Rate of development of forensically-important Diptera in southern Brazil

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ABSTRACT. Rate of development of forensically-important Diptera in southern Brazil. Dipteran larvae were collected from rabbit (Oryctolagus cuniculus L.) carcasses during the four seasons in 2005 in the southernmost state of Rio Grande do Sul, Brazil. The larvae were fed ground beef at ambient temperatures following collection from carcasses. The development of each species under these conditions was estimated. The most abundant species in the carcasses were Lucilia eximia (Wiedemann) and Chrysomya albiceps (Wiedemann) (Calliphoridae), and they were found in all seasons. The data were fitted to a linear model that describes the relationship between temperature and linear developmental rating. These two species are primary forensic indicators in southern Brazil. Other species such as Hemulucilia semidiaphana (Rondani) (Calliphoridae), Synthesomyia nudiseta (Wulp), Muscina stabulans (Fallen) (Muscidae), and Fannia puto (Wiedemann) (Fanniidae) were forensically less important because they only occurred in high frequency in certain seasons and during the first days of carcass decomposition.

KEYWORDS. Calliphoridae; degree-day; developmental period; Muscidae.

RESUMO. Taxa de desenvolvimento de Diptera de importância forense no sul do Brasil. Larvas de dipteronous foram coletadas em carcaças de coelho-doméstico (Oryctolagus cuniculus L.) ao longo das quatro estações de 2005 no extremo-sul do Rio Grande do Sul, Brasil. As larvas foram alimentadas com carne bovina moída e acondicionadas em temperatura ambiente. O desenvolvimento de cada espécie foi acompanhado nestas condições. As espécies mais abundantes na carcaça foram Lucilia eximia (Wiedemann) e Chrysomya albiceps (Wiedemann) (Calliphoridae) em todas as estações. Os dados foram ajustados a um modelo linear da relação entre a taxa de desenvolvimento destas espécies e a temperatura média diária do ambiente. Estas duas espécies são as principais indicadoras forense no sul do Brasil. Outras espécies como Hemulucilia semidiaphana (Rondani) (Calliphoridae), Synthesomyia nudiseta (Wulp), Muscina stabulans (Fallen) (Muscidae) e Fannia puto (Wiedemann) (Fanniidae) foram menos importantes porque ocorreram em alta abundância em determinadas estações do ano no decorrer dos primeiros dias de decomposição.

PALAVRAS-CHAVE. Calliphoridae; desenvolvimento; grau-dia; Muscidae.

Forensic indicator insects are those organisms that use a carcass as a resource for their immature stages of development. Entomological data are more reliable than visual inspection of the level of decomposition, especially when the post mortem interval (PMI), the time interval between death and the discovery of the cadaver, is more than three days (Keh 1985; Smith 1986; Goff & Odom 1987; Catts & Haskell 1991, Catts & Goff 1992; Byrd & Castner 2000).

There are two possible methods to estimate the PMI with entomological data: determination of the minimum age for development of the immatures, and identification of the species associated with the stages of decomposition (Greenberg & Kunich 2002; Gunn 2009). The best way to determine the PMI has been the use of model development in terms of accumulated degree hours (ADH) or accumulated degree days (ADD), a process known as thermal summation (Higley et al. 1986; Worner 1992; Higley & Haskell 2000).

The application of this method requires good fits of the data of development in function of temperature in linear regression models since this time interval may change because of many factors, such as temperature, humidity, photoperiod, rainfall, oviposition circadian behavior, generational superposition, heat generated by the grouping of larvae, geographic position, and the effect of drugs and toxins (Greenberg 1990; Goff et al. 1991; Turner & Howard 1992; Goff et al. 1997; Higley & Haskell 2000; Carvalho et al. 2001; Donovan et al. 2006).

Two other sources of PMI errors refer to the planning itself of the experiment. The first relates to temperatures close to the limits that insects tolerate. Permanency and development of insects in carcasses are determined by the upper limits of temperature, around 33-38°C and lower limits of temperature, normally between 6 and 10°C. Upper temperature limits may produce a loss of water as well as denaturing of proteins necessary for metabolism, while lower temperatures may cause an increase in crystals originating from the corporeal liquids that cause rupture of tissue. These limits cause an alteration in the relation between the development rate of the immatures and the temperature of the exposure of the carcass (Denlinger & Yocum 1998; Higley & Haskell 2000).

Another source of error relating to PMI is the influence of fluctuating temperature versus constant temperature (Byrd & Allen 2001). Many measurements of development of Diptera...
of forensic importance occur under controlled temperatures (Greenberg 1991; Byrd & Butler 1998; Marchenko 2001; Grassberger & Reiter 2001). It is necessary to adjust rates of development of carrion flies due to fluctuating temperatures of the environment to give more robustness to linear regression models that represent this relationship. For this we chose three objectives: i) to determine the species colonizing carcasses in the area of research; ii) to chose the species which indicated the PMI based on the colonizing period of the carcasses and their development; and iii) estimate the model representing the relationship between the rate of development and temperature during the cyclical rhythms of this intrinsic factor.

MATERIAL AND METHODS

A domestic rabbit (Oryctolagus cuniculus L. 1758) carcass was exposed every three months during 2005 at the Federal University of Pelotas campus (UFPel), Capão do Leão County, State of Rio Grande do Sul. (31°48’08”S and 52°25’11”W). Each animal was killed by cervical dislocation and put in a cage which excluded larger necrophagous organisms, modified as per Monteiro-Filho & Penereiro (1987). Larvae were collected daily, in three hour intervals between 11am and 2 pm, until the carcasses reached the dry state, according with the decomposition stages proposed by Rodriguez & Bass (1983). We followed the pattern of succession of the species in the carcass by time of decomposition and season of the year. Larvae were stored in containers with ground beef and placed in large flasks with wet soil, taken from the place of collection, for pupation of the collected larvae. These containers were placed in a room at the Laboratory of Insect Biology, Department of Microbiology and Parasitology, UFPel, without temperature control and photoperiod, for emergence of adults.

The development period was determined from the date of larval collection until the emergence of the adults. For each individual the average room daily temperature it was exposed to for completion of development was recorded. Each day corresponded to one measure of larval development. The period was transformed on rate of development. The independent variables (average temperature to which each larva was exposed) and dependent variables (average of rate of development) were analyzed by regression model, followed by residual analyses. All analyses were done using the free R software (R Development Group 2006).

Adults were identified according to Carvalho & Ribeiro (2000), Carvalho & Couri (2002), Carvalho & Mello-Patiu (2008) and Wendt & Carvalho (2009), and the vouchers were placed in the Collection of Invertebrates at the National Institute of Amazon Research (Instituto Nacional de Pesquisas da Amazonia (INPA)).

RESULTS

During the year calliphorid larvae were predominant in the carcasses in all seasons, especially the immature stages of Chrysomya albiceps (Wiedemann, 1819) and Lucilia eximia (Wiedemann, 1819). Besides these two, ten other species may be considered as forensic indicators for the southernmost part of Brazil, especially Hemilucilia semiapipana (Rondani, 1850) (Calliphoridae), Peckia (Pattonella) resona (Lopes, 1935) (Sarcophagidae), Synthesiomyia nudiseta (Wulp, 1898), Muscina stabulans (Fallen, 1825) (Muscidae) and Fannia pusio (Wiedemann, 1819) (Fanniidae). These larvae may be important because of the frequency in which they were collected and because they were present in the first two days of carcass exposure (Table I).

The appearance of L. eximia and C. albiceps during the year (Table I), allowed us to estimate the influence of environmental temperatures during the developmental period of the immatures of these species. The variables rate of development and species were important to model construction (DF=32, F= 69.577, p<0.01). The model was obtained with high linear significance between rate of development and species (F1,29= 69.58, p<0.01, r2=0.878). In the model the term of interaction indicates that the temperature behaves in a distinct manner between the two species (F1,29= 5.546, p=0.025) (table 2), with bigger changes during the rate of development by temperature period for C. albiceps than for L. eximia (Fig. 1).

DISCUSSION

The two most abundant species were L. eximia and C. albiceps. These two species are forensic indicators from the north and northeastern to the southern areas of Brazil (Monteiro-Filho & Penereiro 1987; Salviano et al. 1996; Moura et al. 1997; 2005; Souza & Linhares 1997; Carvalho et al. 2000; Carvalho & Linhares 2001; Oliveira-Costa et al. 2001; Andrade et al. 2005; Souza et al. 2008; Souza & Ferreira-Kepler 2009). In Argentina, L. eximia, is found in carcasses, C. albiceps, is not. Together with Calliphora vicina Robineau-Desvoidy, 1830 and Lucilia sericata (Meigen, 1826), they are forensic indicators for the Province of Buenos Aires (Oliva 1997, 2001; Centeno et al. 2002).

Among the 12 species found in the carcasses, only S. crassipalpis was not observed as being necrophagous in previous research done in Brazil. There are citations in Argentina of this species found in domestic pork carcasses (Oliva 1997; Centeno et al. 2002). The other species which frequently occurs in carcasses in Argentina is M. stabulans (Oliva 1997; Centeno et al. 2002) which in Brazil was observed only once (Freire 1923).

Among the 12 species found, there are no data regarding the complete life cycle of L. eximia. As for the others, data refer to the estimations obtained for the development of immatures in constant temperatures, which are in disagreement with results observed in this work (Wells & Kurahashi 1994; Aguiar-Coelho & Milward-de-Azevedo 1995; D’Almeida & Mello 1995; Queiroz & Milward-de-Azevedo 1996; Milward-de-Azevedo et al. 1996; Paes et al. 1997; Marchenko 2001; Mascarini & Prado 2002; Gabre et al. 2005; Krüger et al. 2008) (Table I). With the exception of estimates for S. crassipalpis, S. chlorogaster and H. segmentaria (Bonatto 1996; Krüger et al. 2003; Thyssen 2005), which are close to
The data obtained in ambient temperature, the remainder of the species presents a development period larger than what would be expected from ambient temperatures.

This may be explained by three possibilities. The type and size of the carcass may explain the variation found for the large numbers of these two species according to the geographic location. In the Southeast of Brazil the experiments were done with models based on domestic pigs, animals with more biomass than the rats or the rabbits which were the models used in the South. In different tissues there is the possibility of a different rate of development among the species mentioned (Kaneshsjrajah & Turner 2004). For the carcasses with a different quantity of biomass, the larger the carcass the faster the development. This is because the abundance of larvae is greater, increasing the internal temperature of the carcass, thus accelerating the development of the Diptera (Greenberg 1991; Turner & Howard 1992; Greenberg & Tantawi 1993). In carcasses of less than 1kg, C. albiceps are not commonly found (Moura et al. 1997), possibly because they need higher temperatures, and that is why they colonize larger corpses to complete their development.

Another possibility refers to optimal development conditions. The species of Chrysomya originated in the Middle East Region (Prado & Guimarães 1982), where temperatures are higher and this may mean pre-adaptation to conditions requiring higher temperatures. When these temperatures become very low, species of this genus don’t appear in carcasses (Tantawi et al. 1996). In the Pelotas region in the southern-most part of Brazil, C. albiceps is more abundant in the warmer months of the year, during spring and summer, whereas L. eximia is drastically less frequent in the summer (Vianna et al. 1997, 2004).

The decrease in abundance of L. eximia and increase of C. albiceps may also be explained by a third possibility. The predatory habit of third-instar larvae of C. albiceps may help it to obtain the necessary resources for the process of pupation, through predation of larvae of other necrophagous Diptera (Denno & Cothran 1975). Chrysomya albiceps is highly predatory and this may account for the change of location and reduction of populations of necrophagous native species (Faria et al. 1999; Faria & Godoy 2001; Andrade et al. 2002; Faria et al. 2004).

The spatial and temporal variation may occur because of intrinsic factors (Hwang & Turner 2005). Investigating the stochastic influence on population dynamic of C. albiceps and L. eximia, Serra et al. (2007) showed that these species exhibit smaller oscillation amplitudes on environment, pushing the populations closer to stability without risk of extinction caused by biological invasion of blowflies. This stability of population equilibrium depends essentially on survival and fecundity (Godoy et al. 2001; Silva et al. 2003; Gião & Godoy 2006), despite the probability that C. albiceps is the only successful species in the presence of other blowflies (Faria et al. 1999). According to these authors, extrinsic factors would have low probability of interference in the selections of occurrence of these two species, reinforcing the hypothesis that biotic factors, such as competition and predation were the most important mechanisms in regulating these populations (Ulliett 1950). The spatial synchrony between third-instar larvae of C. albiceps and L. eximia occurs during spring, the period prior to the reduction of frequency of adults of L.
eximia in the summer (Vianna et al., 1997), which implies the possibility of regulation of the populations of L. eximia for C. albiceps during this period of the year.

Immature C. albiceps develop at a higher velocity in fluctuating temperatures (Fig. 1) than in constant temperatures (Marchenko 2001). The same may occur with L. eximia, even though there are no data relating to its complete development for more than one generation.

Fluctuating temperatures should be considered as sources of error in data using ADD (Tachibana & Numata 2004), even though there are difficulties in its estimation (Ames & Turner 2003). Grassberger & Reiter (2002) affirmed that larger differences in the period of development in different reports because of temperature are not necessarily attributed to variation in the experimental method (extrinsic factors, constant versus fluctuating temperatures), but to geographic adaptation (intrinsic factors). This may not occur for C. albiceps and other cosmopolitan species. The developmental period from the egg to adult of C. albiceps at 30°C, estimated by Marchenko (2001) in the old USSR, is very near to the estimation by Aguiar-Coelho & Milward-de-Azevedo (1995) in Rio de Janeiro, Brazil. Even in less distinct biogeographic regions, the period was very close to 9.4 days in the USSR and 9 days in Brazil.

Fluctuating temperatures increased the rate of development of Calliphora vomitoria (Linnaeus, 1756), Protophormia terraenovae (Robineau-Desoidy, 1830), L. sericata and Hydrotaea rostrata compared to constant temperatures (Davies & Ratcliffe 1994; Dadour et al. 2001), even though Greenberg (1991) verified that fluctuating temperatures diminished this speed in L. sericata, which generated some controversy according to Donovan et al. (2006).

Another factor is that fluctuating temperatures in the laboratory may not match those observed in the field, because in the majority of cases the variation of the photoperiod is not measured (Nabity et al. 2007). In the field with fluctuating temperatures, the emergence period (the point of definition of the total development period) may correspond to the interactions of the photoperiod (gate effect) with the temperature, determining the rhythm of development (Hweise 1967).

Degree-day estimates typically are based on weather service data, but these data can vary strikingly from the thermal environment occupied by insects, particularly forensic species that depended of carcass (Higley & Haskell 2000).

We conclude that L. eximia and C. albiceps are the major species of forensic importance in southern Brazil. According to our results, we assume that fluctuating temperatures cause a great effect on PMI based on these species when compared to constant temperatures. Fluctuating temperatures speed the developmental period of these species as represented by model as a function of temperature.

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REFERENCES


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Table II. Analysis of Variance (ANOVA) of linear regression between the rate of development of Lucilia eximia and Chrysomya albiceps and mean temperature of exposure in °C (MT) in the region of Pelotas, southern Brazil.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F-value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
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<tr>
<td>MT:species</td>
<td>1</td>
<td>0.0009729</td>
<td>0.0009729</td>
<td>5.546</td>
<td>0.0255</td>
</tr>
<tr>
<td>Residuals</td>
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<td>0.0001754</td>
<td>0.0050872</td>
<td>0.0112036</td>
</tr>
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<td></td>
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<tr>
<td>Species</td>
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<tr>
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<td>0.0000000</td>
<td>0.0000000</td>
</tr>
</tbody>
</table>

Residual standard error: 0.01324 on 29 degrees of freedom;
Multiple R-squared: 0.878, Adjusted R-squared: 0.8654,
F-statistic: 69.58 on 3 and 29 DF, p-value: 2.341e-13
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Oswaldo Cruz 91: 187–196.


