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Development Period of Forensic Importance Calliphoridae (Diptera: Brachycera) in Urban Area Under Natural Conditions in Manaus, Amazonas, Brazil

Alex Sandro Barros-Souza✉, Ruth Leila Ferreira-Keppler & Daniela de Brito Agra

1. Instituto Nacional de Pesquisas da Amazônia - INPA - Coordenação de Pesquisas em Entomologia, e-mail: souza.alex83@gmail.com (Autor para correspondência✉), ruth@inpa.gov.br, agra.daniela@gmail.com.

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Abstract. In order to describe the development period of forensically-important Calliphoridae species (Diptera: Brachycera) under natural conditions in Manaus, Amazonas, Brazil, two experiments were carried out at *Campus* II of National Institute for Amazon Research (INPA), Manaus, Amazonas, one in the rainy season and the other one in the less rainy season. Pig carcasses (25kg each) were used as attractive to oviposition of the blowflies. Calliphoridae females were collected and the eggs were placed into containers with ground beef. The reared species and development time from egg to adult (in days) in the rainy season and less rainy season, respectively, were: *Chrysomya albiceps* (Wiedemann) 14.5 days and 9.4 days, *C. megacephala* (Fabricius) 10.7 and 9.4, *Hemilucilia segmentaria* (Fabricius) 11.5 and 10.7, *Lucilia eximia* (Wiedemann) 19.7 and 14.3 and *Paralucilia paraensis* (Mello) reared only in the less rainy season with 11.8 days. This is a first record of the development time of *P. paraensis*.

Keywords: *Chrysomyia*; Forensic Entomology; *Hemilucilia*; *Lucilia*; *Paralucilia*.

Período de Desenvolvimento sob Condições Naturais de Espécies de Calliphoridae (Diptera: Brachycera) de Importância Forense em Área Urbana, Manaus, Amazonas, Brasil

Resumo. Para descrever o tempo de desenvolvimento dos imaturos de Calliphoridae sob condições naturais, dois experimentos foram realizados no *Campus* II do Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, um na estação chuvosa e o outro na estação menos chuvosa. Cadáveres de porcos domésticos (25kg cada) foram utilizados como substrato atrativo para a ovipostura dos califorídeos. Fêmeas grávidas de Calliphoridae foram coletadas e os ovos foram transferidos para potes plásticos contendo placas de Petri com carne bovina moída. As espécies criadas, com respectivo tempo de desenvolvimento de ovo a adulto (em dias), na estação mais chuvosa e menos chuvosa, foram: *Chrysomya albiceps* (Wiedemann), 14,5 e 9,4 dias, *Chrysomya megacephala* (Fabricius), 10,7 e 9,4, *Hemilucilia segmentaria* (Fabricius), 11,5 e 10,7 *Lucilia eximia* (Wiedemann) 19,4 e 14,3 e *Paralucilia paraensis* (Mello), 11,8 dias, essa criada somente na estação menos chuvosa. Este é o primeiro registro do tempo de desenvolvimento de *P. paraensis*.

Palavras-Chave: *Chrysomyia*; Entomologia Forense; *Hemilucilia*; *Lucilia*; *Paralucilia*.

Entomologia Forense

Data concerning immature's development time of necrophagous Diptera help estimate the *postmortem* interval (PMI), the interval that corresponds to the time between death and discovery of the cadaver (Byrd & Castner 2003). This period is influenced by several factors such as temperature, overlapping of different specie's generations, heat generated by the mass of larvae and even drug effects (Souza & Kirst 2010). These factors, when not given the proper attention, can generate mistakes in the PMI estimative. Nevertheless, entomological data are more precise especially for PMIs superior to three days (Byrd & Castner 2003). The most influent factors in the variation on the development period of immature insects are temperature and relative humidity, to what insects answer by reducing or accelerating their development cycle (Souza & Kirst 2010), which goes from egg to adult.

Studies related to the knowledge of the ontogenetic cycle of forensically important species are needed, for data obtained from different geographic regions may not be comparable and interfere in the determination of the PMI, since changes on the physical factors interfere in the specie's biology (Turchetto & Vanin 2004).

Most existing studies have been carried out in laboratories under constant temperatures. The present study, however, brings the first information on the development time of five Calliphoridae species exposed to ambient temperatures in urban areas in two seasonal periods at the municipality of Manaus, Amazonas.

MATERIAL AND METHODS

Study Area. The experiments were carried out at *Campus* II of the National Institute for Amazon Research – INPA (03°5'47"S e 59°59'22"W), East-Zone, in an urban forest fragment at the municipality of Manaus, AM, in January and October of 2008.

According to Ribeiro & Adis (1984) the climate at the region where the city is located is classified as equatorial hot and humid, corresponding to the tropical climate. The average temperature is 26.7°C, relative humidity mean 83% and precipitation of 2.291.8 mm, with an average of 190 rainy days per year. The Manaus region shows only two seasons over the year: rainy season (December to May), with March as the rainiest month, and the dry season (June to November), with August as the month with the least precipitation.

Experimental Design. Domestic pig models (*Sus scrofa* L.) weighing 25 kg were used, one in each season (rainy and less rainy). The animals were taken alive to a secondary forest area,

killed by cervical dislocation and left at ambient conditions. The cadaver was put inside a cage measuring 160 x 40 x 68 cm wrapped with a 3 x 3 cm spaced, which allowed insects to enter but prevented the access by medium size an big necrophagous vertebrates.

To observe the development time of five species of Calliphoridae: *Chrysomya albiceps* (Wiedemann), *Chrysomya megacephala* (Fabricius), *Hemilucilia segmentaria* (Fabricius), *Lucilia eximia* (Wiedemann) and *Paralucilia paraensis* (Mello), females that initiated the oviposition process on the pig carcass were captured with BD Falcon™ conical tubes. This procedure was adopted to avoid the collection of females in which the vitellogenesis had not been completed. The females were individualized for oviposition in test tubes containing about 20 gr of ground beef, removed from refrigeration 24 hours before being offered. After, the eggs were transferred to plastic bottles containing 50 gr of ground beef, until the eclosion. After that, the larvae were transferred to Petri dishes in 92 lots of 30 individuals each, to avoid competition and temperature rises caused by the mass of larvae, which could influence the development time. Since the larval growth is expected to be very fast, and observations must be conducted in short time intervals, the larvae were observed in intervals of one hour for the first 36 hours of the process.

To avoid the substrate drying and protein loss the same initial amount of ground beef (50 gr) was re-offered every two days, until the postfeeding stage. When the larvae reached the third stage they were transferred to Petri dishes containing 50 gr of ground beef. The dishes were inserted in transparent plastic pots containing vermiculite, to wait for pupation and posterior adult emergence. During this developmental stage observations were carried out every three hours. When the larvae started the pupation process, observations were conducted in intervals of four hours (9:00 to 21:00h). All this process took place in wood cages measuring 120 x 50 x 30 cm wrapped in nylon screens. The bottles were placed in shelves inside the cage, thus, eggs and larvae developed in fluctuating ambient temperatures, accumulating all the temperature and humidity variations throughout sampled days.

The larval stages were characterized according to the number of spiracular openings (Greenberg & Szyska 1984). Identification of the adults followed the keys by Amat *et al.* (2008), Carvalho & Mello-Patiu (2008) and the descriptions of Mello (1969).

After emergence, adults were killed by cooling and transferred to flasks with 70% alcohol. Vouchers were placed in the Collection of Invertebrates at INPA.

To verify if there was a significant difference in the development time between samples in each season and to verify if there was a difference between the development times, an ANOVA was conducted.

The influence of temperature and humidity in the development of the larvae and pupae was verified with a Linear Regression. Temperature and humidity data from the period comprising the development time of each species during the experiment was used, and a Pearson's Linear Correlation test was carried out to verify the possibility of using one or both the variables in the analysis. All data were processed in the software PAST 2.11 (Hammer *et al.* 2001).

Data relative to temperature and relative humidity of the air were obtained through the LBA (Large Scale Biosphere-Atmosphere Experiment in Amazonia) meteorological station, located at *Campus* II of INPA near to the study area and with a thermohygrometer at the site of study.

RESULTS

The data obtained from meteorological station, not show significant difference between the those registered at the experiment site ($F= 3.8382$, $df= 23.287$, $p= 0.062$) and we opted to use data from the meteorological station for the analysis of development time. The average temperature and relative humidity of the air during the experiment in January were 25.87 ± 1.27°C and 82.57 ± 6.40%, respectively, while in October the averages were 28.03 ± 1.60°C and 71.24 ± 6.23%.

On the first day of the carcass exposure, ovipositions of *Lucilia eximia* were observed, which happened until the third day of decomposition in the rainy period and until the second day in the less rainy season. Oviposition of *C. albiceps* was observed from the first day until the third day. On the the remaining species, the oviposition was observed only from the second day of decomposition. *C. megacephala* was observed ovipositing on the second and third days, and *H.segmentaria* and *P. paraensis* were observed only on the second day of decomposition. *H. segmentaria* and *C. megacephala* showed this same behavior on both seasons and *P. paraensis* was collected only in the less rainy season.

Regarding the development time, eggs eclosions occurred between 10 and 14 hours after oviposition (Figures 1 to 5). The duration of the first and second larval stages are similar in all

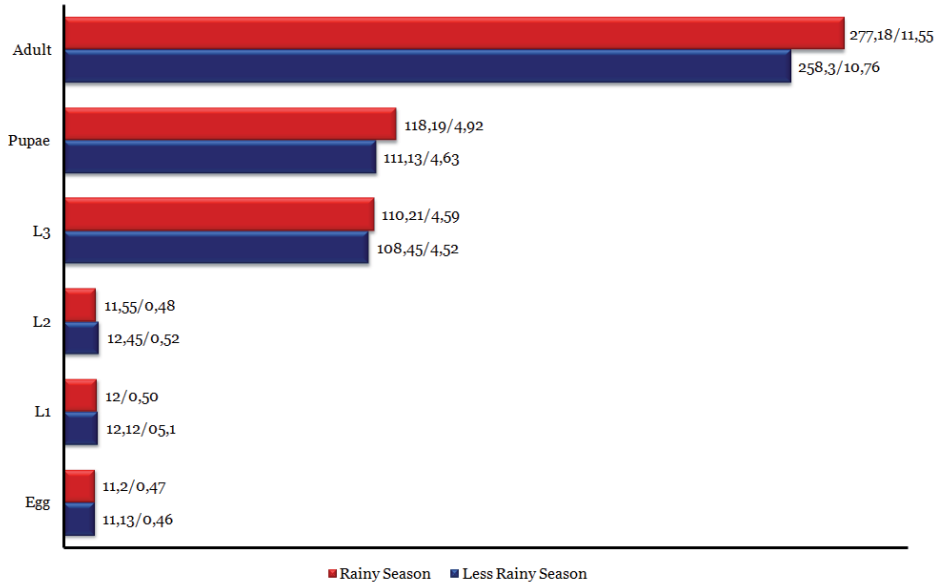

Figure 1. Variation in the mean development time (hours/days) of *Hemilucilia segmentaria* collected in swine in an urban area of the municipality of Manaus, AM, in 2008, in relation to the seasonal period.

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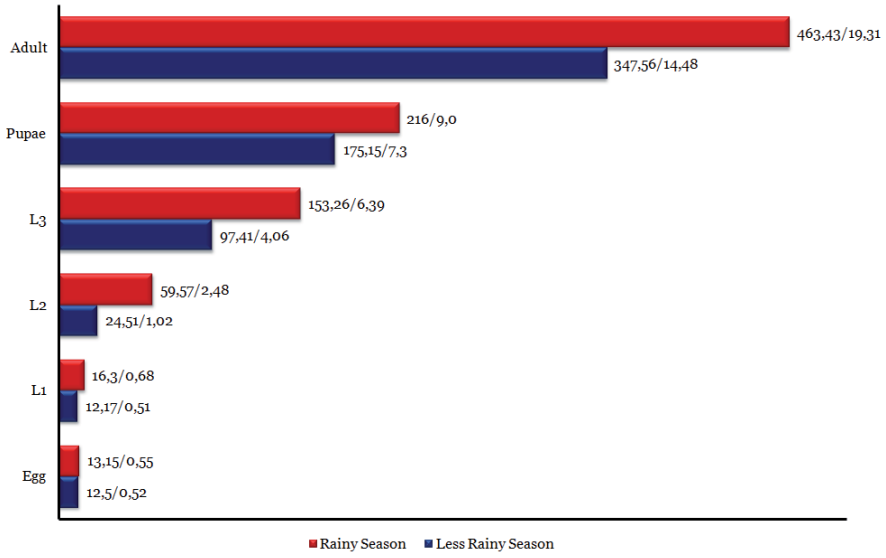

Figure 2. Variation in the mean development time (hours/days) of *Lucilia eximia* collected in swine in an urban area of the municipality of Manaus, AM, in 2008, in relation to the seasonal period.

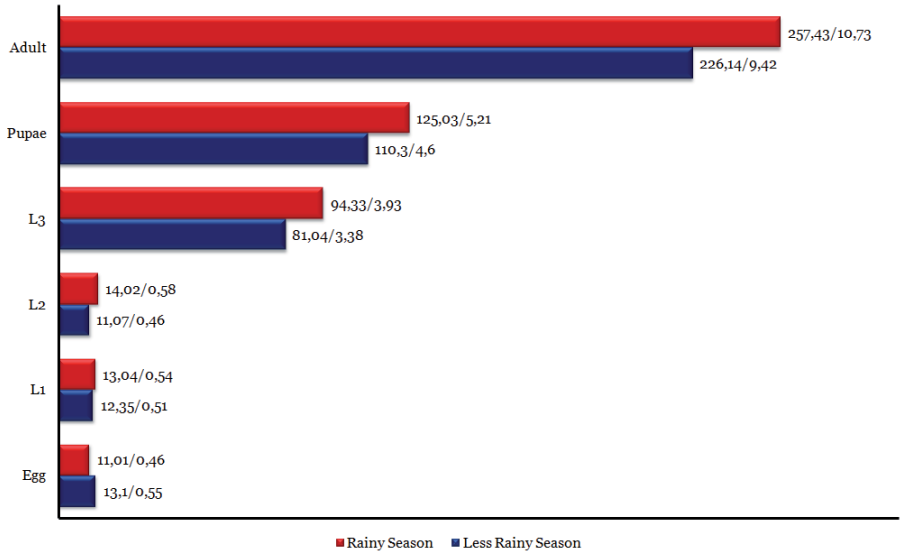

Figure 3. Variation in the mean development time (hours/days) of *Chrysomya megacephala* collected in swine in an urban area of the municipality of Manaus, AM, in 2008, in relation to the seasonal period.

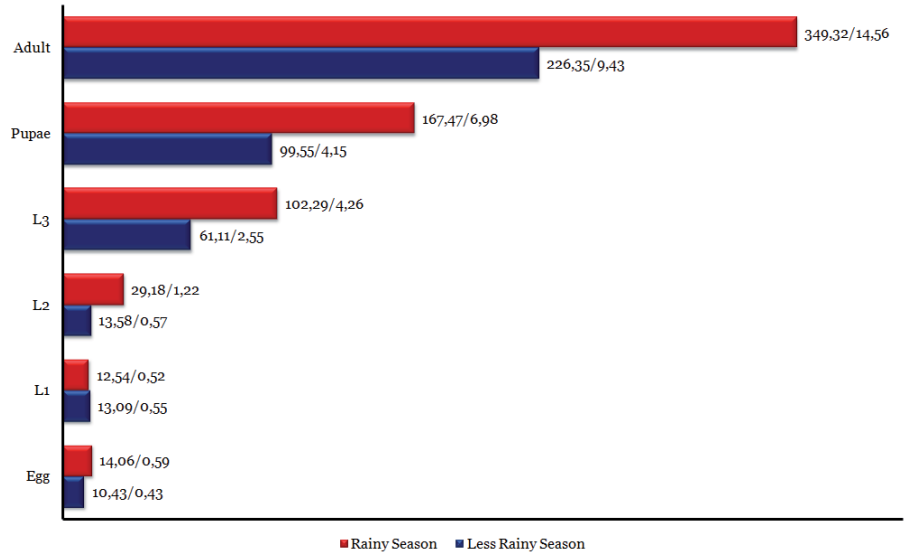

Figure 4. Variation in the mean development time (hours/days) of *Chrysomya albiceps* collected in swine in an urban area of the municipality of Manaus, AM, in 2008, in relation to the seasonal period.

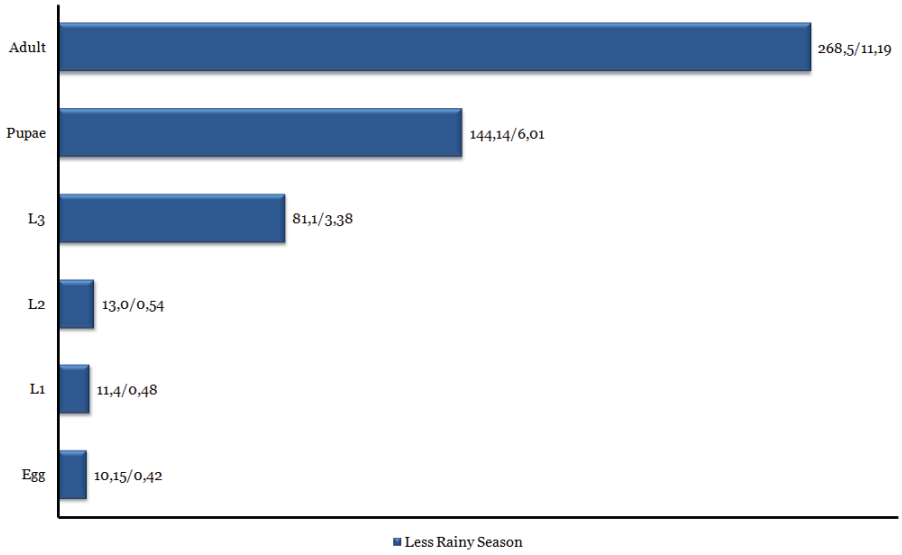

Figure 5. Variation in the mean development time (hours/days) of *Paralucilia paraensis* collected in swine in an urban area of the municipality of Manaus, AM, in 2008, in the less rainy season.

sampled species (Figures 1 to 5), but from the third stage and pupal stage, the variation is more visible. Such variation is what causes the difference in the total development time (Figures 1 to 5).

The development time of samples did not show significant difference between the treatments *C. albiceps* (F= 0.023, df= 7.526, p= 0.882), *C. megacephala* (F= 0.158, df= 5.564 , p= 0.705), *H. segmentaria* (F= 0.442, df= 6.425, p= 0.529), *L. eximia* (F= 0.216, df= 7.978, p= 0.654) and *P. paraensis* (F= 0.355, df= 7.363, p= 0.568), but it occurs between the two studied seasons, this difference was found only for: *C. albiceps* (F= 70.231, df= 16.574, p= 0.001), *C. megacephala* (F= 11.458, df= 12.672, p= 0.003), *H. segmentaria* (F= 6.831, df= 9.810, p= 0.001) and *L. eximia* (F= 82.001, df= 16.883, p= 0.001).

Pearson's linear correlation between temperature and humidity in the rainy season showed strong negative correlation (r= -0.767, p=0.009), while in the less rainy season it showed weak negative correlation (r= -0.260, p= 0.467). Due to variation of the abiotic variables between seasons, a linear regression analysis was conducted with both variables, to verify their influence in the duration of the total development time of all studied species.

In the rainy season both variables showed significant relation with the duration of species development time (Table 1), a pattern not observed in the less rainy season (Table 1), where only the relative humidity showed influence in the duration of the process. This can be explained by the low variation in temperature during the less rainy season, when this variable remained practically stable unlike relative humidity, which presented greater variation during sampled days.

The fact that in the less rainy period only relative humidity showed influence on the development time can be due the fact that, in this season, relative humidity was the only variable that presented a strong correlation with another variable, precipitation (r= 0.665, p= 0.008). This indicates that the sparse rains that occurred in this period caused peaks of humidity, somehow had some influence on the development time. In this experiment precipitation becomes a secondary variable, since its direct action was not tested. Although this experiment was carried out in an open environment, it was under the direct action of only temperature and humidity, since the cages that were used to follow the development time were placed in areas protected from the direct action of precipitation.

The minimum and maximum development time showed a visible difference between the two seasons (Table 2). With such variations in the developmental stages, the accumulated degree days (ADD) calculations had different values for each species (Table 3).

The sex ratio observed was around 0,50, or 1:1 (Table 4). This value was only different for *C. albiceps*, whose females emerged on both seasons. The collected eggs presented high viability, close to 100% (Table 4) in all studied species.

Table 1. Linear regression analysis between duration of the development time and abiotic variable in each season, for Calliphoridae species associated to pig decomposition of exposed in an urban area of the municipality of Manaus, AM, in 2008.

Species	Variable/ Seasonal Period	F	p
Chrysomya albiceps	T/LR	0.302	0.602
	RH/LR	12.877	0.007
	T/R	19.058	0.002
	RH/R	38.787	<0.001
Chrysomya megacephala	T/LR	0.582	0.527
	RH/LR	8.792	0.017
	T/R	12.337	0.008
	RH/R	20.182	0.002
Hemilucilia segmentaria	T/LR	0.452	0.525
	RH/LR	5.551	0.044
	T/R	22.712	0.001
	RH/R	14.907	0.005
Lucilia eximia	T/LR	0.481	0.512
	RH/LR	7.856	0.023
	T/R	31.825	<0.001
	RH/R	6.493	0.033
Paralucilia paraensis	T/LR	0.326	0.583
	RH/LR	8.714	0.016

LR= Less Rainy; R= Rainy; T= Temperature; RH= Relative Humidity.

Table 2. Variation in the development time of Calliphoridae species associated to pig decomposition of exposed in an urban area of the municipality of Manaus, AM, in 2008.

Species	Minimum Time (hours)	Maximum Time (hours)	Seasonal Period
Chrysomya albiceps	185h 30	259h 33	LR
	289h 46	403h 05	R
Chrysomya megacephala	212h 15	240h 55	LR
	216h 43	262h 20	R
Hemilucilia segmentaria	250h 02	266h 58	LR
	241h 40	312h 56	R
Lucilia eximia	314h 26	383h 06	LR
	451h 39	504h 27	R
Paralucilia paraensis	252h 19	282h 10	LR

LR= Less Rainy, R= Rainy.

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Table 3. ADD* and ADH* for each developmental stage of Calliphoridae species associated to the decomposition of swine exposed in an urban area of the municipality of Manaus, AM, in 2008.

Species		Seasonal Period	Stage					
			Egg	L1	L2	L3	Pupae	Adult
Chrysomya albiceps	ADH	R	190,60	199,80	463,09	1623,34	2657,75	5543,71
		LR	188,05	236,01	244,85	1101,81	1794,89	4081,09
	ADD	R	7,94	8,33	19,30	67,64	110,74	230,99
		LR	7,84	9,83	10,20	45,91	74,79	170,05
Chrysomya megacephala	ADH	R	174,73	206,94	222,50	1497,02	1984,23	4085,41
		LR	200,13	222,67	199,59	1461,15	1988,71	4077,30
	ADD	R	7,28	8,62	9,27	62,38	82,68	170,23
		LR	8,34	9,28	8,32	60,88	82,86	169,89
Hemilucilia segmentaria	ADH	R	177,74	190,44	183,30	1721,10	1875,68	4398,85
		LR	200,67	218,52	224,47	1987,09	2006,74	4657,15
	ADD	R	7,41	7,94	7,64	71,71	78,15	183,29
		LR	8,36	9,11	9,35	82,80	83,61	194,05
Lucilia eximia	ADH	R	176,95	258,68	945,38	2432,24	3427,92	7354,63
		LR	207,35	201,40	423,89	1738,27	3139,92	6248,48
	ADD	R	7,37	10,78	39,39	101,34	142,83	306,44
		LR	8,64	8,39	17,66	72,43	130,83	260,35
Paralucilia paraensis	ADH	LR	183,00	205,54	235,65	1462,23	2598,84	4841,06
	ADD	LR	7,63	8,56	9,82	60,93	108,29	201,71

LR= Less Rainy; R= Rainy; ADH= Accumulated Degree Hour; ADD= Accumulated Degree Days
* Values individually calculated for each studied stage. This way, to estimate the PMI it is necessary to add the ADD or ADH of the considered stage to the previous stages according Oliveira-Costa & Queiroz (2007), considering the minimum temperature threshold of 10°C according to Higley & Peterson (1994).

Table 4. Total eggs used and emerged adults of Calliphoridae species collected in swine exposed in an urban area of the municipality of Manaus, AM, in 2008, with the respective sex ratio.

Species	N	N E	Sex Ratio	Seasonal Period
Chrysomya albiceps	300	300	-	LR
	300	300	-	R
Chrysomya megacephala	300	297	0,52	LR
	300	300	0,52	R
Hemilucilia segmentaria	300	300	0,51	LR
	300	298	0,50	R.
Lucilia eximia	300	300	0,50	LR
	300	300	0,55	R
Paralucilia paraensis	360	356	0,51	LR

LR = Less Rainy, R = Rainy, N= Number of eggs analyzed, NE= Number of adults emerged.

DISCUSSION

The species considered of greater potential for forensic use in the two experiments carried out in an urban area of Manaus, are *L. eximia* and *C. albiceps*. That was due the great number of immature individuals developing on the carcasses and of the number of visiting adults, a fact that has also been observed in most studies conducted in urban areas in Brazil (Salviano *et al.* 1996; Moura *et al.* 1997; Souza & Linhares 1997; Carvalho & Linhares 2001; Souza *et al.* 2008; Krüger *et al.* 2010).

The observed sex ratio is very similar to what is expected, which is 1:1, half males and half females in the offspring. The only exception was *C. albiceps*, which on both seasons, only females were originated from the collected eggs. This happens because *C. albiceps* is a monogenic species, which means its offspring is thelygenic (only females) or arrhenogenic (only males), and the sex ratio is controlled at the population level (Ullerich 1958). This fact has also been observed by Queiroz *et al.* (1996) and Serra *et al.* (2007).

The development time differed from the one found by Fraga (2004), who studied the development time of *H. segmentaria* and *Lucilia eximia* under natural conditions in a Forest Reserve of a peri-urban area in Manaus.

When the total values of the species development (*op cit.*) were compared to the ones verified in our studies conducted in an urban area, these species presented shorter development time, despite average temperatures are similar. This can be explained by the location of the study area, since in urban areas both temperature and humidity are usually greater than in peri-urban areas, forming the so-called "urban heat island" (Oke 1987). That may have been the determinant factor for the difference found between the studies.

The total development time of the species observed in this study differed little when compared to studies carried out at temperatures between 25°C and 27°C in laboratory (Liu & Grenberg 1989; Marchenko 2001; Gabre *et al.* 2005; Thyssen 2005), and at semi-controlled temperatures (Souza *et al.* 2008,

Vélez & Wolff 2008, Krüger *et al.* 2010). What in fact differed was the duration of the two first larval stages, which were shorter. However, the third stage and the pupal period were longer, which made the total development time become similar to studies conducted in laboratory.

Usually, fluctuating temperatures increase the speed of development in necrophagous species when compared to constant temperatures (Dadour *et al.* 2001; Grassberger & Reiter 2001). Also, the effects of competition tend to strongly influence the duration of the development time, being able to influence the duration of developmental instars, since the initial instars are the most susceptible to variations (Tarone & Foran 2008). This fact was also observed in this study, where species tended to have shorter development period on initial developmental instars. This can be a behavior adopted to avoid competition with *C. albiceps,* which is an extremely competitive, predator species (Faria *et al.* 1999, Faria & Godoy 2001) who, besides not having any preference as for the species to be predated (Faria *et al.* 1999), also has a total development time shorter than the species collected. That can result in species tending to shorten the duration of their initial instars to avoid competition with the final instars of *C. albiceps*.

Another factor observed by Nabity *et al.* (2007) is that fluctuating temperatures in laboratory do not match the ones observed on the field, where the period of development can answer to an interaction between photoperiod and temperature, determining the rhythm of development.

In that sense, it is extremely important to conduct studies on development without temperature control, so that there is better understanding of the development-temperature relation, increasing this way the precision in the ADD calculations and therefore, the PMI calculation.

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