# Effect of Temperature on the Life Cycle of Euspilotus azureus (Coleoptera: Histeridae), a Predator of Forensic Importance

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# Effect of temperature on the life cycle of *Euspilotus azureus* (Coleoptera: Histeridae), a predator of forensic importance

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**Abstract**

*Euspilotus azureus* (Sahlberg, 1823) (Coleoptera: Histeridae) is one of the most frequently sampled species of Histeridae on carcasses in South America. Determining its physiological responses to different temperatures is essential for providing development rate equations to estimate the period of insect activity and postmortem interval of the body. Thus, to evaluate the influence of temperature on their life cycle and to understand their developmental threshold, experiments were conducted under laboratory conditions at 6 different temperatures (10, 15, 20, 25, 30, and 35 °C). All *E. azureus* life stages were responsive to thermal variations. Eggs of *E. azureus* did not hatch at extreme temperatures (10 and 35 °C) and the development time from egg to adult was longer at low temperatures (15 and 20 °C). Their biological responses, such as mean number of eggs produced, the number of eggs per egg mass, and development time at different temperatures, indicate that 20 to 25 °C is optimal for growth. The results suggest that *E. azureus* shows tolerance and adaptability in its ontogenetic stages over a wide temperature range, which is common in generalist species. Also, this study provides the development rate equation for the species, which is applicable to studies of forensic entomology.

Key Words: development time; histerid; medico-legal entomology; phenotypic plasticity.

**Resumo**

*Euspilotus azureus* (Sahlberg, 1823) (Coleoptera: Histeridae) é um dos histerídeos mais frequentemete coletados em carcaças na América do Sul. Suas respostas fisiológicas em diferentes temperaturas são imprescindíveis para obtenção de equações de desenvolvimento aplicáveis na estimativa do intervalo pós-morte e/ou período de atividade do inseto no cadáver. Com o objetivo de avaliar a influência da temperatura no ciclo de vida e conhecer os seus limiares térmicos, os experimentos foram desenvolvidos em seis temperaturas (10, 15, 20, 25, 30, e 35 °C). Como esperado, todos os estágios se mostram amplamente responsivos às variações térmicas. Os ovos não eclodiram em temperaturas extremas (10 e 35 °C). O tempo de desenvolvimento de ovo a adulto e a longevidade foram maiores em temperaturas mais baixas. A média de oviposição, ovos por postura e tempo de desenvolvimento indicam que as temperturas de 20–25 °C são as mais favoráveis para a espécie. Os resultados sugerem que os estágios ontogenéticos de *E. azureus* apresentam tolerância e adaptabilidade em uma amplitude de temperaturas, o que é comum em espécies generalistas. Além disso, o presente estudo provém equações de desenvolvimento para a espécie aplicáveis na entomologia forense.

Palavras Chave: entomologia médico-legal; histerídeo; plasticidade fenotípica; tempo de desenvolvimento

The use of carrion insects for postmortem interval (PMI) estimation is a common application of forensic entomology (Wells & LaMotte 2010). The maximum PMI ($PMI_{max}$) can be calculated mainly by the successional pattern of insects on the carcass, and the minimum ($PMI_{min}$) by the developmental rate of immatures collected, usually through accumulated degree-day models (Catts 1990; Tabor et al. 2004; Wells & LaMotte 2010). However, both approaches need previously known biological data available in the literature. Among the insect groups, flies (Diptera) and beetles (Coleoptera) are most studied for their use in PMI estimation worldwide (Grassberger & Reiter 2001; Richardson & Goff 2001; Grassberger et al. 2003; Midgley & Villet 2009; Velásquez & Viloria 2009, 2010; Lecheta et al. 2015). Dipteran species are more often used for time of death estimation through the $PMI_{min}$ approach than coleopterans, and the potential use of beetle development time is largely neglected (Midgley et al. 2009; Midgley & Villet 2009; Bajerlein et al. 2011). Thus, not all entomological resources available on a death scene are being used to their fullest potential, which could jeopardize and mislead the PMI estimation.

Coleopterans are commonly collected on carcasses in Brazil, and several studies highlight Histeridae as one of the most-recorded families in forensic research (Monteiro-Filho & Penereiro 1987; Carvalho et al. 2000; Carvalho & Linhares 2001; Mise et al. 2007, 2010; Corrêa et al. 2014). Histerid larvae and adults are voracious predators that are sampled on carcasses throughout the decay process, feeding on the immature stages of Diptera (Nuorteva 1970). *Euspilotus azureus* (Sahlberg, 1823) (Coleoptera: Histeridae) stands out as the most-collected histerid in faunistic surveys associated with carcasses in Brazil (Souza and Linhares 1997; Mise et al. 2007, 2010). It is well known that *E. azureus* has a wide geographical distribution, occurring in different environments with huge variations in temperature (Mazur 2011; Aballay et al. 2012; Degallier et al. 2012). However, its behavior on carcasses, and other biological aspects (life cycle, fertility, egg viability, mortal-

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ity, thresholds) at different temperatures, are poorly known. Besides enriching our knowledge about the life history of *E. azureus*, this information can be important for estimating the PMI by using the developmental rate of the immature stages. The objectives of this study were to determine the influence of temperature on the life cycle parameters of *E. azureus*, and to obtain the developmental rate equations for use in PMI calculation.

## Materials and Methods

The study was carried out at the Department of Zoology, Universidade Federal do Paraná, from Jul 2011 to May 2013. Specimens of *E. azureus* were collected from fragments of chicken or pork. The collected beetles were raised on larvae of *Sarconesia chlorogaster* (Wiedemann) (Diptera: Calliphoridae). A breeding stock of *S. chlorogaster* was established using the method described by Lecheta et al. (2015).

For evaluating the reproductive variables, 30 males and 30 females of *E. azureus* from the field were placed in the experimental arena (250 ml plastic pot) containing 200 g of sifted commercial topsoil (Terra Vegetal, Vitaplan, Santo Grande, São Paulo, Brazil) (Fig. 1), kept at 25 °C, with a 12:12 h L:D photoperiod. Because of their different consumption capacities, males of *E. azureus* were fed daily with 1 second instar *S. chlorogaster* larva, and females were provided with 3 larvae.

To study the developmental parameters of *E. azureus*, a virgin male and female from the breeding stock were paired in a new experimental arena (as mentioned above). At this time, the male was separated from the female after mating and kept in a different container until death. The female was kept in the same copulation arena to which another 200 g of sifted commercial topsoil was added to provide a substrate for oviposition, and moistened filter paper to avoid dehydration. The same methodology was used for each temperature tested (10, 15, 20, 25, 30, and 35 °C) and the reproductive variables of 30 couples per temperature were tested. The eggs from each mated female were placed in 100 ml plastic pots with moistened filter paper and sifted topsoil and maintained at the respective temperatures. The numbers of eggs and egg masses per pair, the duration of each instar, and mortality rates were recorded. At 15 °C, 122 individuals were monitored from egg to adult; 138 at 20 °C; 282 at 25 °C; and 150 at 30 °C.


**Fig. 1.** Experimental arena (250 ml pot) containing 200 g of sifted commercial topsoil, where *Euspilotus azureus* were paired under controlled conditions (15, 20, 25, and 30 °C; photoperiod 12:12 h L:D; RH 65 ± 10%).

All data collected in the aforementioned experiments were analyzed using the R statistical software (R Development Core Team 2015 - R 3.2.3 for Windows) program. To evaluate if there were differences in the duration of the *E. azureus* ontogenetic stages, fertility, and survival rate between temperatures, an adjusted generalized linear model (GLM) with family distribution Gaussian (inverse) was considered. An adjusted GLM with family Gamma (identity) and a Tukey test ($P \leq 0.05$) for a posteriori comparison were made to evaluate the sex ratio. Both GLM and correlation tests were used to evaluate if there were variations in the developmental rate of *E. azureus* at different temperatures. The analysis was performed for each immature stage (egg, 1st, 2nd instar larva, and pupa) using development time as the response variable and temperature as the predictor variable, as described by Lecheta et al. (2015). The libraries used on GLM tests were MASS (Venables & Ripley 2002) and effects (Fox 2003), and the library used on the a posteriori test was multcomp (Hothorn et al. 2008).

## Results

In this study, we observed that *E. azureus* females oviposit daily; the female burrowed and reached the bottom of the experimental arena to lay the egg masses. The extreme temperatures (10 and 35 °C) played a significant role in egg hatch; the eggs did not hatch, were excluded from the analysis. The temperature influenced the duration of all immature stages and the egg-adult duration, differing significantly among temperatures ($F = 71.51$; df = 691; $P < 0.01$) (Table 1). Mean egg incubation periods at different temperatures ranged between 5.5 ± 0.6 (± SD ) and 1.8 ± 0.4 d, while at 25 °C it took 2.1 ± 0.5 for the egg hatch. There were 2 larval instars (L1 and L2) with the average span of 5.5 ± 0.7 and 13.2 ± 2 d at 25 °C, respectively. The pupal stage ranged between 8.3 ± 1.4 d to 15.4 ± 0.8 d. As expected, at 15 °C the developmental period of *E. azureus* life stages was longer when compared to other temperatures, and it had the longest interval from egg to adult emergence (Table 1).

High temperatures had greater effect on egg and L1 stages than on the other developmental stages. When comparing the percent survival from egg–L1 among the 4 temperatures tested, at 30 °C only 71% of the eggs hatched into L1, whereas at the other temperatures the viability was over 90% (Table 2). The L1-L2 survival was higher at 20 °C (94%) than 25 °C (81%) and 30 °C (78%) ($P < 0.01$). The percent survival of L2–pupa and pupa–adult did not vary statistically among temperatures ($P = 0.823$). Only 77% of the L2 individuals became pupa, and all pupa turned into adults in this experiment. The adult longevity was not different between sexes at 15, 20, and 25 °C ($P = 0.695$), but female longevity (47.1 ± 11.8 d) (± SD ) was greater than males (37.4 ± 13.4 d) at 30 °C ($P < 0.05$) (Table 1). When comparing among temperatures, the longevity of males was statistically the same at 20 °C (41 ± 13.5 d), 25 °C (38 ± 15.6 d), and 30 °C (37.4 ± 13.4), but it was longer at 15 °C (46 ± 19.5 d) than at 25 and 30 °C ($P < 0.01$). The longevity of females was shorter at 25 °C (39.5 ± 14 d) than 15 (44.3 ± 18.9 d) and 30 °C (47.1 ± 11.8 d) ($P < 0.01$) (Table 1). The sex ratio was equivalent at 30 °C; however, more males emerged (57%) at 20 and 25 °C and females (57%) at 15 °C. The proportion of males increased from 0.43 (15 °C) to 0.57 (25 °C), whereas the proportion of females decreased from 0.57 (15 °C) to 0.43 (25 °C) (Table 3), so the correlation between sex ratio and temperature was positive in males and negative in females.

**Table 1.** Mean duration (days ± SD) of various developmental stages of *Euspilotus azureus* under laboratory conditions maintained at 4 temperatures, photoperiod of 12:12 h L:D and RH 65 ± 10%.

| Ontogenetic stages | 15 °C | 20 °C | 25 °C | 30 °C |
|---|---|---|---|---|
| Egg | 5.5 ± 0.6 a | 4.5 ± 0.9 b | 2.1 ± 0.5 c | 1.8 ± 0.4 d |
| 1st instar (L1) | 9.4 ± 0.7 a | 7.5 ± 2 b | 5.5 ± 0.7 c | 5.4 ± 1.8 c |
| 2nd instar (L2) | 24.3 ± 2.9 a | 22.2 ± 3 b | 13.2 ± 2 c | 10.4 ± 2.9 d |
| Pupa | 15.4 ± 0.8 a | 14.5 ± 0.8 b | 10.6 ± 1.3 c | 8.3 ± 1.4 d |
| Egg–Adult | 54.8 ± 3.2 a | 48.8 ± 3.9 b | 31.5 ± 2.8 c | 26 ± 3.4 d |
| Male longevity | 46 ± 19.5 aA | 41 ± 13.5 abA | 38 ± 15.6 bA | 37.4 ± 13.4 bA |
| Female longevity | 44.3 ± 18.9 acA | 40.7 ± 14.5 abA | 39.5 ± 14 bA | 47.1 ± 11.8 cB |

Means within a row followed by the same small letters are not significantly different. Adult longevity that differed between sexes are followed by different capital letters ($P < 0.05$).

The fertility parameters of *E. azureus* at different temperatures (viability of the eggs, number of eggs deposited, number of eggs per egg mass, and eggs laid per female) are presented in Table 4. The egg viability was about 90% at all temperatures, except at 30 °C, where the rate dropped to 70%. Mean oviposition was significantly higher at 25 and 30 °C than at the lower 2 temperatures. The mean number of eggs per egg mass was highest (5.8 ± 1.4) at 25 °C, differing statistically from all the other temperatures. The number of eggs per female was low at 15 °C when compared to 25 and 30 °C. Values of each fertility parameter suggest that the optimum temperature for *E. azureus* is about 25 °C (Table 4).

The development rate (1/*D*) line and the adjusted time of growth curves for each immature stage, and for total development from egg to adult of *E. azureus,* are shown in Figure 2. Development rate and temperature were positively correlated for all stages: egg ($R^2 = 0.8992$, $P = 0.0342$), L1 ($R^2 = 0.8782$, $P = 0.04147$), L2 ($R^2 = 0.8992$, $P = 0.03419$), pupa ($R^2 = 0.8970$, $P = 0.03493$) and egg to adult ($R^2 = 0.8992$, $P = 0.0342$) (Table 5). The minimum threshold temperature ($t_0$), thermal constant (*K* in degree-d), and developmental rate equations for each immature stage of the species were calculated and presented in Table 5. The equations had a good fit, indicating good data suitability to the linear model, and the temperature explained 87 to 89% of the developmental rate variation, depending on the ontogenetic stage (Table 5). The parameters $t_0$ and *K* were 9.31 °C and 37.75 d °C for egg hatch; 4.2 °C and 17.68 d °C for the L1; 5.96 °C and 25.74 d °C for the L2; 3.78 °C and 26.20 d °C for pupae; 9.31 °C and 37.58 °C for the total development (egg to adult emergence) (Table 5). Overall, the minimum development threshold was similar for all developmental stages (mean 5.8 °C), except the L1 (9.31 °C).

## Discussion

Temperature had a broad influence on the biology of *E. azureus*, including life-stage duration, number of eggs per egg mass, survival, and development time. Understanding these biological parameters can help determine the species dynamics and the interaction of *E. azureus* with the carcass or conspecifics, and bring more accuracy to PMI estimation. Despite the fact that development does not continue under high and low constants temperatures, these are not necessarily lethal temperatures under variable conditions, such as the field. In the field there are refuge sites and various microenvironmental abiotic and biotic factors that may vary significantly, affecting the occurrence and persistence of a species. However, despite the considerations cited above, the species phenotypic plasticity or thresholds can be reliably tested, achieving results similar to those in the natural environment, when comparing different temperatures using this methodology (Jarosík et al. 2004). Based on the results of the current study and reported previously (Bajerlein et al. 2011; Vanlaerhoven & Anderson 1999), histerid eggs seem to be susceptible to thermal variation. *Margarinotus striola succicola* (Thomson) and *Saprinus semistriatus* (Scriba) (Coleoptera: Histeridae) oviposit eggs on the ground near the carcass (Bajerlein et al. 2011) where the soil temperature is cooler due to the lack of larval mass (Vanlaerhoven & Anderson 1999). A similar behavior was observed in the present study where the female *E. azureus* oviposited on the bottom of the arena, after digging the soil above it, corroborating the observation on *Phelister panamensis* LeConte (Coleoptera: Histeridae) (Summerlin et al. 1991). It suggests that the eggs of *E. azureus* are sensitive to thermal variation; and that other factors such as moisture and the presence of natural enemies could influence the oviposition site temperature as well.

The finding that egg viability is lower at 30 °C can possibly be explained by the increased occurrence of pathogenic fungi in the breeding site at the higher temperature. Fungal contamination is one of the major problems affecting the breeding of insects (Sikorowski & Lawrence 1994) and was considered as a determinant factor for Curculionidae species (Jordão et al. 1997). Insects exposed to stress, such as lack of food or exposure to high temperatures, may be more susceptible to the action of pathogens, which is indicated by a change in their life cycle and the development of their ontogenetic stages (Sikorowski & Lawrence 1994; Rampelotti et al. 2007). Another possibility is that eggs dehydrate severely at high temperatures. Browning (1953) observed *Gryllulus commodus* Walker (Orthoptera: Gryllidae) egg weight at different temperatures and noticed there was significant water loss from the eggs at higher temperatures, affecting egg viability. Although insect eggs have the chorion as an external protective structure that prevents

**Table 2.** Survivability (proportion surviving ± SD) of the developmental stages of *Euspilotus azureus* under laboratory conditions maintained at 4 temperatures, and with photoperiod of 12:12 h L:D and RH of 65 ± 10%.

| Ontogenetic stages | 15 °C | 20 °C | 25 °C | 30 °C |
|---|---|---|---|---|
| Egg–L1 | 0.95 ± 0.06 ab | 0.91 ± 0.05 a | 0.98 ± 0.02 b | 0.71 ± 0.06 c |
| L1–L2 | 0.85 ± 0.09 abc | 0.94 ± 0.04 b | 0.81 ± 0.11 c | 0.78 ± 0.09 c |
| L2–Pupa | 0.81 ± 0.1 a | 0.86 ± 0.08 a | 0.74 ± 0.1 a | 0.71 ± 0.09 a |
| Pupa–Adult | 1.0 ± 0 a | 1.0 ± 0 a | 1.0 ± 0 a | 1.0 ± 0 a |

Means within a row followed by the same letter are not significantly different ($P < 0.05$).

**Table 3.** Sex ratio (proportion male or female ± SD) of *Euspilotus azureus* adults under laboratory conditions maintained at 4 temperatures, and with photoperiod of 12:12 h L:D and RH of 65 ± 10%.

| Sex | 15 °C | 20 °C | 25 °C | 30 °C |
|---|---|---|---|---|
| Male | 0.43 ± 0.14 aA | 0.57 ± 0.19 abA | 0.57 ± 0.11 bA | 0.47 ± 0.11 abA |
| Female | 0.57 ±0 .13 aB | 0.43 ± 0.19 bB | 0.43 ± 0.14 bB | 0.53 ± 0.11 abA |

Means within a row followed by the same small letters are not significantly different. Means that differed between sexes are followed by different capital letters ($P < 0.05$).

mechanical damage and dehydration, extreme conditions can affect their viability and development (Tuft 1950). Females of *M. striola succicola* and *S. semistriatus* oviposit in the soil near the carcass (Bajerlein et al. 2011), which can minimize exposure to predators and carcass conditions, such as the warming caused by the larval mass of Diptera. This particular oviposition behavior may be one among several mechanisms established by many species for protecting their more vulnerable ontogenetic stages (egg, early larva).

The number of *E. azureus* larval instars observed in the study is not usual for Coleoptera, but they are common for histerid beetles (Summerlin et al. 1981; Summerlin et al. 1991; Lawrence 1991; Kovarik & Passoa 1993; Caterino & Tishechkin 2006), and other forensically relevant beetles, such as *Sciodrepoides watsoni watsoni* (Spence) (Coleoptera: Leiodidae) (Kilian & Madra 2015). The duration of the larval stages of *E. azureus* marginally differed from other histerid species, such as *P. panamensis*. When reared at 25 °C, the average duration for *P. panamensis* L1 and L2 stages was 3.0 ± 0.7 and 8.1 ± 1.6, respectively (Summerlin et al. 1991). This difference between species can be explained by the kind of environments they inhabit; *P. panamensis* inhabit cattle dung and prey on horn flies maggots *Haematobia irritans* (L.) (Diptera: Muscidae), whereas *E. azureus* occurs on carcasses. The period of resource availability may be correlated to the duration of larval instars occurring in such environments. Cattle dung is a more ephemeral resource than a carcass, and *P. panamensis* immature stage is shorter than that of *E. azureus*.

As with any ectothermic organism, *E. azureus* also relies on external sources of heat, and its metabolism can drop or increase depending on abiotic conditions (Gotthard 2001; Jarosík et al. 2004). Studies on the forensically important beetle *Attagenus fasciatus* (Thunberg) (Coleoptera: Dermestidae) have shown that the immature stage responds more dramatically to environmental changes than the adults (Ali et al. 2011). The same was observed for the development time of the predatory beetles *Harmonia axyridis* (Pallas) (Lamana & Miller 1998; Castro et al. 2011), *Nephus includens* (Kirsch), and *N. bisignatus* (Boheman) (Coleoptera: Coccinellidae) (Kontodimas et al. 2004). The responsiveness of egg to adult development also was observed in other histerid species including *P. panamensis* (Summerlin et al. 1991), *Hister coenosus* Erichson, and *H. incertus* Marseul (Coleoptera: Histeridae) (Summerlin et al. 1981) reared at high temperatures (25–30 °C). Their longevity ranged from 23 to 36 d, similar to *E. azureus* (31.5 ± 2.8 d) at 25 °C. This convergence of results with the present study suggests that, although having different evolutionary histories, these species may have the same response tendencies. When comparing the immature stages with the adult of *E. azureus*, we may observe immature carcass-dependency, not only for food but mainly for protection against dehydration and thermal variation due to its phenotypic and mobility limitations. Despite the presence of the chorion on eggs and some sclerotized parts on larvae and pupae, their soft integument is more vulnerable than the full sclerotized body of the adults (Costa & Ide 2006). Thus, immature stages must physiologically adapt to adverse conditions, responding noticeably to microvariations, which makes them potentially valuable for using in PMI estimations.

The beetle fauna associated with cadavers consists predominantly of predators (Goff 1991) and, despite the plentiful availability of food, there is a great deal of competition between species (Bajerlein et al. 2011). However, because the predator species are abundant at different stages of decomposition (Hanski 1986), it is possible to estimate the time of death on the basis of entomological succession ($PMI_{max}$) from knowledge of the occurrence and persistence of predators and rates of decomposition (Bajerlein et al. 2011; Matuszewski et al. 2010). Bajerlein et al. (2011) suggested that because the biology of necrophilic predators has been poorly studied, inferences about its use in estimating the PMI from development time are underrated, but not impossible. The $PMI_{min}$ can be determined using predatory larvae of beetles collected on a cadaver by using successional data combined with the life cycle, both of which are available in the literature. In this context, the main contribution of the present study is the model based on immature age determination that can be applied in death cases for estimating the period of entomological activity on the body.

The mathematical model adopted in this article assumes that the insect development rate and environmental temperature have a linear relationship, which is commonly addressed in forensic entomological studies (Amendt et al. 2011). It is well known that as the temperature drops, the insect development gradually decreases until it reaches a point where it ceases; this point is the minimum threshold of development ($t_0$) (Richards and Villet 2008; Higley & Haskell 2010). Information on the developmental threshold of individual species is critical for using the models in the $PMI_{min}$ calculation as there may exist interspecific, and sometimes intraspecific variations (Lecheta et al. 2015). For instance, the overall (egg to adult) minimum development threshold for *E. azureus* was 9.3 °C, whereas for larvae it was was 4.2 (L1) and 5.9 °C (L2), and the pupa was the most cold-tolerant stage (3.8 °C).

The optimum temperature for a species is characterized by rapid development, and a greater number of offspring, thus improving their

**Table 4.** Biological parameters of *Euspilotus azureus* under laboratory conditions maintained at 4 temperatures, and with photoperiod of 12:12 h L:D and RH of 65 ± 10%.

| Parameters | 15 °C | 20 °C | 25 °C | 30 °C |
|---|---|---|---|---|
| Egg viability | 0.9 ± 0.3 a | 0.9 ± 0.3 a | 0.9 ± 0.2 a | 0.7 ± 0.1 b |
| Oviposition | 4.3 ± 1.1 a | 6.1 ± 2.1 a | 10 ± 2.7 b | 9 ± 2.21 b |
| Eggs per egg mass | 3.4 ± 0.8 a | 4.3 ± 1.3 b | 5.8 ± 1.4 c | 3.9 ± 1.2 ab |
| Eggs per female | 15.1 ± 5.6 a | 23.2 ± 7 ab | 28 ± 8.5 b | 27.3 ± 8.3 b |

Means within a row followed by the same letter are not significantly different ($P < 0.05$).

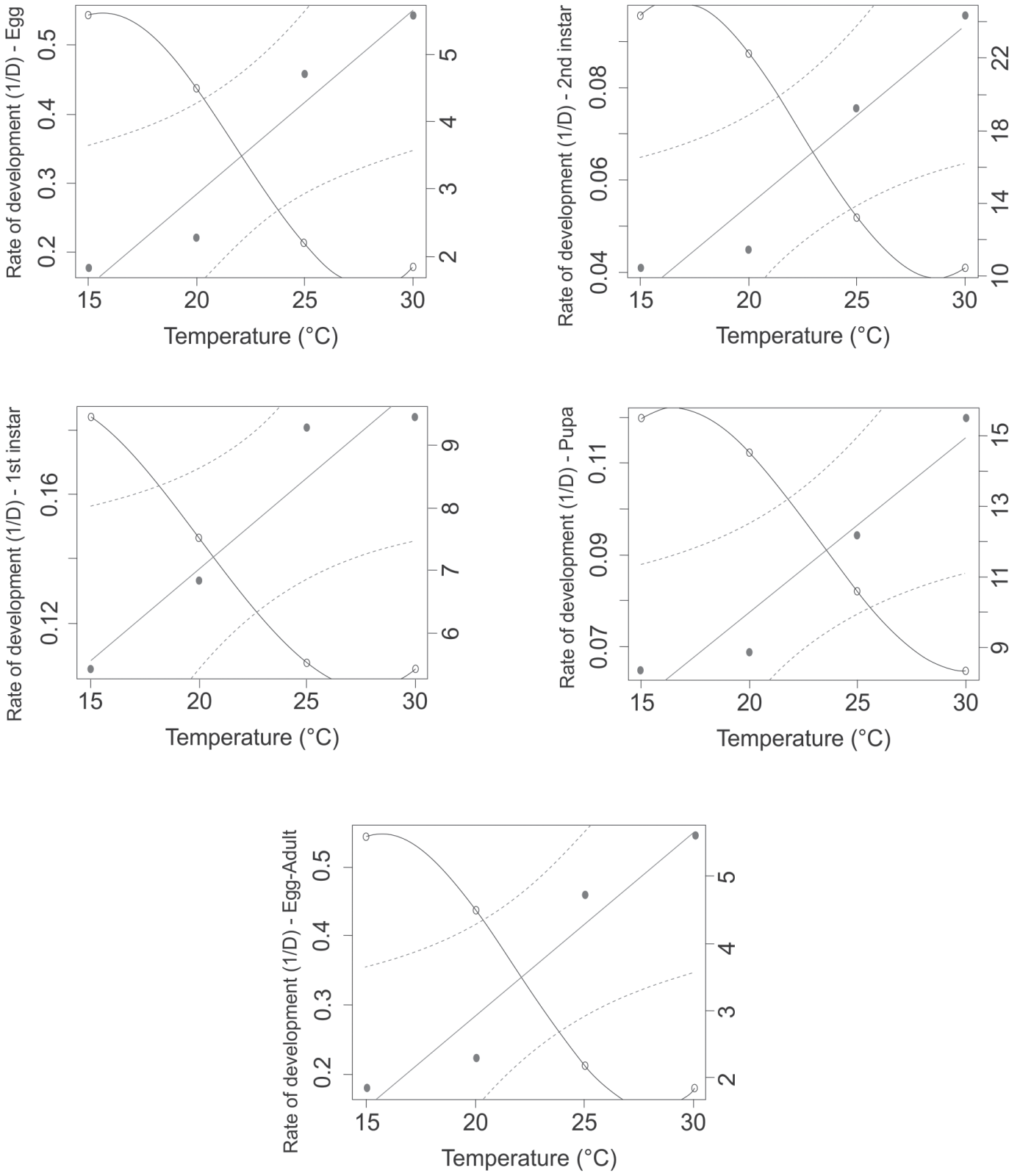


**Fig. 2.** Development rate (continuous line in grey) with a 95% confidence intervals and duration (in d, black line) of (a) egg development period, (b) first instar larvae, (c) second instar larvae, (d) pupa and (e) egg to adult of *Euspilotus azureus*, reared under controlled conditions (15, 20, 25, and 30 °C; photoperiod 12:12 h L:D; RH 65 ± 10%).

fitness (Roy et al. 2002). However, in assessing fitness, it is necessary to consider several responses of the insect, such as fertility, the rate of development, survival, and longevity. Not always do all responses converge on a single temperature. Sometimes only the majority of results display good performance at a common temperature, as for each parameter there is an optimum. Based on the longevity, survival rates, egg viability, number of eggs laid per female and per egg mass, the optimal temperature for *E. azureus* seemed to be 20–25 °C. Knowing

**Table 5.** Minimum threshold temperature ($t_0$ in °C), thermal constant ($K$ in degree-d), regression formulae of developmental rate ($1/D$) with the temperature of the death scene (T) and correlation ($R^2$) of each developmental stage of *Euspilotus azureus* reared at 4 temperatures, and with photoperiod of 12:12 h L:D and RH 65 ± 10%.

| | $t_0$ | $K$ | Development regression equation | $R^2$ |
|---|---|---|---|---|
| Egg | 9.31 | 37.75 | $1/D$ = -0.2477882 + (0.02660511 x T) | 0.8992 |
| 1st instar larva | 4.20 | 17.68 | $1/D$ = 0.023776590 + (0.005654038 x T) | 0.8782 |
| 2nd instar larva | 5.96 | 25.74 | $1/D$ = -0.023159818 + (0.003884116 x T) | 0.8992 |
| Pupa | 3.78 | 26.20 | $1/D$ = 0.001064126 + (0.003815587 x T) | 0.8970 |
| Egg to Adult | 9.31 | 37.58 | $1/D$ = -0.24778825 + (0.02660511 x T) | 0.8992 |

the thermal limits and developmental patterns of the species may help improve estimating $PMI_{min}$ in different contexts or environments.

Although the temperature is an important abiotic factor, it is not the only one that influences the use of insects in a forensic perspective. The different environments where the carcasses occur (Hanski 1986) show different moisture content, vegetation composition, soil, amount of light, and altitude, determining the rate of decomposition factors and consequently, attraction to insects (Matuszewski et al. 2010). For example, Gerard & Ruf (1997) observed that temperature influenced the biology of *Anthrenocerus australis* Hope (Coleoptera: Dermestidae), but other abiotic factors such as relative humidity and photoperiod affect the life cycle to a similar extent. Thus, complementary research controlling different abiotic factors is necessary, given that the conditions affecting the rate of decomposition of the carcass and the occurrence and abundance of the insect fauna remain the same (Hanski 1986). Such information will be important for applicability of *E. azureus* in forensic cases in regions with diverse biomes.

In summary, this study documented the effects of temperature on development time of *E. azureus*. Although temperature limits development under extreme conditions (10 or 35 °C), the species shows tolerance and adaptability over a wide temperature range, which is common among generalist species. Life cycle aspects of this study provide information for future forensic researches and case reports. The important outcome of this study was the development rate equations which may find applicability in PMI estimation, where immatures of *E. azureus* are sampled. The combination of these data with the temperature of the death scene and the time of arrival of the species in the carcass (which are available in the literature) may help determine more precise PMI estimation.

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