Research article

The role of temperature in shaping *Culex acharistus* mosquitoes life history traits in its southern limit of distribution (Patagonia-Argentina)

M.G. Grech [a,b,*], M.L. Miserendino [a,b], W.R. Almirón [c,d]

[a] *Centro de Investigación Esquel de Montaña y Estepa Patagónica (CIEMEP), CONICET and Universidad Nacional de la Patagonia San Juan Bosco, Esquel, Chubut, Argentina*
[b] *Facultad de Ciencias Naturales y Ciencias de la Salud, Universidad Nacional de la Patagonia San Juan Bosco, Sede Esquel, Esquel, Chubut, Argentina*
[c] *Universidad Nacional de Córdoba. Facultad de Ciencias Exactas, Físicas y Naturales, Centro de Investigaciones Entomológicas de Córdoba, Córdoba, Argentina*
[d] *Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET, Instituto de Investigaciones Biológicas y Tecnológicas (IIBYT), Córdoba, Argentina*

ARTICLE INFO

ABSTRACT

There is substantial evidence showing that temperature have a great impact on insects behavior, phenology and life histories. Because of mosquito global importance as disease vectors, in temperate regions where climatic conditions could be only borderline suitable for mosquito development, there is a growing interest in understanding the effect of temperature shifts on vital statistics to more accurately define how such changes could impact distribution and abundance patterns, as well as disease transmission cycles. We determined the role of ambient temperature under fluctuating conditions in shaping *Culex acharistus* (Diptera: Culicidae) life history traits, and estimated its development threshold and physiological time, in its southern limit of distribution in the Argentine Patagonia region. Four horizontal life tables were conducted under natural fluctuating temperature range in Esquel city (42°S – 71°W; 563 m a.s.l.), during spring-summer (17°C), summer (15.4°C), summer-autumn (12.7°C) and autumn-winter (5.6°C) seasons. Larvae, pupae and adult traits were recorded. The mean duration of the experiments varied between 28 to ≅100 days for spring-summer and autumn-winter seasons. Only during the cold season experiment pupae experienced the most severe temperatures and freeze-thaw cycles, and failed to reach adult stage. We found that larva and pupa development time, adult emergence time and longevity significantly increased with decreasing temperatures, while larval survival was greatest at an intermediate temperature and decreased toward low and high values. Also, protandry was observed and males emerge 2 days before females across seasons. Temperature development threshold and physiological time estimated for larva + pupa were 5.98°C and 211.24°C-days. Our study contributes to a growing body of knowledge by examining the effect of

* Corresponding author. Centro de Investigación Esquel de Montaña y Estepa Patagónica (CIEMEP), CONICET and Universidad Nacional de la Patagonia San Juan Bosco. Esquel, Chubut, Argentina.
E-mail addresses: mgrech@comahue-conicet.gob.ar (M.G. Grech), mlmiserendino@comahue-conicet.gob.ar (M.L. Miserendino), wralmiron@unc.edu.ar (W.R. Almirón).

seasonal changes in temperature on mosquito life history traits. Results obtained here can be applied as useful parameters in the development of population dynamic models, improving current mosquito control strategies in cold-temperate regions.

1. Introduction

Insects are ectotherms, and therefore their body temperature, basic physiologic functions, life history and population dynamics are almost entirely dependent on that temperature of the surrounding environment [1–4]. The range over which insects can survive is limited above and below by lethal temperatures, and within this range lies the activity threshold [5,6]. There is substantial evidence showing that temperature have a great impact on mosquito life and disease transmission cycles [7–11]. Worldwide increasing temperatures have been associated with increased rates of immature survival and development, and the abundance of adults and feeding activity of mosquitoes [8,12–14]. However, the effect of extremely high temperatures beyond a certain threshold over an extended period has been shown to be complex and nonlinearly, affecting negatively immature and adult mosquito life history [15,16]. Because of mosquito global importance as disease vectors, there is a growing interest in understanding the limits of their thermal tolerance and the local adaptation to temperate climates of species constantly expanded towards temperate regions [17–19].

Climate change and global warming may be the most pervasive and rapidly increasing threat, and is expected to have a severe impact on biodiversity and ecosystems [4,20]. Shifting patterns of temperature and precipitation due to climate change, combined with a variety of interacting anthropogenic stressors (e.g. degradation of land and habitat loss, pesticides, pollution), pose novel challenges on insects communities [1,21]. Global changes are likely to result in alterations of insects behavior, phenology and life histories, and species distribution and abundance [4]. Climate change is expected to affect the distribution and seasonal dynamic of mosquito populations, with implications for disease incidence, period of transmission, spread and persistence [22–24].

Culex acharistus Root, 1927 (Diptera: Culicidae) is known from Colombia, Brazil, Chile, Argentina and Uruguay [25]. It was first described by Ref. [26], from specimens collected in Agua Limpia (Brazil). In Colombia, *Cx. acharistus* was collected in high mountain areas (2546–2642 m a.s.l) of Salento [27]; whereas, in Chile it is distributed in northern, central and southern regions (29–41° S; 70–73° O) [28,29]. In Argentina it is widely distributed through northern and central areas, up to Chubut province (22–46° S; Fig. 1) [31,32], and was mainly found in both, natural and artificial breeding sites [33]. *Culex acharistus* females have been incriminated as
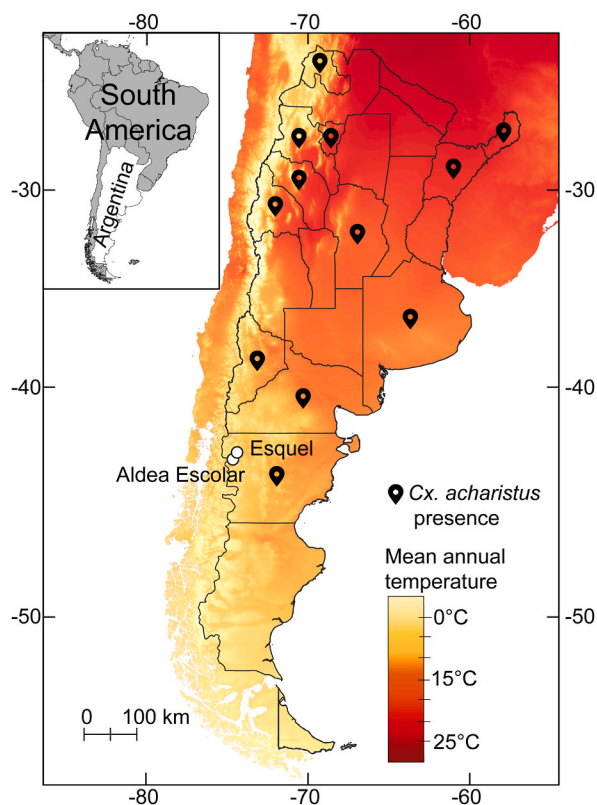
Fig. 1. Map of Argentina showing *Culex acharistus* distribution by provinces, and mean annual temperature variation across the country. Locations of Aldea Escolar and Esquel cities (Chubut province) are shown, were experimental material was collected and procedure was conducted, respectively. Temperature was derived from data produced by WorldClim v.2 [30].

potential vector of Eastern Equine Encephalitis Virus during the 1988 epizootic in Chaco province [34], and found naturally infected with Bunyamwera Virus in Formosa Province [35]. Knowledge on the effect of temperature shifts on life history traits is needed to more accurately define how such changes could impact abundance and distribution patterns of mosquito and vector-borne diseases. Here, we determined the role of ambient temperature under fluctuating conditions in shaping *Cx. acharistus* life history traits, and estimated its development threshold and physiological time, in its southern limit of distribution in the Argentine Patagonia region.

2. Material and methods

2.1. Study area

The study area was located in Esquel city (42°S – 71°W; 563 m a.s.l.), northwestern of Chubut Province, Argentine Patagonia (Fig. 1). The climate is cold temperate and rainfall is mainly concentrated in winter months, receiving 461.5 mm of mean annual precipitation. The mean summer temperature is 14 °C (mean maximum of 21 °C; mean minimum of 7 °C). During winter the mean temperature is 3.4 °C (mean maximum of 8.3 °C; mean minimum of −1.5 °C), with heavy snow (Precipitation and temperature data collected during the period: 1981–2010) [36].

2.2. Culex acharistus mass rearing and experimental procedure

To evaluate the effect of temperature on *Cx. acharistus* life history traits four horizontal life tables were conducted under natural fluctuating temperature range. Cohorts were reared at different natural thermal profiles recorded in Esquel city, in the CIEMEP (Centro de Investigación Esquel de Montaña y Estepa Patagónica) laboratory facilities. The laboratory setup consists of an open-room ensuring climatic conditions similar to the surrounding environmental conditions. The room was made of three concrete walls and the roof, exposed to ambient temperature, humidity and lighting conditions, and provide a shelter from direct rain and snow. During each study period, temperature was allowed to vary freely and daily maximum, minimum, and average temperatures were recorded with a data logger (HOBO 8 K Pendant Temperature/Alarm; logging rate: 30 min). Three cohorts of *Cx. acharistus* were reared in each study period at: 1) 17 ± 0.5°C (end of spring-beginning of summer season: November–December 2016, mean minimum: 12.3 ± 0.4°C, mean maximum: 22.5 ± 0.6°C, amplitude of fluctuation: 10.2°C); 2) 15.4 ± 0.5°C (summer season: February–March 2018, mean minimum: 11.3 ± 0.5°C, mean maximum: 20 ± 0.7°C, amplitude of fluctuation: 8.7°C); 3) 12.7 ± 0.4°C (end of summer-beginning of autumn season: March–April 2017, mean minimum: 9.4 ± 0.4°C, mean maximum: 16.9 ± 0.5°C, amplitude of fluctuation: 7.5°C); 4) 5.6 ± 0.4°C (autumn-beginning of winter season: April–July 2018, mean minimum: 3.7 ± 0.4°C, mean maximum: 8.1 ± 0.4°C, amplitude of fluctuation: 4.4°C) (Fig. 2).

Culex acharistus mass rearing was performed from field egg rafts originally collected from Aldea Escolar city (Chubut Province, Argentina), a semi-urban area located ≅ 30 Km far away from Esquel city (Fig. 1). Egg rafts were collected from natural breeding habitats and transfer to the laboratory, each raft in a well in 24-well tissue culture plates. Newly emerged larvae were randomly selected from 10 to 15 egg rafts that hatched synchronously the day before to each experiment, and separated into cohorts of 40 larvae each. To ensure egg rafts collected from Aldea Escolar city were only *Cx. acharistus*, ten larvae by raft were reared to the fourth-instar larvae for taxonomic identification according to available keys [37], being kept each larvae in a small plastic flask containing distilled
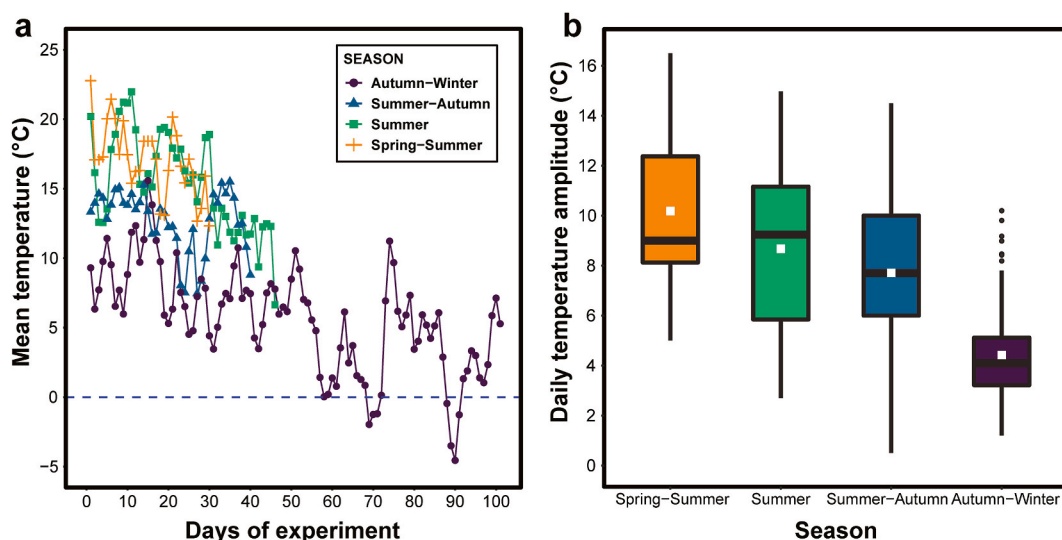

Fig. 2. Temperature variation. a) Natural thermal profiles recorded in Esquel city, Argentine Patagonia, and b) daily temperature amplitude, during each study period (Spring-Summer, Summer, Summer-Autumn and Autumn-Winter).

water and added 0.25 mg daily/larva liver powder.

Four cohorts of 40 larvae of *Cx. acharistus* were established during each study period. Each cohort was kept in a 500 ml dechlorinated water-filled plastic tray (16.5 cm length, 12 cm width, 4.5 cm height), and fed with 0.25 mg daily/larva of liver powder [38]. Water surface of larval trays was skimmed daily using a strip of filter paper to avoid subsequent fungal and bacterial development. When larvae reached the pupal stage they were picked from trays, and transferred into individually labelled, mesh-covered plastic flasks containing 30 ml of dechlorinated water (3.5 cm in diameter and 6.5 cm height), until adult emergence. Adults were kept alive in individually labelled mesh-covered containers, provided with only water-soaked cotton pad, until all had died.

Each cohort was monitored daily, counting the number of larvae alive in each cohort and categorized them visually according to the instars. First and second instar larvae were considered together as one age class (larva 1 + 2). To estimate the duration of each instar larvae and the pupal stage development, the number of days spent in each instar larvae or pupal stage was recorded in each cohort (the presence of exuviae indicated changes of instar/stage). Larval exuviae and dead larvae/pupae were removed and counted. Total larva and pupa development time was recorded as the sum of days spent in each stage. Longevity of pupae surviving to adulthood (longevity success) and longevity of pupae that failed to reach adult stage (longevity failure) were discriminated. The number of living and dead males and females was recorded daily, and adult longevity was estimated. Survival was expressed as the percentage of individuals that reached the next instar larvae or pupal stage.

2.3. Data analysis

Descriptive summary measures were obtained (mean ± standard error) in order to assess the variation of immature and adult life history traits across seasons. The effect of season on larva and pupa development time and survival was analyzed using generalized linear models (GLM) [39]. Six separate models that included factor season (4 levels: Spring-Summer, Summer-Autumn, Autumn-Winter and Summer) as a fixed effect were tested. The response variables total larva, total pupa and total larva + pupa development time were modeled assuming a Gaussian distribution and log link function. A Beta distribution and logit link function were used for total larva, total pupa and total larva + pupa survival. Since the survival response variables were expressed as proportions, and assumes the extremes 0 and 1 values, we applied the following transformation described by Ref. [40]:

$$(\Upsilon \times (\eta - 1) + 0.5)/\eta$$

where, Υ is the response variable survival, and η the sample size. Residual plots were examined for model validation following the protocol described by Ref. [39]. The percentage of explained deviance by each model was estimated as a measure of goodness of fit. Also pseudo-R^2 values for survival models are shown. Modelling was performed in R software, Version 4.1.0 [41], via the RStudio software, Version 1.4.1106 [42], using R stats [41] and betareg [40] packages.

To better understand the longevity of pupae surviving to adulthood and the longevity of pupae that failed to reach adult stage across seasons, three univariate GLM models were performed. Factor treatment (two levels: longevity success and longevity failure) was included as a fixed effect, and the response variable mean of longevity was modeled with a Gaussian distribution and log link function. Statistical analyses were conducted using R software in RStudio, and R stats package.

GLM with a Gaussian distribution and log link function was employed to test the effects of season (3 levels: Spring-Summer, Summer-Autumn and Summer), sex (male and female), and their interactions on mean adult emergence time and longevity. An automatic backward step-wise approach was applied for model selection (likelihood ratio test) using the command "drop1". Statistical analyses were conducted using R software in RStudio, and R stats package.

Temperature development threshold was estimated by linear regression of development rate as function of mean temperature. The linear model expressed below evaluated the relationship between development rate of *Cx. acharistus* larva + pupa and temperature:

$$r = a + bT$$

where, *r* is the development rate expressed as the reciprocal of development time, *T* is the mean ambient temperature (°C), while the intercept (a) and slope (b) are model parameters. The value of the x-axis obtained by extrapolation of the straight line (below which no development occurs) was determined as the development thermal threshold [43]. Statistical analysis was conducted using R software in RStudio, and R stats package.

Physiological time was estimated as the cumulative product of total development time multiplied by the mean ambient temperature above the temperature development threshold:

$$K = t(T - c)$$

where, *K* is the physiological time measured in degree-days, *t* is the time from start of development to completion (larva + pupa), *T* is the mean ambient temperature, and *c* is the estimated temperature development threshold [8].

All R codes and data are available on figshare (https://doi.org/10.6084/m9.figshare.20338245) [44].

3. Results

### 3.1. Temperature fluctuation: natural thermal profiles

Natural fluctuating temperature profiles across seasons are shown in Fig. 2a. The mean temperature ranged from 5.6°C during autumn-winter season to 17°C in spring-summer. Large temperature fluctuations were recorded at high mean temperatures, whereas small fluctuations occurred at low mean temperatures, with an observed decrease in temperature amplitude from 10.2°C (spring-summer) to 4.4°C (autumn-winter) (Fig. 2b). The highest maximum temperature was 27.5°C, being recorded during spring-summer. Minimum temperatures were mostly above 0°C except for autumn-winter season, where the lowest minimum temperature was −6.4°C, being this autumn-winter experiment the longest and lasted ≅100 days. After day 50, mean temperature dropped below 0°C, and freezing temperatures and snowfalls were recorded. At least three consecutive freeze events occurred, of 2, 4 and 7 days of duration each one, at mean temperatures ≅1.6, −1.2 and −1.9°C, respectively (Fig. 2a).

3.2. Culex acharistus life history traits: larvae and pupae – adult transition

Larva and pupa life history traits obtained across seasons are shown in Supplementary Table S1 and Fig. 3. The mean larval, pupae and larva + pupa development time differed significantly between seasons (Table 1). The mean larval development time ranged from 15.3 to 48.3 days, at spring-summer and autumn-winter, respectively. The mean pupal development time was ≅4 days at 15.4 and 17°C, and took up to 4 days longer at the end of summer-beginning of autumn season (7.6 days). Total development time (larva + pupa) followed a similar pattern, increasing from ≅20 days at 15.4 and 17°C, to 32 days at 12.7°C (Supplementary Table S1, Fig. 3a).

A significant effect of season on larval, pupal and larva + pupae survival was observed (Table 1). Larvae reached the pupal stage during the four study periods, being mean larval survival greatest at 17°C (73%), showing lower values toward the autumn-winter (10%) (Fig. 3b). Summer and summer-autumn seasons yielded higher pupal survival values (averaging ≅90%). No pupal survival was observed during the cold season experiment; whereas, the highest larva + pupa survival was recorded at the end of summer-beginning of autumn (62%).

At high mean temperatures, longevity of pupae surviving to adulthood was significantly greater than longevity failure, being ≅4 and 2 days, respectively (Table 2; Supplementary Table S2). During summer-autumn, similar trends were observed for both groups (longevity success and failure: 8 days). Then, during the cold season experiment, 100% of pupae failed to reach adult stage and only longevity failure values were recorded (Fig. 3b). Individuals spent a mean of 24 days as pupae, but then died.
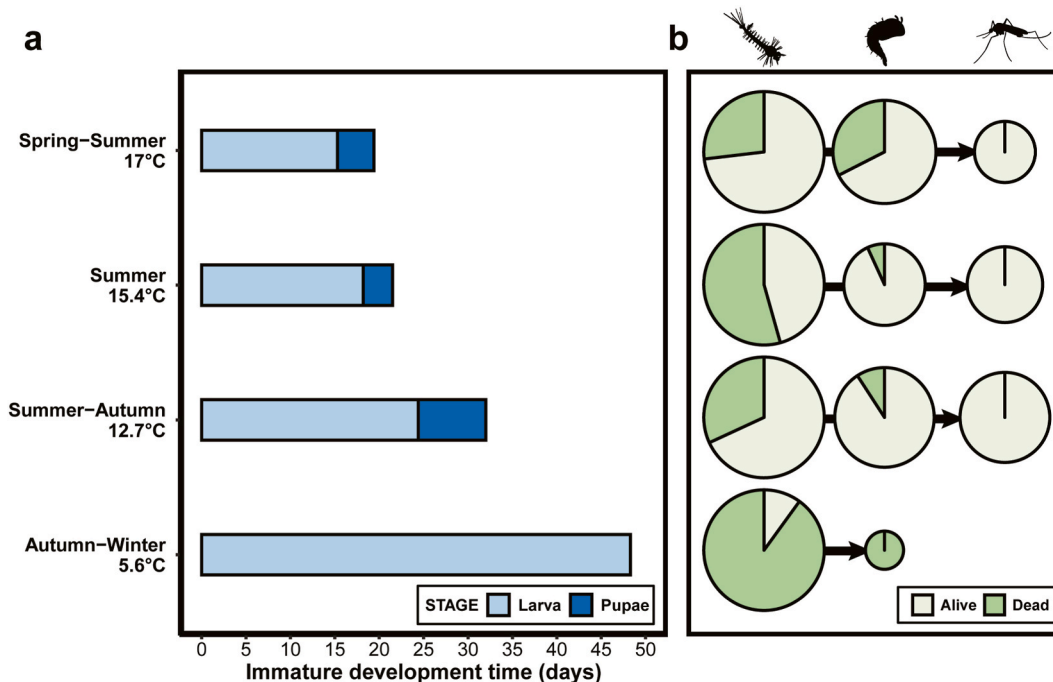
Fig. 3. Larva and pupa life history traits. a) Staked bar graph showing mean development time for larva (light blue) and pupa (dark blue), and b) number of dead (dark green) and alive (light green) larvae, pupae and adults of *Culex acharistus*, during each study period (Spring-Summer, Summer, Summer-Autumn and Autumn-Winter). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1

–GLM results. Generalized linear models results for the effect of season on immature development time and survival. Explanatory variables, parameter estimates (β) (±SE), t or z- and p-values, explained deviance or pseudo-R^2 are shown for each model.

Model	Explanatory variables	β ± SE	t or z-value	p-value	Explained deviance	Pseudo-R^2
Total larva development time	Intercept	15.3 ± 0.4	39	<0.001	99.7%	–
	Summer-Autumn	9 ± 0.6	16.2	<0.001		
	Autumn-Winter	32.9 ± 0.6	59.3	<0.001		
	Summer	2.8 ± 0.6	5	<0.001		
Total pupa development time	Intercept	4.1 ± 0.3	16.5	<0.001	95%	–
	Summer-Autumn	3.5 ± 0.4	10	<0.001		
	Summer	-0.8 ± 0.4	-2.2	0.052		
Total larva + pupa development time	Intercept	19.4 ± 0.5	37.4	<0.001	97.4%	–
	Summer-Autumn	2.5 ± 0.7	17	<0.001		
	Summer	2.1 ± 0.7	2.8	0.021		
Total larva survival	Intercept	1 ± 0.2	4.2	<0.001	–	85%
	Summer-Autumn	-0.2 ± 0.3	0.5	0.6		
	Autumn-Winter	-3±0.4	-8	<0.001		
	Summer	-1.1 ± 0.3	-3.7	<0.001		
Total pupa survival	Intercept	0.7 ± 0.2	2.9	0.004	–	93%
	Summer-Autumn	1.4 ± 0.4	3.3	<0.001		
	Autumn-Winter	-3.6 ± 0.5	-7	<0.001		
	Summer	1.5 ± 0.4	3.5	<0.001		
	Intercept	-0.02 ± 0.2	-0.1	0.9	–	94%
Total larva + pupa survival	Summer-Autumn	0.5 ± 0.3	1.8	0.08		
	Autumn-Winter	-3±0.4	-7	<0.001		
	Summer	-0.3 ± 0.3	-0.9	0.3		

-The reference category in the model is Season_Spring-Summer.
-Number of experimental units (cohorts of 40 larvae) used in each model: n = 16.

Table 2

–GLMM results. Generalized linear models results for the effect of treatments (success and failure) on mean longevity of pupae at three seasons. Explanatory variables, parameter estimates (β) (±SE), t - and p-values, and explained deviance are shown for each model.

Model	Explanatory variables	β ± SE	t-value	p-value	Explained deviance
Longevity Summer	Intercept	3.8 ± 0.3	12.6	<0.001	83.5%
	Longevity_failure	-2.3 ± 0.5	-5.1	0.004	
Longevity Summer-Autumn	Intercept	7.8 ± 0.6	12.9	<0.001	15%
	Longevity_failure	0.9 ± 0.9	0.9	0.4	
Longevity Spring-Summer	Intercept	4.1 ± 0.2	24.5	<0.001	92.5%
	Longevity_failure	-2.1 ± 0.2	-9	<0.001	

-The reference category in the model is Longevity_success
-Number of experimental units (each pupa) used in each model: n = 74 (Longevity Summer), n = 109 (Longevity Summer-Autumn) and n = 116 (Longevity Spring-Summer).

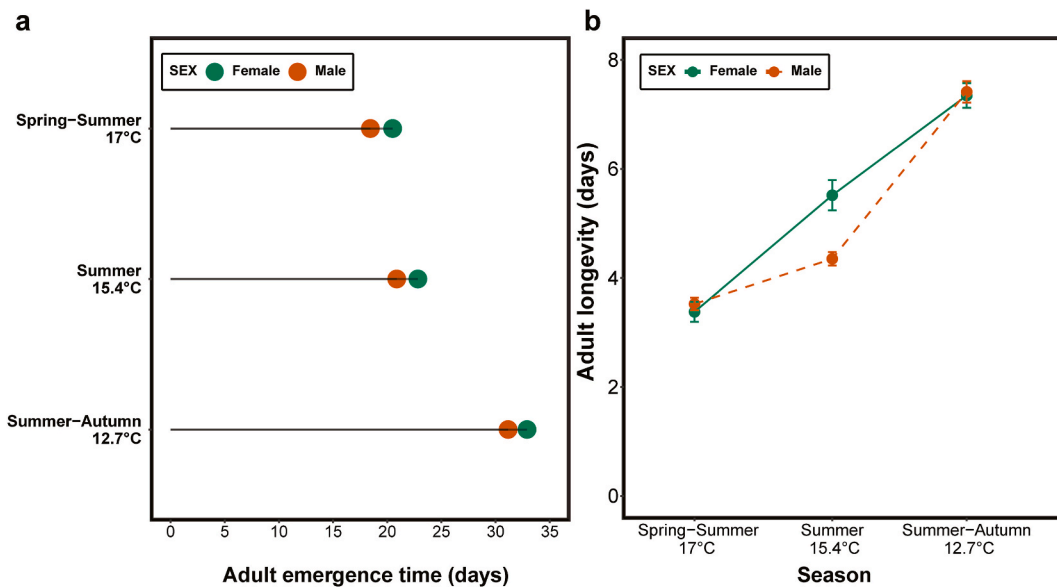### 3.3. Culex acharistus life history traits: males and females adults

Male and female adults emerged before at higher temperatures compared to lower temperatures, but had a much shorter longevity (Supplementary Table S3 and Fig. 4). Time from hatching to adult emergence significantly increased with decreasing temperatures, and males always emerged ≅ 2 days before than females (Table 3 and Fig. 4a). A significant effect of season by sex interaction was observed for adult longevity (Table 3). Longevity was higher as temperature decreased, and only at summer season the longevity of females (5.5 days) was significantly greater than that of males (4.4 days) (Fig. 4b).

3.4. Temperature development threshold and physiological time

Linear regression of the development rate (1/development time) as function of temperature was significant (t = 13; p = 1.4e^{-07}). Temperature development threshold and physiological time estimated for larva + pupa were 5.98°C and 211.24°-days, respectively (Supplementary Fig. S1).

4. Discussion

The results of our study showed the role of ambient temperature under fluctuating conditions in shaping immature and adult *Cx. acharistus* life history traits on its southern limit of distribution. We found that larva and pupa development time, adult emergence time and longevity significantly increased with decreasing temperatures, while larval survival was greatest at an intermediate temperature and decreased toward low and high values. We also estimated two important developmental parameters for larva + pupa, the

Fig. 4. Adult life history traits. a) Mean adult emergence time, and b) adult longevity (±SE), for male and female *Culex acharistus* at each study period (Spring-Summer, Summer and Summer-Autumn).

Table 3
–GLMM results. Generalized linear models results for the effect of season and sex on mean adult emergence time and longevity. Explanatory variables, parameter estimates (β) (±SE), t - and p-values, and explained deviance are shown for each model.

Model	Explanatory variables	β ± SE	t-value	p-value	Explained deviance
Adult emergence time (days)	Intercept	20.4 ± 0.6	32.5	<0.001	94%
	Summer-Autumn	12.6 ± 0.8	16.3	<0.001	
	Summer	2.4 ± 0.8	3.01	0.006	
	Male	-1.9 ± 0.6	-3.05	0.006	
Longevity of adults (days)	Intercept	3.5 ± 0.2	14.5	<0.001	94%
	Summer-Autumn	4 ± 0.3	11.5	<0.001	
	Summer	2.1 ± 0.3	6	<0.001	
	Male	-0.06 ± 0.3	-0.2	0.9	
	Summer-Autumn*Male	-0.03 ± 0.5	-0.07	0.9	
	Summer*Male	-1.1 ± 0.5	-2.3	0.03	

-The reference category in the model is Season_Spring-Summer and Sex_female
-p-values from drop1 function for Adult emergence time: Season (3.4e-15) and Sex (0.002).
-p-values from drop1 function for Longevity of adults: Season*Sex (0.02).
-Number of experimental units (cohorts of 40 larvae) used in each model: n = 12.

temperature development threshold and physiological time.

4.1. Natural autumn-winter thermal fluctuations and pupal survival

The mean duration of the experiments varied between 28 and 44 days for spring-summer, summer and summer-autumn seasons, being the cold season experiment the longest ($\cong$ 100 days). During the autumn-winter pupae experienced the most severe temperatures and freeze-thaw cycles, and failed to reach adult stage. Small plastic flasks containing pupae with dechlorinated water were completely frozen from the top to the bottom, for at least three consecutive periods. However, in nature, the size and water volume of *Culex* larval habitats from Patagonia is usually bigger than that of the present study [45], and immature mosquitoes could became protected from a freeze event. Aquatic habitats during cold season commonly develop a layer of surface ice, insulating the underlying water from frigid air temperatures and windchill. Thus, submerged insects are likely to experience warmer winter temperatures than terrestrial insects [46]. Cold-stressed pupae from our study were able to restore their locomotion after the end of each cold period, but then all individuals perished later during the development. Pupae were not able to repair the injuries and did not metamorphosed into adults. Delayed mortality, a mortality which does not occur immediately upon cold stress but instead later during ontogenesis, was also observed in cryopreserved drosophilid fly larvae, where the majority of individuals failed to proceed in ontogenesis [47]. Cold injury occurs at the molecular level in terms of damage to proteins, nucleic acids and biological membranes. The damage occurs as a result of the interaction between temperature, ice formation and secondary effects (e.g. osmotic stress, concentration of solutes, cellular

dehydration and oxidative stress) [48]. The ability to tolerate or even actively repair the injuries is generally attributed to a cold-induced regulation of the activity of cellular protective systems [49,50].

4.2. Linking temperature and life history traits

Larva and pupa development time significantly increased with decreasing temperatures, from spring-summer to autumn-winter. The same relationship was observed in other *Culex* species under constant [7,51], or fluctuating [52] laboratory temperature regimens. Shorter mean development times than the present study were recorded for *Cx. quinquefasciatus*, *Cx. pipiens* and *Cx. herperi* (12.6 and 16.5, 17.4 and 20.9 days, respectively) [7,51–53]. In general, temperature amplitude reported by these authors were higher than the present study, with higher maximum (25–34°C) and minimum (15–16.6°C) temperatures. Only, under a constant temperature of 7°C immatures of *Cx. pipiens* failed to reach pupa stage [51], and the mean development time for each instar larvae were similar than those reported in our cold season experiment. The larva and pupa survival values recorded in this study were highest at an intermediate temperature of 12.7°C, and decline towards both, lower and higher temperatures. A similar pattern was described for *Cx. quinquefasciatus* and *Cx. pipiens* immature survival being greatest at 25°C; while lower values below 50% were recorded at extreme low and high temperatures [7,51]. Only for *Cx. quinquefasciatus* under fluctuating temperature regimen, immature survival increased linearly with increasing temperatures, between 32 and 88% in the range of 16–25.2°C [52].

In accordance with previous studies in other *Culex* species [7,51,53–55], results here showed that time from hatching to adult emergence and adult longevity significantly decreased with increasing temperatures. Thus, increasing temperatures could lead to a more rapid population proliferation; however, adults will survive for a shorter period. In the present study, males emerge 2 days before females across seasons. Protandry is widespread among animals and particular common among insects [56], and has been documented in many mosquito species belonging to *Aedes* [57], *Culex* [58], *Wyeomyia* [59] and *Toxorhynchites* [60]. In short-lived animals in seasonal environments, with discrete generations, it has been argued that males maturing before females may gain a fitness advantage by increasing their mating opportunities (direct selection hypothesis). Alternatively, the sex difference in age at maturity may also be a side-effect of sexually dimorphic selection on body size (indirect selection hypothesis) [56]. Trade-offs in development time versus mass at eclosion has been reported for male and female mosquitoes, with smaller and fast-growing males, and females maximizing mass (positively related to fecundity) but taking longer to develop [61].

4.3. Temperature development threshold and physiological time

Development rate, temperature development threshold and physiological time are fundamental features of ectothermic organisms, and are essential elements for developing population dynamics models [8,62]. According with our results, once the ambient temperature became greater than the estimated development thermal threshold of 5.98°C, it will be necessary 211.24°-days to complete the larval + pupal development of *Cx. acharistus*. Temperature development threshold reported for *Cx. quinquefasciatus* was usually higher than the present study, and varied between 9.5, 10 and 10.9°C [52,63,64]. That species is present throughout Argentina (22–41°S), except its southernmost end [31,32]. However, for *Cx. pipiens* and *Cx. eduardoi*, both species widely distributed through northern and central areas, up to southern Patagonia region (36–52°S) [31,32], the estimated development thresholds were lower of 5 and 5.7°C, respectively [51,65]. Reductions in thermal threshold and increases in physiological time have also been recorded for insect species with increasing latitude or altitude [43,62]. Thus, understanding these parameters is of the utmost important in explaining adaptations of insect life histories to resources that vary seasonally [62].

5. Conclusions

Our study reveals that seasonal changes in temperature had a pronounced effect on larvae, pupae and adult *Cx. acharistus* life history traits from central Patagonia. Understanding these relationships is needed to better define the potential impact of temperature changes on mosquito species distribution, abundance, morphology, behavior and phenology. Regional simulations of climate change under A2 and B2 IPCC emission scenarios, for the period 2081–2090 over southern South America, predicted a warming in the range of 1.5–5.5°C (A2) and 1–4°C (B2), with minimum changes in the mean temperature projected for higher latitudes south of 35°S in the Patagonia region. Projections showed an increase more or less constantly of surface air temperature throughout the entire Patagonia region ($\cong$ 1–3°C of warming), and a significant decrease in annual precipitation in north-west Patagonia [66]. Consequently, our study suggest that a shift in climate towards hotter conditions in Patagonia may decrease *Cx. acharistus* larva and pupa development time and time from hatching to adult emergence, and lead to a more rapid population proliferation. However, adult longevity and survival will decreased with increasing temperatures. Future studies should experimentally test for the effects of hotter conditions, similar to those projected for Patagonia, on mosquito life history traits. That will be particularly important in understanding ecological responses of different life stages to variations and extremes in environmental temperature. Also, the potential use of species distribution models to monitor the distribution pattern of *Cx. acharistus* and other *Culex* or *Aedes* species of sanitary importance in Patagonia, or to mapping potential vector-borne diseases spread in the region, could became important in a context of environmental change and its ecological consequences. Photoperiod, not addressed in the present study, may also contribute to the observed patterns of immature development time and survival, and adult emergence time and longevity, and should be taken into consideration. During spring-summer, summer-autumn, autumn-winter and summer photoperiod was 14:56–9:03, 11:38–12:21, 9:45–14:14 and 13:02–10:58 h of light-dark [67], respectively. The parameters obtained in the present study constitute baseline data for the region, and can be used as useful parameters in the development of population dynamic models, improving current mosquito control strategies in cold-temperate

regions.

Author contribution statement

Marta Gladys Grech, Dr: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper. María Laura Miserendino: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper. Walter Ricardo Almirón: Conceived and designed the experiments; Wrote the paper.

Funding statement

Data availability statement

Data associated with this study has been deposited at Figshare Repository https://doi.org/10.6084/m9.figshare.20338245.

Declaration of interest's statement

The authors declare no competing interests.

Acknowledgements

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e13696.

References

[1] C.A. Deutsch, J.J. Tewksbury, R.B. Huey, K.S. Sheldon, C.K. Ghalambor, D.C. Haak, P.R. Martin, Impacts of climate warming on terrestrial ectotherms across latitude, Proc. Natl. Acad. Sci. USA 105 (2008) 6668–6672.
[2] B.J. Sinclair, C.M. Williams, J.S. Terblanche, Variation in thermal performance among insect populations, Physiol. Biochem. Zool. 85 (2012) 594–606.
[3] B.J. Sinclair, L.E. Coello Alvarado, L.V. Ferguson, An invitation to measure insect cold tolerance: methods, approaches, and workflow, J. Therm. Biol. 53 (2015) 180–197.
[4] F. Johansson, G. Orizaola, V. Nilsson-Örtman, Temperate insects with narrow seasonal activity periods can be as vulnerable to climate change as tropical insect species, Sci. Rep. 10 (2020) 8822.
[5] S.L. Chown, S.W. Nicolson, Insect Physiological Ecology: Mechanisms and Patterns, Oxford University Press, Oxford, 2004.
[6] D.L. Denlinger, R.E. Lee, Insect Low Temperature Biology, Cambridge University Press, Cambridge, 2010.
[7] L.M. Rueda, K.J. Patel, R.C. Axtell, R.E. Stinner, Temperature-dependent development and survival rates of *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae), J. Med. Entomol. 27 (1990) 892–898.
[8] A.N. Clements, The Biology of Mosquitoes, in: Development, Nutrition and Reproduction, vol. I, Chapman & Hall, London, 1992.
[9] G. Kuno, Review of the factors modulating dengue transmission, Epidemiol. Rev. 17 (1995) 321–335.
[10] O.J. Brady, M.A. Johansson, C.A. Guerra, S. Bhatt, N. Golding, D.M. Pigott, H. Delatte, M.G. Grech, P.T. Leisnham, R. Maciel-de-Freitas, L.M. Styer, D.L. Smith, T.W. Scott, P.W. Gething, S.I. Hay, Modelling adult *Aedes aegypti* and *Aedes albopictus* survival at different temperatures in laboratory and field settings, Parasites Vectors 6 (2013) 351.
[11] R. Bellone, A.B. Failloux, The role of temperature in shaping mosquito-borne viruses transmission, Front. Microbiol. 11 (2020), 584846.
[12] W. Tun-Lin, T.R. Burkot, B.H. Key, Effects of temperature and larval diet on development rates and survival of the dengue vector *Aedes aegypti* in north Queensland, Australia, Med. Vet. Entomol. 14 (2000) 31–37.
[13] E.A.P.A. Costa, E.M.M. Santos, J.C. Correia, C.M.R. Albuquerque, Impact of small variations in temperature and humidity on the reproductive activity and survival of *Aedes aegypti* (Diptera, Culicidae), Rev. Bras. Entomol. 54 (2010) 488–493.
[14] J. Couret, M.Q. Benedict, A meta-analysis of the factors influencing development rate variation in *Aedes aegypti* (Diptera: Culicidae), BMC Ecol. 14 (2014) 3.
[15] D.A. Ewing, C.A. Cobbold, B.V. Purse, M.A. Nunn, S.M. White, Modelling the effect of temperature on the seasonal population dynamics of temperate mosquitoes, J. Theor. Biol. 400 (2016) 65–79.
[16] P. Jia, L. Liang, X. Tan, J. Chen, X. Chen, Potential effects of heat waves on the population dynamics of the dengue mosquito *Aedes albopictus*, PLoS Neglected Trop. Dis. 13 (2019), e0007528.
[17] M.S. De Majo, P. Montini, S. Fischer, Egg hatching and survival of immature stages of *Aedes aegypti* (Diptera: Culicidae) under natural temperature conditions during the cold season in Buenos Aires, Argentina, J. Med. Entomol. 54 (2017) 106–113.
[18] G. Ruedas, H. Peña, A. Brisco, K.K. Fujioka, J.W. Wekesa, Life histories and other biological characteristics enabling the establishment of *Aedes albopictus* in the San Gabriel Valley, California, J. Am. Mosq. Control Assoc. 34 (2018) 93–98.
[19] S. Fischer, M.S. De Majo, C.M. Di Battista, P. Montini, V. Loetti, R.E. Campos, Adaptation to temperate climates: evidence of photoperiod-induced embryonic dormancy in *Aedes aegypti* in South America, J. Insect Physiol. 117 (2019), 103887.
[20] R.J. Wilson, R. Fox, Insect responses to global change offer signposts for biodiversity and conservation, Ecol. Entomol. 46 (2021) 699–717.
[21] C.A. Halsch, A.M. Shapiro, J.A. Fordyce, C.C. Nice, J.H. Thorne, D.P. Waetjen, M.L. Forister, Insects and recent climate change, Proc. Natl. Acad. Sci. USA 118 (2021), e2002543117.

[22] A.K. Githeko, S.W. Lindsay, U.E. Confalonieri, J.A. Patz, Climate change and vector-borne diseases: a regional analysis, Bull. World Health Organ. 78 (2000) 1136–1147.

[23] J. Rocklöv, R. Dubrow, Climate change: an enduring challenge for vector-borne disease prevention and control, Nat. Immunol. 21 (2020) 479–483.

[24] L.I. Couper, J.E. Farner, J.M. Caldwell, M.L. Childs, M.J. Harris, D.G. Kirk, N. Nova, M. Shocket, E.B. Skinner, L.H. Uricchio, M. Exposito-Alonso, E.A. Mordecai, How will mosquitoes adapt to climate warming? Elife 10 (2021), e69630.

[25] G.C. Rossi, Nuevos registros y actualización del listado de mosquitos (Diptera: Culicidae) del área de la Represa de Salto Grande, Uruguay, Bol. Soc. Zool. Uruguay. 23 (2014) 51–59.

[26] F.M. Root, Studies on Brazilian mosquitoes III. The genus *Culex*, Am. J. Hyg. 7 (1927) 574–598.

[27] D. Rosero-García, J.D. Suaza-Vasco, J. Barajas-Galindo, S. Uribe-Soto, Mosquito fauna (Diptera: Culicidae) of high Andean mountain ecosystems in Colombia, Int. J. Mosq. Res. 4 (2017) 10–24.

[28] C.R. González, M.I. Jercic, L. Muñoz, The mosquitoes from Chile (Diptera: Culicidae), Acta Entomol. Chil. 29 (2005) 31–35.

[29] C.R. González, C. Reyes, M.I. Jercic, V. Rada, M. Saldarriaga, C. Pavletic, A. Parra, Manual de culícidos (Diptera: Culicidae) de la zona norte y centro de Chile, incluyendo Isla de Pascua. Segunda Edición. Santiago, Chile, Instituto de Salud Pública, 2016. Ministerio de Salud. Available in: https://www.ispch.cl/sites/default/files/ManualCulicidosV02.pdf.

[30] S.E. Fick, R.J. Hijmans, Worldclim 2: new 1-km spatial resolution climate surfaces for global land areas, Int. J. Climatol. 37 (2017) 4302–4315.

[31] G.C. Rossi, Annotated checklist, distribution, and taxonomic bibliography of the mosquitoes (Insecta: Diptera: Culicidae) of Argentina, Check List. 11 (2015) 1712.

[32] R.E. Campos, M. Laurito, Culicidae (Diptera) Species from Argentina and Uruguay, 2022 [May 3, 2022], https://biodar.unlp.edu.ar/culicidae/.

[33] R.E. Campos, R.M. Gleiser, Mosquitos que crían en microambientes acuáticos naturales, in: C.M. Berón, R.E. Campos, R.M. Gleiser, L.M. Díaz-Nieto, O. D. Salomón, N. Schweigmann (Eds.), Investigaciones sobre mosquitos de Argentina, Universidad Nacional de Mar del Plata, Mar del Plata, 2016, pp. 119–141.

[34] G. Avilés, S. Medeot, G. Díaz, T.I. Bianchi, E. Bakos, J. Daffner, W.R. Almirón, M. Brewer, M.S. Sabatini, Estudios en caballos y mosquitos durante una epizootia por virus de la Encefalitis Equina del Este (EEE) en una zona subtropical de Argentina. 12 Reunión Científica, Sociedad Argentina de Virología, 1989.

[35] R. Gallardo, G. Albrieu, M. Laurito, A. Mansilla, M. Beranek, M.S. Contigiani, L. Spinsanti, Primera evidencia de circulación de virus BUNYAMWERA ORTHOBUNYAVIRUS en la provincia de Formosa, XX Jornada de Investigación Científica. Córdoba, Argentina, 2019.

[36] Servicio Meteorológico Nacional Argentina, 2022. Clima de Argentina. Estadísticas de largo plazo. Available in: https://www.smn.gob.ar/estadisticas.

[37] R.F. Darsie, The mosquitoes of Argentina: part I Keys for identification of adult females and fourth stage larvae in English and Spanish (Diptera: Culicidae), Mosq. Systemat. 17 (1985) 153–253.

[38] E.J. Gerberg, D.R. Barnard, R.A. Ward, Manual for mosquito rearing and experimental techniques, J. Am. Mosq. Contrl. Assoc. Bull. 5 (1994) 1–98.

[39] A.F. Zuur, E.N. Ieno, N.J. Walker, A.A. Saveliev, G.M. Smith, Mixed Effects Models and Extensions in Ecology with R, Springer, New York, 2009.

[40] F. Cribari-Neto, A. Zeileis, Beta regression in R, J. Stat. Software 34 (2010) 1–24.

[41] R Core Team, R: a Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2021. www.R-project.org/.

[42] RStudio Team, RStudio: Integrated Development for R. RStudio, PBC, Boston, MA, 2021. URL, www.rstudio.com/.

[43] A. Campbell, B. Frazer, N. Gilbert, A. Gutierrez, M. Mackauer, Temperature requirements of some aphids and their parasites, J. Appl. Ecol. 11 (1974) 431–438.

[44] Grech, M.G, Data and R code for Culex acharistus, Figshare (2022), https://doi.org/10.6084/m9.figshare.20338245.

[45] M.G. Grech, L.M. Manzo, L.B. Epele, M. Laurito, A.N. Claverie, F.F. Ludueña-Almeida, M.M. Miserendino, W.R. Almirón, Mosquito (Diptera: Culicidae) larval ecology in natural habitats in the cold temperate Patagonia region of Argentina, Parasites Vectors 12 (2019) 214.

[46] M.V. Moore, R.E. Lee Jr., Surviving the big chill: overwintering strategies of aquatic and terrestrial insects, Am. Entomol. 37 (1991) 111–118.

[47] T. Štětina, P. Hůla, M. Moos, P. Šimek, P. Šmilauer, V. Košta, Recovery from supercooling, freezing, and cryopreservation stress in larvae of the drosophilid fly, *Chymomyza costata*. Sci. Rep. 8 (2018) 4414.

[48] J. Rozsypal, Cold and freezing injury in insects: an overview of molecular mechanisms, Eur. J. Entomol. 119 (2022) 43–57.

[49] B.J. Sinclair, C.J. Klok, S.L. Chown, Metabolism of the sub-Antarctic caterpillar *Pringleophaga marioni* during cooling, freezing and thawing, J. Exp. Biol. 207 (2004) 1287–1294.

[50] L. Lalouette, C.W. Williams, F. Hervant, B. Sinclair, D. Renault, Metabolic rate and oxidative stress in insects exposed to low temperature thermal fluctuations, Comp. Biochem. Physiol. 158 (2011) 229–234.

[51] V. Loetti, N. Schweigmann, N. Burroni, Development rates, larval survivorship and wing length of *Culex pipiens* (Diptera: Culicidae) at constant temperatures, J. Nat. Hist. 45 (2011) 2207–2217.

[52] M.G. Grech, P.D. Sartor, W.R. Almirón, F.F. Ludueña-Almeida, Effect of temperature on life history traits during immature development of *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae) from Córdoba city, Argentina, Acta Trop. 146 (2015) 1–6.

[53] V. Loetti, N. Burroni, P. Prunella, N. Schweigmann, Effect of temperature on the development time and survival of preimaginal *Culex hepperi* (Diptera: Culicidae), Rev. Soc. Entomol. Argent. 67 (2008) 79–85.

[54] S.S. Andreadis, O.C. Dimotsiou, M. Savopoulou-Soultani, Variation in adult longevity of *Culex pipiens* f. *pipiens*, vector of the West Nile Virus, Parasitol. Res. 113 (2014) 4315–4319.

[55] A.T. Ciota, A.C. Matacchiero, A.M. Kilpatrick, L.D. Kramer, The effect of temperature on life history traits of *Culex* mosquitoes, J. Med. Entomol. 51 (2014) 55–62.

[56] T. Teder, A. Kaasik, K. Taits, T. Tammaru, Why do males emerge before females? Sexual size dimorphism drives sexual bimaturism in insects, Biol. Rev. 96 (2021) 2461–2475.

[57] C.A. Kleckner, W.A. Hawley, W.E. Bradshaw, C.M. Holzapfel, I.J. Fisher, Protandry in *Aedes sierrensis*: the significance of temporal variation in female fecundity, Ecol. 76 (1995) 1242–1250.

[58] M.V. Loetti, N.E. Burroni, N. Schweigmann, A. de Garin, Effect of different thermal conditions on the pre-imaginal biology of *Culex apicinus* (Philippi, 1865) (Diptera: Culicidae), J. Vector Ecol. 32 (2007) 106–111.

[59] C.M. Holzapfel, W.E. Bradshaw, Protandry: the relationship between emergence time and male fitness in the pitcher-plant mosquito, Ecol. 83 (2002) 607–611.

[60] L.P. Lounibos, R.L. Escher, D. Duzak, E.A. Martin, Body size, sexual receptivity and larval cannibalism in relation to protandry among *Toxorhynchites* mosquitoes, Oikos 77 (1996) 309–316.

[61] D.A. Yee, S.A. Juliano, S.M. Vamos, Seasonal photoperiods alter developmental time and mass of an invasive mosquito, *Aedes albopictus* (Diptera: Culicidae), across its north-south range in the United States, J. Med. Entomol. 49 (2012) 825–832.

[62] F. Taylor, Ecology and evolution of physiological time in insects, Am. Nat. 117 (1981) 1–23.

[63] W.R. Almirón, M.E. Brewer, Winter biology of *Culex pipiens quinquefasciatus* Say, (Diptera: Culicidae) from Córdoba, Argentina, Mem. Inst. Oswaldo Cruz 91 (1996) 649–654.

[64] M. Mogi, Temperature and photoperiod effects on larval ovarian development of New Zealand strains of *Culex quinquefasciatus* (Diptera: Culicidae), Ann. Entomol. Soc. Am. 85 (1992) 58–66.

[65] V. Loetti, N. Schweigmann, N. Burroni, Temperature effects on the immature development time of *Culex eduardoi* Casal & García (Diptera: Culicidae), Neotrop. Entomol. 40 (2011) 138–142.

[66] M.N. Nuñez, S.A. Solman, M.F. Cabré, Regional climate change experiments over southern South America. II: climate change scenarios in the late twenty-first century, Clim. Dynam. 32 (2009) 1081–1095.

[67] Salida y Puesta del Sol, Salida y puesta del sol en Esquel, 2022. Available in: https://salidaypuestadelsol.com/sun/esquel.