
Effect of temperature on the development time and survival of preimaginal *Culex hepperi* (Diptera: Culicidae)

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Efecto de la temperatura sobre el tiempo de desarrollo y la supervivencia preimaginal de *Culex hepperi* (Diptera: Culicidae)

■ **RESUMEN.** El objetivo de este trabajo fue analizar el efecto de la temperatura sobre el tiempo de desarrollo y la supervivencia preimaginal de *Culex (Culex) hepperi* Casal y García, 1967. Los individuos fueron criados en laboratorio, desde el primer estadio larval hasta la emergencia del adulto, a cinco temperaturas constantes: 15, 20, 25, 30 y 33°C. El tiempo total de desarrollo se relacionó de manera inversa con la temperatura entre 15 y 25°C. No se detectaron diferencias entre sexos en el tiempo de desarrollo y ningún individuo alcanzó el estado adulto a 33°C. En los estadios larvales III y IV, el tiempo requerido para mudar al siguiente estadio fue mayor a 30°C. La supervivencia más alta se registró a 20°C. Los estadios más avanzados fueron menos resistentes a las temperaturas por encima y por debajo de los 20°C. De acuerdo con el modelo no lineal de Brière, los umbrales de desarrollo inferior y superior fueron 2.6 y 33°C, respectivamente. Nuestros resultados sugieren que el efecto de la temperatura depende del estadio de desarrollo de *C. hepperi*.

PALABRAS CLAVE. Mosquito. Phytotelmata. Temperatura constante.

■ **ABSTRACT.** The aim of this research was to evaluate the effect of temperature on the development time and survival of *Culex (Culex) hepperi* Casal and García, 1967. Individuals were reared in the laboratory, from the first larval stage to adult emergence, at five constant temperatures: 15, 20, 25, 30, and 33°C. The total development time was inversely related to temperature between 15 and 25°C. No differences were observed in the development time between sexes and no adults emerged at 33°C. In the larval stages III and IV, the time required for molting to the next stage increased at 30°C. The highest survival was recorded at 20°C. The more developed stages were less resistant to temperatures above and below 20°C. According to the nonlinear model of Brière, the lower and upper development thresholds were 2.6 and 33°C, respectively. Our results suggest that the effect of temperature depends upon the stage of development of *C. hepperi*.

KEY WORDS. Mosquito. Phytotelmata. Constant temperature.

INTRODUCTION

The biological performance of preimaginal mosquitoes results from the interaction between the intrinsic attributes of the species and the environmental conditions of the breeding site, with temperature being one of the most important abiotic factors affecting the development, growth, and survival of immatures (Clements, 1992).

There is a positive linear relationship between temperature and developmental rate of preimaginal stages of mosquitoes within a certain thermal range, and the relationship becomes sigmoidal when a wider range is considered. Many nonlinear models have been proposed to describe the relationship in the preimaginal stages of arthropods. These models vary with respect to parameter number and basic assumptions about the temperature effect near the threshold. Some of them are descriptive models that allow a descriptive interpretation of parameters (e.g. Logan *et al.*, 1976; Bri e *et al.*, 1999); others are biophysical models based on enzyme reaction rates theory (Sharpe & DeMichele, 1977; Schoolfield *et al.*, 1981). On the other hand, the optimum temperature for development does not necessarily coincide with that for survival. There are several reports of the effect of breeding temperature on development time and survival of different immature mosquito species (e.g. Rueda *et al.*, 1990; Su & Mulla, 2001). Knowledge of the effect of temperature on the development time and survival of the different mosquito species provides baseline information essential for studies on population dynamics, spatial-temporal distribution and epidemiology.

Culex (Culex) hepperi Casal and Garc a, 1967 is a phytotelmic species whose immature stages have been found only in the leaf axils of unidentified species of *Eryngium* sp. (Belkin *et al.*, 1968; Casal & Garc a, 1967a) and *Eryngium pandanifolium* Cham. and Schlecht (Campos & Lounibos, 1999). So far, *C. hepperi* is one of the three species of the genus *Culex (Culex)* that breed exclusively in the axils of *Eryngium* species in Argentina (Casal & Garc a, 1967a; Casal & Garc a, 1967b). In Argentina, studies on

mosquitoes breeding in phytotelmata are scarce (e.g. Marti *et al.*, 2007; Campos & Lounibos, 1999). In particular for *C. hepperi*, little is known about its distribution (Mitchell & Darsie, 1985; Campos & Maci a, 1998; Belkin *et al.*, 1968) and ecology (Campos & Lounibos, 1999), and there is no available information on the influence of temperature on the development time and survival of its preimaginal stages.

Consequently, the objective of the present study is to evaluate the effect of the breeding temperature on the development time and survival of immatures of *C. hepperi* under experimental conditions. The hypothesis tested is that breeding temperature affects development time and survivorship of *C. hepperi*. It was predicted that within the range of temperatures tested, the immatures of *C. hepperi* reared at cooler temperatures take longer to emerge as adults than those reared at warmer temperatures. We also expected greater survival at intermediate temperatures than at extreme ones.

MATERIAL AND METHODS

Mosquito collection and rearing.

Mosquito eggs used in this study were collected from the leaf axils of *Eryngium* sp. growing naturally in the Delta of the Paran  River (34 11'S, 58 29'W), Argentina. The region is characterized by a mean annual temperature of 17 C, mean temperatures ranging between 6 and 30 C and a mean annual precipitation of 931 mm. Egg rafts were maintained until eclosion in individual plastic containers with tap water. All eggs were collected between April-June 2003, and during the same period the treatments were conducted. Within 12 h of eclosion, between 10 and 15 first instar larvae were randomly selected from each raft and individually placed in labeled plastic cups (3 cm in diameter, 5 cm high) with tap water. They were reared until adult emergence in thermal baths (Loetti *et al.*, 2001) at one of the following constant temperatures: 15, 20, 25, 30 and 33 C; the total number of first instar larvae tested in each one was:

65, 48, 37, 68 and 84, respectively. Larvae were kept under a photoperiod of L14:D10 and fed according to Gerber's methodology (1970) for *Culex pipiens* L.

The presence of exuviae, mortality and date of imago emergence were recorded daily at the same hour of the day to estimate the development time and survival of each immature stage (ecdysis to ecdysis). The adults were killed by freezing (-18°C), and then sexed under stereoscopic microscope. Fourth-instar exuviae and females were determined using keys to confirm species identity (Darsie, 1985; Rossi *et al.*, 2002) and species description (Casal & García, 1967a).

Data analysis. Differences in the development time (in days) of each immature stage among different experimental temperatures were tested with the nonparametric Kruskal-Wallis test, and the Dunn test was used for *post-hoc* comparisons (Zar, 1999).

For each temperature, the total development time was compared between sexes using the Mann-Whitney U-test (Zar, 1999).

The nonlinear model of Brière (Brière *et al.*, 1999) was used to quantify the relationship between temperature and development rate. The expression of the Brière model is:

$$R(T) = aT(T - T_0)(T_L - T)^{1/m}$$

$R(T)$ is the rate of development (1/development time) at temperature T , T = temperature (°C), T_0 = low-temperature development threshold (lower threshold), T_L = lethal temperature (upper threshold), a = empirical constant and m = shape parameter interpreted as the capacity of an insect species to develop and survive at high temperatures. This capacity is greater when $m < 2$. The parameters of this model were estimated from the total development rate (the reciprocal of time required for adult emergence) recorded for each experimental temperature.

For each temperature, the overall survival was calculated as the number of individuals that reached the adult stage divided by the number of larvae I at the beginning of each treatment. The survival of each preimaginal stage was calculated as the number of

individuals surviving to the next stage divided by the number of survivors in the previous stage. The results obtained from each trial were compared using the test of proportions for independent samples (Fleiss, 1981).

RESULTS

1. Preimaginal development time.

Culex hepperi showed a significant decrease in the number of days required for total development between 15-25°C, but no significant decrease was recorded between treatments of 25 and 30°C (Table I).

The relationship between the breeding temperature and the development time varied among the different immature stages. Between 15 and 25°C the time required for molting to the next stage decreased gradually with temperature. Thus, for all stages the development time at 25°C was between one-half and one-fourth that at the lowest temperature (15°C). However, at the warmest temperatures (30 or 33°C) only the development time of first instar larvae continued diminishing, while the time of larvae III and IV showed a significant increase (Table I).

The development time of males and females was similar regardless of the breeding temperature (15°C: U (N: 8, N: 11) = 43.00, 20°C: U (N: 20, N: 26) = 248.50, 25°C: U (N: 10, N: 10) = 31.00, 30°C: U (N: 5, N: 7) = 12.00; $P > 0.1$).

Fitting to the Brière model. The development rate of *C. hepperi* as a function of the experimental temperatures (15-33°C) was very well described by the model (Figure 1; $R^2 = 0.878$; $P < 0.001$). The lethal temperature (T_L) estimated by the model was 33.7°C, which was higher than the temperature to which there was not adults emergency (33°C). Thus, the model was fitted with the upper threshold temperature (T_L) determined empirically (33°C). The lower threshold temperature (T_0) estimated by the model was 2.3°C, and values of parameters a and m were 4.5169×10^{-5} and 2.1957, respectively. The estimated optimum

Table I. Duration of the development stages (days) of *Culex hepperi* at five constant temperatures.

Preimaginal stage	Rearing temperature (°C)	n	Median* (days)
Larva I	15	63	8.0a
	20	46	3.0b
	25	35	3.0b
	30	67	2.0c
	33	29	2.0c
Larva II	15	62	4.0a
	20	46	3.0b
	25	35	2.0c
	30	64	2.0c
	33	16	2.0c
Larva III	15	62	5.0a
	20	46	3.0bc
	25	35	3.0b
	30	50	4.0c
	33	0	
Larva IV	15	44	10.0a
	20	46	6.0b
	25	23	5.5b
	30	17	7.0a
	33	0	
Pupa	15	38	7.0a
	20	46	4.0b
	25	20	3.0c
	30	12	3.0c
	33	0	
Total†	15	38	33.0a
	20	46	19.0b
	25	20	16.0c
	30	12	15.5c

* For each development stage, medians with a same letter were not significantly different ($P > 0.05$; Dunn Test).

† From larva I to adult emergence.

temperature was 27.1°C, with a development time of 14.7 days.

2. Preimaginal survival. The proportion of larvae I of *C. hepperi* reaching the adult stage varied according to the different experimental temperatures ($\chi^2 = 70.299$; d.f. = 3; $P < 0.001$). The highest survival was recorded at 20°C ($\chi^2 = 44.913$; d.f. = 1; $P < 0.001$), with 96% of larvae I (46/48) reaching the adult stage. Survival was about 55% at 15 and 25°C (38/65 and 20/37, respectively), and this value was significantly higher than the one recorded at 30°C ($\chi^2 = 25.904$; d.f.

= 1; $P < 0.001$), with only 18% of larvae I (12/68) reaching the adult stage. No adult emergence was observed at 33°C.

The effect of the breeding temperature on survival varied among different developmental stages. More than 90% of larvae I survived between 15-30°C, and this same pattern was observed for larvae II; these were the only stages that molted at 33°C. The survival of larvae III was high between 15-25°C and decreased significantly at 30°C. The survival of larvae IV and pupae was 100% only at 20°C, whereas it decreased significantly for the rest of the temperatures (Fig. 2)

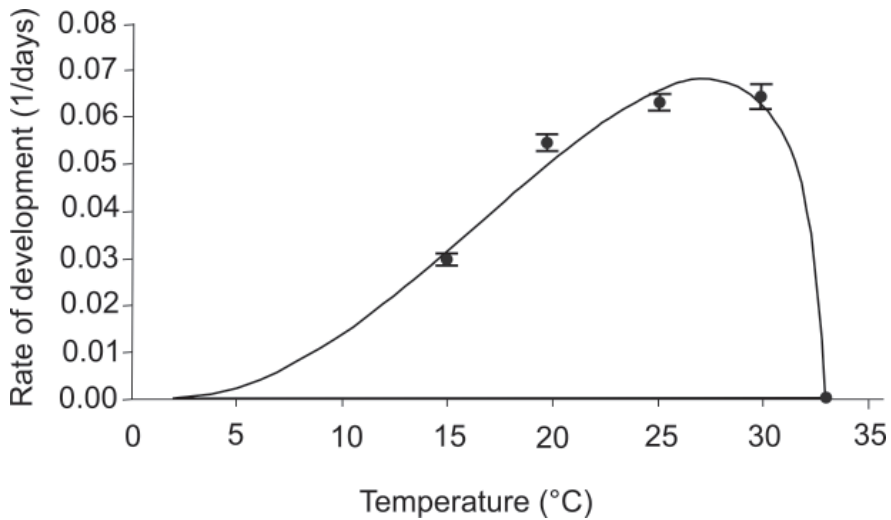


Fig. 1. Temperature-dependent rate (mean \pm SEM) of development of *Culex hepperi* from first instar larva to adult emergence: Observed data (●) fitted to the Briere model (solid line).

DISCUSSION

Our results indicate that the time required for *C. hepperi* to reach the adult stage from larva I is inversely related to water temperature within the range 15-25°C, but not above that range. A similar relationship has been reported for different mosquito species (e.g. Brust, 1967; Rueda *et al.*, 1990). This pattern of progressive decrease in development time with increasing temperature seems to change for larval stages III and IV, which showed a longer development time at 30°C than at lower temperatures. According to Sharpe & DeMichele (1977), extreme temperatures affect the catalysis of enzymes involved in the development of poikilotherms. In this sense, our results could be interpreted as an indirect evidence of the effect of unsuitable thermal conditions on the enzymatic activity during immature stage development. On the other hand, this laboratory result could indicate a limited adaptation of *C. hepperi* to zones of very warm temperatures, an assumption that seems to agree with the distribution of this species (Mitchell & Darsie, 1985; Campos & Maciá, 1998).

Both sexes had the same development time within the temperature range studied. In mosquitoes, males usually emerge before

females (Christophers, 1960; Clements, 1992), a phenomenon known as protandry. The no observation of protandry in *C. hepperi* could be either a specific feature like in *Culiseta inornata* (Williston, 1893) and *Aedes nigromaculis* (Ludlow, 1906) (Brust, 1967), or simply an artifact resulting from the experimental conditions (constant temperature and photoperiod), as previously observed for *Culex apicinus* Philippi (Loetti *et al.*, 2007).

The nonlinear model of Briere indicated that the highest development rate occurs between 25-30°C, with the optimum temperature being 27.1°C. According to the values of the lower and upper threshold temperatures (2.3 and 33°C, respectively), this species seems to tolerate a relatively wide thermal range. Our laboratory observations agree with the field observations reported by Campos & Lounibos (1999), who collected immatures of *C. hepperi* every month for a year in a nearby locality at the south of the Delta of the Paraná River (ca. 150 km). Although this species could develop all-year-round at least at this latitude (34°S), the estimated value of m parameter (2.20) suggested that low temperatures seem to be the most favorable for immatures of *C. hepperi*.

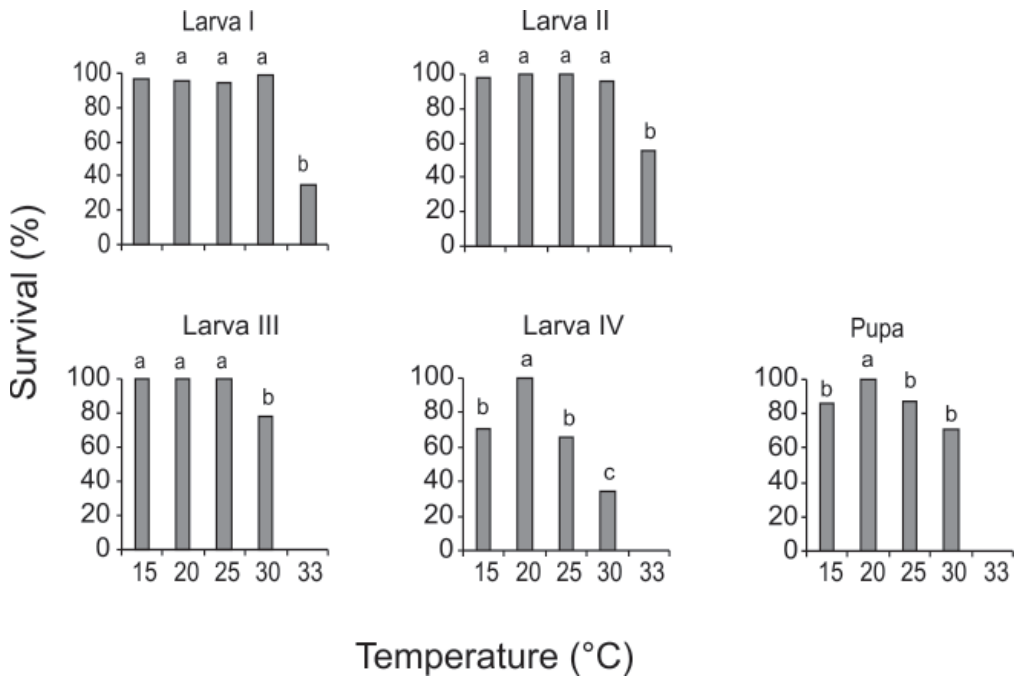


Fig. 2. Effect of temperature on the survival of preimaginal stages of *Culex hepperi*. The survival was calculated as the number of individuals surviving to the next stage divided by the number of survivors in the previous stage. Different letters indicate significant differences only within the individual plots ($P < 0.05$).

As for survival, 20°C appears to be the optimum temperature for *C. hepperi* based on the highest proportion of immatures that reached the adult stage. Survival values of each immature stage showed a significant decrease above and below 20°C for the more advanced stages (larva IV and pupa). In addition, only larvae I and II succeeded in molting at 33°C. The tolerance of mosquitoes to high temperatures tends to decrease with development, and this fact depends on the exposure time and the mosquito species (Forattini, 1962). Our results suggest that the different immature stages tolerate different temperatures, with the more advanced stages showing the lowest tolerance to non-optimal temperatures. However, we should be cautious about the interpretation of this finding because the effect of exposure time to high temperatures was not evaluated in the present study.

In brief, the most favorable temperatures for *C. hepperi* immatures in terms of highest

survival and development rate seem to be in the range of 20-27.1°C. There were harmful effects at 30°C and lethal at 33°C. Although under natural conditions water temperatures may not remain high (i.e., 30-33°C) for a long period of the day, data obtained from this laboratory study could be used as indicators of the capacity of *C. hepperi* to tolerate different breeding temperatures. These results can be of interest in the face of a climatic change scenario. Shifts in the physiology, distribution and phenology of some species seem to be explained by the current climatic and atmospheric trends (Hughes, 2000). If the warm temperatures have a negative effect on the preimaginal biology of this species, then an increase of the ambient temperature could cause a displacement of its distribution toward higher latitudes. Clearly, a better knowledge about this phytotelmic mosquito and its habitat is needed to assess these topics.

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