment of appearance of the pupae. They reported a maximum time of 144 to 198 hours, which corresponds to the lower value of the interval obtained in the present study. However, this study reported a longer time for the emergence of the adults when compared to the same authors. This discrepancy could be sustained by the difference between the temperatures (27°C in Wells and Kurahashi and 23.13°C in this study). In Medellín, this species is commonly encountered, showing a preference for urban areas.

C. nigribasis was reared at 10.62°C, with a development time of 111 days. Studies carried out with other species with the purpose of evaluating the effects of temperature on development, clearly show how a decrease in temperature can decrease metabolism, increasing the time intervals for the immature stages (Anderson, 2000; Byrd & Allen, 2001; Grassberger & Reiter, 2001; Grassberger & Reiter, 2002a; Grassberger & Reiter, 2002b; Ames & Turner, 2003; Grassberger *et al.,* 2003). Hence, it is essential to consider that this species belongs to the Andean region (including paramo region) (Baumgartner & Greenberg, 1985) and it is part of the cadaverous entomofauna of these regions, becoming an important forensic indicator.

There are many differences between this study and others treating the same issue. This disparity can be given by the experimental conditions, such as the food source and the photoperiod. Not only do we have to consider differences in the methods (extrinsic factors), but we also have to take into account intrinsic factors such as geographic adaptation, temperature regimes, feeding and density. Variations in these parameters exist between different geographic populations of the same species (Grassberger & Reiter, 2002a). The extrapolation of results obtained in the laboratory for field conditions should be carried out with caution given that lab conditions can eliminate competitors and have optimal abiotic conditions, such as controlled temperature, (Anderson, 2000) which produces higher viability (Krüger *et al.,* 2003). Because the present study worked with species commonly found in the studied areas and the semicontrolled conditions allow the influence of the regional temperatures, the development data from this work becomes an important database for the application of forensic entomology, considering that it is important that results used in the solution of forensic cases, be obtained from field conditions, where temperatures have cyclic fluctuations modifying development times in different ways when compared with constant temperatures (Introna *et al.,* 1989). Furthermore, they better resemble the conditions of a body found in decomposition.

It is also important to consider the effects of the preservative solutions. It is known that the use of 70% alcohol produces shrinkage of the larvae, modifying their size and the estimation of age using length (Tantawi & Greenberg, 1993). Even so, it is important to consider that samples obtained in Colombia from the crime scenes are preserved in alcohol and our results could be applied.

Additionally, this study shows that rearing these species in field conditions can be successful, and that the results obtained could easily be used in forensic entomology, providing valuable information in the determination of the postmortem interval.

CONCLUSION

In general, developmental times from oviposition to emergence might differ depending on many factors. Geographic variations and climate conditions can influence adaptations explaining the differences in the development at different temperatures. High temperatures accelerate the growth and development, whereas low temperatures slow it down. Alternatively, the rearing in field conditions allows a more precise approximation to the conditions of growth in a real forensic case. However, more studies are necessary to understand the behavior during the development of the immature stages, like the degree-days estimates and the knowledge of the intrinsic and extrinsic factors that could affect the development of the forensically important species.

RESUMEN

La familia Calliphoridae es ampliamente conocida por liderar la colonización de los cadáveres y sus tasas de desarrollo son frecuentemente utilizadas para estimar el intervalo postmortem. Este estudio presenta el crecimiento larval de cinco especies de Calliphoridae de importancia forense en Colombia, considerando que la cría se dio en condiciones de campo semicontroladas en los lugares donde las masas de huevos fueron colectadas. Mostramos también, los intervalos de longitud y el tiempo empleado en alcanzar todos los estadios inmaduros para Lucilia eximia *y* Cochliomyia macellaria *en dos lugares con diferentes condiciones climáticas y* Chrysomya albiceps, Chrysomya megacephala *y* Calliphora nigribasis, *en un solo lugar. En general, las altas temperaturas producen una aceleración en el desarrollo de las especies criadas en dos sitios diferentes, mientras que bajas temperaturas para* C. nigribasis, *alargaron el tiempo utilizado para completar el desarrollo. Las diferencias entre este estudio y otros, pueden estar dadas por las condiciones experimentales presentadas en campo sin los controles estrictos que son posibles durante la cría en laboratorio.*

Palabras-claves: *Lucilia eximia, Cochliomyia macellaria, Chrysomya* spp., *Calliphora nigribasis,* crecimiento larval.

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